THE USE OF SNAPPING TURTLE EGGS AS AN INDICATOR OF CONTAMINANT EXPOSURE IN COASTAL WETLANDS OF THE GREAT LAKES – ST. LAWRENCE BASIN

Presented to

The Great Lakes Coastal Wetland Consortium

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By

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Executive Summary

- Eggs of the common snapping turtle are excellent indicators of wetland health and contaminant bioavailability. Snapping turtle eggs provide excellent temporal and spatial trends information concerning organochlorine pesticides (e.g., DDT), polychlorinated biphenyls (PCBs), dioxins and furans. In addition, the eggs of this species are capable of providing information for such trends concerning newly emerging chemicals of concern (e.g., polybrominated diphenyl ethers (PBDEs).

- The snapping turtle has been ranked seventh out of 25 vertebrate species used as indicators of persistent organic pollutants (Golden and Rattner 2003).

- The health of snapping turtles has been adversely affected by contaminant exposure in the Great Lakes Basin (Bishop et al., 1991; Bishop et al., 1998).

- Snapping turtles inhabit many types of wetlands when suitable habitat is available, and have small home ranges with limited movement. These characteristics make this species a good reflector of local (point) sources of contaminants, as well as different chemical mixtures (e.g., Aroclors) of contaminants, in a wide variety of wetland types. They are also excellent indicators of the bioaccumulation of chemicals through the food chain.

- In a monitoring program, the annual collection of data is preferred to less frequent sampling in terms of providing the most robust data/information in the fewest number of years (Hebert and Weseloh, 2003).

- Multiple state and provincial agencies, volunteers, and paid staff with one coordinating agency, will have to be involved to adequately cover multiple wetlands/sites across a wide geographical area such as the Great Lakes Basin or even both Canadian and American sides of one of the Great Lakes. The extent of each agency's participation in this monitoring plan will have to be discussed

individually. Snapping turtles lay their eggs during the same two week period (usually the second and third weeks) of June regardless of their location in the Great Lakes Basin. They have not been found on the northern shores of Lake Superior.

- **A pilot project for the first three years of the program** is proposed for Lake Ontario or Lake Erie, with each of the three appropriate wetland types (lacustrine, riverine, barrier-protected) represented relative to their known or anticipated contaminant levels (high, medium and low; alternatively use a high-low contaminant classification); a total of 9 sites will be needed for this pilot study. Lake Michigan may be considered as an alternative for this pilot project but will obviously negate the bi-national aspect of the pilot monitoring program. Protected lacustrine, drowned river-mouth riverine, and barrier beach lagoon wetlands should contain high densities of snapping turtles, but, open lacustrine, connecting channel, delta riverine wetlands, and barrier-protected swale complexes, will have much lower densities of snapping turtles making sampling difficult. In order to achieve a good representation of lake-wide contaminant patterns, coastal wetlands should be located throughout the lake's shoreline although some clustering is likely to occur.

- **Subsequent to the pilot project,** an assessment of the data should be completed to determine if the type of wetland affects the contaminant concentrations found in snapping turtle eggs. If no effect of wetland type is found on these concentrations, then this factor should be removed from the experimental design. In order to determine an overall assessment of contaminant trends on a lake-wide basis, four locations (two Canadian, two American) within each of the three contaminant concentration categories (high, medium, low) should be selected on each of lakes Michigan, Huron, Erie and Ontario. This experimental design will provide a total of 48 sites and data for a bi-national assessment of the contaminants trends in coastal wetlands across all the Great lakes except Lake Superior. The number of sites may be reduced by only using sites that are of high or low levels of contamination; the total number of sites would be 32 using this design.

Alternatively, if wetland type does affect the contaminants levels in snapping turtle eggs, then a different experimental design will have to be employed to determine trends in contaminant levels using snapping turtle eggs. Wetland type (3) within each of the contaminant categories (high, medium, low) on each side (Canadian, American) of each of the Great Lakes (4) will result in the monitoring of 72 sites, or 48 sites if the contaminant categories are restricted to high, low classification.

Whether or not the experimental design accounts for wetland type, selected wetlands should be located throughout the basin of each lake, with the realization that some clustering will occur depending on the location of most wetlands.

- **Estimated Project Budget**: Based on 2004 project costs for snapping turtle work by the Canadian Wildlife Service, we estimate that the pilot study will cost approximately $171,025 CDN per year or a total of $513,075 CDN for three years for work completed on Lake Ontario. Sampling and analysis of eggs and data from each field collection site will cost approximately $12,925 CDN per year, but the costs for a full-time person, statistical analysis, and report writing (total $75,000 CDN) must still be accounted for. Following the pilot study, the cost for a basin-wide (four lakes) monitoring plan using snapping turtles is estimated to cost between $0.494 M CDN (32 sites) and $0.701 M CDN (48 sites) regardless of wetland type, or between $0.701 M CDN (48 sites) and $1.012 M CDN (72 sites) when accounting for wetland type. For these basin-wide program budgets, the $75 K CDN for the full-time person (coordinate program, complete statistical analysis and report writing) and eight additional agency co-ordinators ($60.0 K CDN) are included. These budgets may be pro-rated according to the number of sites in each state/provincial jurisdiction.

- A monitoring program for contaminants in snapping turtle eggs must involve the coordination of people, agencies, and groups to insure comparability and robustness of data, and that all protocols are followed in an appropriate manner.

Consequently, the program must follow the approved protocol outlined in the Quality Assurance Project Plan (QAPP) (see Appendix) to ensure the development and implementation of an integrated, bi-national monitoring program. The groups involved in the monitoring activities will coordinate their efforts through the use of this protocol in sampling procedures, sample and data analysis, and reporting methods, to insure a basin-wide, bi-lateral consistency in data collection and methodologies, thereby enhancing the comparability and value of the data in identifying spatial and temporal trends in contaminant levels.

1.0 Introduction

1.1 *Preamble*.

In preparing the documents involving the snapping turtle as a model for monitoring contaminants as requested by the Great Lakes Wetlands Consortium, the Monitoring Plan and the White Paper (Literature Review) have been combined in this report. Section 2.0 of this report outlines the monitoring plan for using snapping turtle eggs to determine trends in chemical concentrations found in coastal wetlands of the Great Lakes Basin, and hence the integrity of these coastal wetlands. Following the monitoring plan in Section 2.0, the White Paper addresses the six criteria previously established by the Consortium, relating to the utility, cost and validity of using snapping turtle eggs for measuring contaminant concentrations. In addition, we have appended the approved Quality Assurance Project Plan (QAPP) document (Project # WETLANDS2-EPA-05, Revision #3) required by the United States Environmental Protection Agency. All criteria for this project as stipulated by the Great Lakes Wetlands Consortium in the Request for Proposal are outlined below in the Introduction.

1.2 *Introduction*

This white paper describes the utility of the common snapping turtle (*Chelydra serpentina serpentina*) as an indicator of persistent organic contaminants in Great Lakes coastal wetlands. It originates from a need to consistently measure and monitor the status of wetland systems in terms of their degradation due to anthropogenic, persistent, organic

chemicals. Our major goal is to present a framework for a sustainable, long-term, basin-wide wetland contaminants monitoring plan. While monitoring chemical parameters in water and sediment generally reflect the degree of pollution, the measurement of contaminant concentrations in tissues of snapping turtles will provide a gauge of toxicant bioavailability in wetland environments. Thus, this white paper also validates the common snapping turtle as an indicator of chemical exposure, particularly local but non-specific sources of contaminants. The snapping turtle provides many advantages for monitoring contaminant levels in wetlands, including its wide geographic distribution, abundance in a variety of wetland systems, longevity, sedentary nature, its potential for bio-accumulating organic contaminants through its diet, and the ability of adult turtles to store high concentrations of polychlorinated biphenyls (PCBs) in their adipose tissue without apparent adverse effects (Meyers-Schöne and Walton, 1994). Moreover, egg samples for analysis of contaminant concentrations may be taken in sufficient quantities without seriously impacting adult populations (Cunnington and Brooks 1996).

The White Paper addresses six criteria that originate from the Request for Proposals (RFP) disseminated by the Great Lakes Commission on behalf of the Great Lakes Coastal Wetlands Consortium. These criteria fall under the "Scope of Work" in the RFP as one of the goals "to test the feasibility of applying indicators in a monitoring plan." The following are a list of questions posed by the Consortium that serve as the basis for the information discussed in this white paper:

- What is the cost of implementing a program using snapping turtle eggs to measure organochlorine contamination and pesticides, as well as the cost and availability of analytical methods to measure other chemicals of concern?
- Are contaminants measurable in snapping turtle eggs? What is the design and methodology best suited to obtain geographic and temporal contaminant trends in coastal wetlands, and how will wetland sites be chosen for the monitoring plan?
- How applicable and reliable is the snapping turtle in terms of measuring/monitoring contaminants in various wetland types across the upper and lower Great Lakes basin?

- What complementary existing research and data are available that is relevant to using the common snapping turtle to monitor contaminant levels?
- Are snapping turtles sensitive in terms of detecting changes in contaminant concentrations of wetlands over time and space?
- How useful is the snapping turtle for a monitoring plan in terms of being able to set endpoint(s) or attainment levels relative to contaminant levels in wetlands of the Great Lakes basin?

2.0 A Proposed Bi-National Monitoring Plan Utilizing the Snapping Turtle as a Sentinel Species for Contaminant Concentrations in Coastal Wetlands of the Great Lakes Basin

This section of the report will outline the proposed plan for monitoring the quality of Great Lakes coastal wetlands in terms of their degradation due to persistent organic contaminants utilizing snapping turtle eggs. The rationale for this plan, and the validation of using the snapping turtle as a basin-wide and within-lake indicator of contaminant bioavailability, are provided in subsequent sections of the White Paper.

In the RFP, the major objective of the snapping turtle monitoring program was to determine spatial and temporal trends in contaminant concentrations in the three types of coastal wetlands (lacustrine, riverine, and barrier-protected system) regardless of the location(s) of contaminant sources. However, the location of a wetland relative to the contaminant source, will determine the levels of contamination within that wetland as well. Consequently, we recommend that several wetlands of each type, at varying distances from contaminant sources, be selected on each lake (with the exception of Lake Superior). Such an approach will provide a better understanding of contaminant trends in different types of coastal wetlands at the larger scale of the individual lake and the basin as a whole. However, there are many coastal wetlands along the shoreline of each lake and the sampling of two or three wetlands of each type would not be properly representative of the coastal wetlands of that lake.

In selecting the wetlands for use in this snapping turtle monitoring plan, three other considerations must also be taken into account: (1) suitable habitat for adult snapping

turtles to inhabit and to lay eggs must be present at the wetland; (2) egg laying by snapping turtles generally occurs during the same 14 d period in the middle of June, regardless of their location within the Great Lakes Basin; (3) snapping turtles are not found along the northern shores of Lake Superior in Canada, nor are they likely to be found since existing wetlands do not have appropriate habitat and the Lake Superior environment is too cold.

In addition, the monitoring program must follow the approved protocol outlined in the Quality Assurance Project Plan (QAPP) (see Appendix). Development and implementation of an integrated, bi-national monitoring program requires that all participants have the most current version of the approved QAPP (Appendix). The groups involved in the monitoring activities will coordinate their efforts through the use of this protocol in sampling procedures, sample and data analysis, and reporting methods, to insure a basin-wide, bi-lateral consistency in data collection and methodologies, thereby enhancing the comparability and value of the data in identifying spatial and temporal trends in contaminant levels.

We recommend the following monitoring plan using snapping turtle eggs to achieve the objective of the Great Lakes Wetlands Consortium:

- Coastal Wetland Selection:
    - **A pilot project for the first three years of the program**: On Lake Ontario or Lake Erie, each of the three appropriate wetland types will be represented (if possible) relative to their contaminant levels (high, medium and low to serve as the reference site); a total of 9 sites will be needed for this pilot study. Lake Michigan may be considered as an alternative for this pilot project.
        - We recommend sampling protected lacustrine, drowned river-mouth riverine, and barrier beach lagoon wetlands as such habitats are likely to contain high densities of snapping turtles. However, open lacustrine, connecting channel, and delta riverine wetlands will have much lower densities of snapping turtles making sampling difficult; sampling of

wetlands and creeks near to these large wetlands may be an alternative. Similarly, barrier-protected swale complexes may also prove difficult as habitat will likely be unsuitable for snapping turtles.

- In order to achieve a good representation of the lake basin, wetlands should be located throughout the lake basin as much as possible. For example, most coastal wetlands in Lake Ontario are located in the eastern basin, so many of the sampling points will be located here. However, it is important that other wetlands of all types be chosen from the other areas throughout Lake Ontario in order to gain an understanding of lake-wide trends in contaminants in coastal wetlands.
- For many wetlands, contaminants levels are unlikely to be known but contaminant concentrations for water and sediment samples are available for many sites through universities and/or government agencies. In addition, selecting sites according to the distance from known contaminant sources (e.g., industry, sewage treatment plants, agricultural inputs; urban vs. rural areas) will aid in determining approximate contaminant levels in a wetland.
- Alternatively, only a reference site and a highly-contaminated site within each wetland type may be selected for the pilot work.

- **Subsequent to pilot project**: Following the pilot project, an assessment of the data should be completed to determine if the type of wetland affects the contaminant concentrations found in snapping turtle eggs. If no effect of wetland type is found on these concentrations, then this factor should be removed from the experimental design. In order to determine an overall assessment of

contaminant trends on a lake-wide basis, four locations (two Canadian, two American) within each of the three contaminant concentration categories (high, medium, low as a reference site) should be selected on each of lakes Michigan, Huron, Erie and Ontario. This experimental design will provide a total of 48 sites and data for a bi-national assessment of the contaminants trends in coastal wetlands. The number of sites may be reduced by only using sites that are of high and low levels of contamination; the total number of sites would be 32 using this design.

Alternatively, if wetland type does affect the contaminants levels in snapping turtle eggs, then a different experimental design will have to be employed to determine trends in contaminant levels using snapping turtle eggs. Wetland type (3) within each of the contaminant categories (high, medium, low) on each side (Canadian, American) of each of the Great Lakes (4) will result in the monitoring of 72 sites, or 48 sites if the contaminant categories are restricted to high, low classification.

Whether or not the experimental design accounts for wetland type, selected coastal wetlands should be located throughout the shoreline of each lake in order to characterize lake-wide contaminant patterns, with the realization that some clustering will occur depending on the location of most coastal wetlands.

- Site Selection: Suitable coastal wetland sites with historical contaminants data for snapping turtle eggs should be included when possible. In addition, all sites should have known high density populations of snapping turtles to insure collection of eggs in a timely manner within the 14 day period. Speaking with local residents, fishers, and fish biologists at universities and state/provincial agencies, is helpful in determining the existence and density of snapping turtles in nearby water bodies.

Herdendorf (2004) provides an excellent classification of the significant coastal wetlands of the Great Lakes; this classification system differs from the one used by the Great Lakes Wetlands Consortium. Below, is a list of possible Canadian wetland sites known to have snapping turtles.

a. St Clair River: St. Clair National Wildlife Area (barrier-protected diked wetland), Walpole Island (riverine delta). Contaminant levels are relatively low compared to other Canadian sites.
b. Detroit River: Turkey Creek – a riverine wetland with high contaminant levels; Canard River Marshes – estuarine/diked wetland, but historically difficult to locate snapping turtle eggs.
c. Lake Erie: Wheatley Provincial Park (barrier-protected but the barrier is washed out quite regularly resulting in a lacustrine wetland each summer), Rondeau Provincial Park, Long Point National Wildlife Area (lacustrine wetland). These sites are moderately to highly contaminated.
d. Niagara River: Lyons Creek – a riverine or diked wetland; water is pumped into the Creek from the Welland Canal. Snapping turtle eggs from this area indicate a point source of PCB contaminants.
e. Lake Ontario: Cootes Paradise – riverine wetland and one of the most contaminated sites. Oshawa Second Marsh (lacustrine), the Bay of Quinte (lacustrine), Lynde Creek although the current existence of snapping turtles in this area is questionable.
f. St. Lawrence River: Upper Canada Bird Sanctuary near Ingleside ON – barrier-protected diked wetland (north side of UCBS) and open lacustrine wetland (west side of UCBS). Contaminant levels were relatively low at this site in 2003.

- Frequency of Collection: Egg samples should be collected yearly for the three year pilot study, and then yearly or once every two years from each site following the pilot study. Preferably, all sites should be collected from within the same year. An

assessment as to the frequency necessary to determine trends should be conducted after the first three collections.

- Site monitoring: Each year, each collection site will be monitored to determine when the snapping turtles commence nesting (usually for 10 – 14 days during the middle of June, depending on the location within the Basin). Eggs must be collected as soon as possible after laying since 99% of nests are predated by raccoons or other mammalian predators within 12 hours of laying; furthermore, embryonic development is minimal at this time.

- Sample Size: At each site, five clutches of eggs should be collected for contaminant analysis. Five eggs taken from throughout each clutch should be collected. In order to minimize sample loss during shipping, the eggs from each clutch may be broken open and the contents put into hexane-rinsed jars. Clutches should be kept separately. The jars (or shipping container) need to be labeled with site location, date of collection, contact information for the collector. The samples from each site need to be shipped immediately after egg collection is complete, to the coordinating agency. The coordinating agency will log the locations and numbers of samples per location, and then forward all of the egg samples to the contract lab for specific contaminant analysis.

- Multiple agencies will have to participate in order to successfully conduct this monitoring program. Discussions with each individual agency will have to be conducted to determine the extent of their participation. Possible agencies include: universities and natural history groups; state and provincial groups (e.g., New York Department of Environmental Conservation (NYDEC), Michigan Department of Natural Resources (DNR), Ohio DNR, Wisconsin DNR, Minnesota DNR, Ontario Ministry of Natural Resources); and federal agencies (e.g., Canadian Wildlife Service, U.S. Fish and Wildlife Service).

- Estimated Project Budget: Based on 2004 project costs for snapping turtle work by the Canadian Wildlife Service, we estimate that the pilot study will cost approximately $171,025 CDN per year or a total of $513,075 CDN for three years for work to be completed on Lake Ontario. The details are provided in the table immediately below and are best estimates only; please note that some costs may have been overlooked. Egg collections and chemical analyses for each site is likely to cost approximately $12,925 CDN per year, but the costs for a full-time person who will act as the main project coordinator and complete the statistical analysis and report writing (total $75,000 CDN), must still be accounted for. Following the pilot study, the cost for a basin-wide (four lakes) monitoring plan using snapping turtles is estimated to cost between $0.494 M CDN (32 sites) and $0.701 M CDN (48 sites) regardless of wetland type, or between $0.701 M CDN (48 sites) and $1.012 M CDN (72 sites) when accounting for wetland type; the $75K CDN for the full-time person, statistical analysis and report writing, as well as the $60 K CDN for hiring eight agency co-ordinators, are included in all of these budgets.

Pilot study: 9 sites on Lake Ontario (all costs are listed in Canadian dollars)

(Lake Erie would require additional contractors, field costs for field collections & as contact for main coordinator)

		per Site		**Per Year**
Contaminants	5 pools of 5 eggs each/site		45 pools/year	
egg preparation ($25/egg)	25 eggs/site	$625		$5,625
OC pesticides ($350/sample)	5 samples/site	$1,750	45 samples*350	$15,750
dioxins ($1200/sample)	1 sample/site	$1,200	9 pools *1200	$10,800
BDEs ($350/sample)	5 samples/site	$1,750	45 samples*350	$15,750
Total mercury ($30/sample)	5 samples/site	$150		$1,350
Total: contaminant analyses		**$5,475**		**$49,275**
Field collection costs				
per diem per person ($150/d * 4 d at each site); 2 people (for safety reasons)		$1,200		$10,800
Food per day ($75/d * 4 d/site) per person; 2 people/site		$600		$5,400
hotels (4 nights/site*$100/d*2 people)		$800		$7,200
Total: field collection costs		**$2,600**		**$23,400**
Travel, vehicle costs				
van rental (14 d * $100/d)		$1,400		$5,600
insurance & gasoline (best estimate only)		$1,000		$4,000
Total: travel, vehicle costs		**$2,400**		**$9,600**
Staffing costs				
1 full-time (overall project co-ordination, statistical analysis, report writing)				$67,500
1 full-time person as agency co-ordinator				$7,500
1 contractor (agency co-ordinator; $150/d*50d)		$1,700		$7,500
Total: staffing costs		**$1,700**		**$82,500**
Miscellaneous costs				
courier costs (btwn sites, lab prep, central lab, reports)		$500		$4,000
Field equipment (containers, vermiculite, water)		$250		$2,250
Total: miscellaneous costs		**$750**		**$6,250**
Grand total costs		**$12,925/site**		**$171,025/year**

- Analytical costs:
  - PCBs, organochlorine (OC) pesticides, polybrominated diphenyl ethers (PBDEs): Currently (2004), the Great Lakes Institute of Environmental Research at the University of Windsor, a contract lab used by the CWS, charges $350 CDN per sample for PCB and organochlorine pesticides and an additional $350 CDN per sample for PBDEs. One sample per clutch is usually analyzed for these contaminants.
  - Non-ortho PCBs, dioxins, and furans: AXYS Analytical, another contract laboratory used by the CWS, currently charges $1200 CDN per sample for non-ortho PCBs, dioxins. One pooled sample per site (sub-samples from all clutches from one site pooled into one sample) is usually analyzed for dioxins, furans and non-ortho PCBs. The Great Lakes Institute of Environmental Research at the University of Windsor does not conduct this type of chemical analysis (Dr. K. Drouillard, University of Windsor, pers. comm.).
  - Total mercury (Hg) (approximately $30 CDN /sample) is also measured but not the biologically important form of methyl-mercury (approximately $100 CDN /sample); one sample per clutch is usually selected for analysis from those sites in which total mercury is a suspected problem. Philip Analytical Services (Halifax, NS) is a contract laboratory that will analyze Hg in wildlife tissues.

- Statistical analysis and reporting of results will be completed after each collection, although the time required for laboratory chemical analysis may not make annual reporting feasible.

- Endpoint for Chemical Monitoring: Monitoring of chemical concentrations using snapping turtle eggs may be ceased when concentrations of toxic chemicals are similar among inland reference site(s) and the various coastal wetland sites located within the Great Lakes Basin. This endpoint definition is used by the CWS in its herring gull chemical monitoring program which has been run since 1974.

The following sections of this report provide the rationale for this monitoring program and the scientific background for using snapping turtle eggs as a means to monitor chemical concentrations in coastal wetlands in the Great Lakes Basin.

2.0 A Proposed Bi-National Monitoring Plan Utilizing the Snapping Turtle as a Sentinel Species for Contaminant Concentrations in Coastal Wetlands of the Great Lakes Basin

This section of the report will outline the proposed plan for monitoring the quality of Great Lakes coastal wetlands in terms of their degradation due to persistent organic contaminants utilizing snapping turtle eggs. The rationale for this plan, and the validation of using the snapping turtle as a basin-wide and within-lake indicator of contaminant bioavailability, are provided in subsequent sections of the White Paper.

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northern shores of Lake Superior in Canada, nor are they likely to be found since existing wetlands do not have appropriate habitat and the Lake Superior environment is too cold.

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- Alternatively, only a reference site and a highly-contaminated site within each wetland type may be selected for the pilot work.

- **Subsequent to pilot project**: Following the pilot project, an assessment of the data should be completed to determine if the type of wetland affects the contaminant concentrations found in snapping turtle eggs. If no effect of wetland type is found on these concentrations, then this factor should be removed from the experimental design. In order to determine an overall assessment of contaminant trends on a lake-wide basis, four locations (two Canadian, two American) within each of the three contaminant

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- St. Lawrence River: Upper Canada Bird Sanctuary near Ingleside ON – barrier-protected diked wetland (north side of UCBS) and open lacustrine wetland (west side of UCBS). Contaminant levels were relatively low at this site in 2003.

- Frequency of Collection: Egg samples should be collected yearly for the three year pilot study, and then yearly or once every two years from each site following the pilot study. Preferably, all sites should be collected from within the same year. An assessment as to the frequency necessary to determine trends should be conducted after the first three collections.

- Site monitoring: Each year, each collection site will be monitored to determine when the snapping turtles commence nesting (usually for 10 – 14 days during the middle of June, depending on the location within the Basin). Eggs must be collected as soon as possible after laying since 99% of nests are predated by raccoons or other mammalian predators within 12 hours of laying; furthermore, embryonic development is minimal at this time.

- Sample Size: At each site, five clutches of eggs should be collected for contaminant analysis. Five eggs taken from throughout each clutch should be collected. In order to minimize sample loss during shipping, the eggs from each clutch may be broken open and the contents put into hexane-rinsed jars. Clutches should be kept separately. The jars (or shipping container) need to be labeled with site location, date of collection, contact information for the collector. The samples from each site need to be shipped immediately after egg collection is complete, to the coordinating agency. The coordinating agency will log the locations and numbers of samples per location, and then forward all of the egg samples to the contract lab for specific contaminant analysis.

- Multiple agencies will have to participate in order to successfully conduct this monitoring program. Discussions with each individual agency will have to be conducted to determine the extent of their participation. Possible agencies include: universities and natural history groups; state and provincial groups (e.g., New York Department of Environmental Conservation (NYDEC), Michigan Department of Natural Resources (DNR), Ohio DNR, Wisconsin DNR, Minnesota DNR, Ontario Ministry of Natural Resources); and federal agencies (e.g., Canadian Wildlife Service, U.S. Fish and Wildlife Service).

- Estimated Project Budget: Based on 2004 project costs for snapping turtle work by the Canadian Wildlife Service, we estimate that the pilot study will cost approximately $171,025 CDN per year or a total of $513,075 CDN for three years

for work to be completed on Lake Ontario. The details are provided in the table immediately below and are best estimates only; please note that some costs may have been overlooked. Egg collections and chemical analyses for each site is likely to cost approximately $12,925 CDN per year, but the costs for a full-time person who will act as the main coordinator and complete the statistical analysis and report writing (total $75,000 CDN) must still be accounted for. Following the pilot study, the cost for a basin-wide (four lakes) monitoring plan using snapping turtles is estimated to cost between $0.494 M CDN (32 sites) and $0.701 M CDN (48 sites) regardless of wetland type, or between $0.701 M CDN (48 sites) and $1.012 M CDN (72 sites) when accounting for wetland type; the $75 K CDN for the full-time person, statistical analysis and report writing, as well as the $60 K CDN for hiring eight agency co-ordinators, are included in all of these budgets.

Pilot study: 9 sites on Lake Ontario (all costs are listed in Canadian dollars)

(Lake Erie would require additional contractors, field costs for field collections & as contact for main coordinator)

		per Site		**Per Year**
Contaminants	5 pools of 5 eggs each/site		45 pools/year	
egg preparation ($25/egg)	25 eggs/site	$625		$5,625
OC pesticides ($350/sample)	5 samples/site	$1,750	45 samples*350	$15,750
dioxins ($1200/sample)	1 sample/site	$1,200	9 pools *1200	$10,800
BDEs ($350/sample)	5 samples/site	$1,750	45 samples*350	$15,750
Total mercury ($30/sample)	5 samples/site	$150		$1,350
Total: contaminant analyses		**$5,475**		**$49,275**
Field collection costs				
per diem per person ($150/d * 4 d at each site); 2 people (for safety reasons)		$1,200		$10,800
Food per day ($75/d * 4 d/site) per person; 2 people/site		$600		$5,400
hotels (4 nights/site*$100/d*2 people)		$800		$7,200
Total: field collection costs		**$2,600**		**$23,400**
Travel, vehicle costs				
van rental (14 d * $100/d)		$1,400		$5,600
insurance & gasoline (best estimate only)		$1,000		$4,000
Total: travel, vehicle costs		**$2,400**		**$9,600**
Staffing costs				
1 full-time (overall project co-ordination, statistical analysis, report writing)				$67,500
1 full-time person as agency co-ordinator				$7,500
1 contractor (agency co-ordinator; $150/d*50d)		$1,700		$7,500
Total: staffing costs		**$1,700**		**$82,500**
Miscellaneous costs				
courier costs (btwn sites, lab prep, central lab, reports)		$500		$4,000
Field equipment (containers, vermiculite, water)		$250		$2,250
Total: miscellaneous costs		**$750**		**$6,250**
Grand total costs		**$12,925/site**		**$171,025/year**

- Analytical costs:
    - PCBs, organochlorine (OC) pesticides, polybrominated diphenyl ethers (PBDEs): Currently (2004), the Great Lakes Institute of Environmental Research at the University of Windsor, a contract lab used by the CWS, charges $350 CDN per sample for PCB and organochlorine pesticides and an additional $350 CDN per sample for PBDEs. One sample per clutch is usually analyzed for these contaminants.
    - Non-ortho PCBs, dioxins, and furans: AXYS Analytical, another contract laboratory used by the CWS, currently charges $1200 CDN per sample for non-ortho PCBs, dioxins. One pooled sample per site (sub-samples from all clutches from one site pooled into one sample) is usually analyzed for dioxins, furans and non-ortho PCBs. The Great Lakes Institute of Environmental Research at the University of Windsor does not conduct this type of chemical analysis (Dr. K. Drouillard, University of Windsor, pers. comm.).
    - Total mercury (Hg) (approximately $30 CDN CDN /sample) is also measured but not the biologically important form of methyl-mercury (approximately $100 CDN /sample); one sample per clutch is usually selected for analysis from those sites in which total mercury is a suspected problem. Philip Analytical Services (Halifax, NS) is a contract laboratory that will analyze Hg in wildlife tissues.

- Statistical analysis and reporting of results will be completed after each collection, although the time required for laboratory chemical analysis may not make annual reporting feasible.

- Endpoint for Chemical Monitoring: Monitoring of chemical concentrations using snapping turtle eggs may be ceased when concentrations of toxic chemicals are similar among inland reference site(s) and the various coastal wetland sites located within the

Great Lakes Basin. This endpoint definition is used by the CWS in its herring gull chemical monitoring program which has been run since 1974.

The following sections of this report provide the rationale for this monitoring program and the scientific background for using snapping turtle eggs as a means to monitor chemical concentrations in coastal wetlands in the Great Lakes Basin.

3.0 Program budget and analytical costs

3.1 *Program Budget*

Based on 2004 project costs for snapping turtle work by the Canadian Wildlife Service, we estimate that the pilot study will cost $171,025 CDN per year or a total of $513,025 CDN for three years. Budget details for the project have been provided in the preceding section, and are based on a best estimate only; some costs may have been overlooked although this was not intentional. Each field collection site is likely to cost approximately $12,925 CDN per year, but the costs for a full-time person, statistical analysis, and report writing (total $75,000 CDN) must be taken into account. Following the pilot study, the cost for a basin-wide (four lakes) monitoring plan using snapping turtles is estimated to cost between $0.494 M CDN (32 sites) and $0.701 M CDN (48 sites) regardless of wetland type, or between $0.701 M CDN (48 sites) and $1.012 M CDN when accounting for wetland type; the $75 K CDN for the full-time person, statistical analysis and report writing, as well as the $60 K CDN for the eight agency co-ordinators, are included in all of these budgets. These budget figures include the estimated costs for hiring of staff (full-time person, one contractor per agency), purchasing of field equipment and materials, travel (hotel, food, gas, vehicles, insurance), courier shipping of egg samples and other materials, statistical analysis of data, and the presentation and reporting of results. Depending on the timeliness of the chemical analysis, statistical analysis and reporting of results should be completed after each collection.

3.2 *Analytical Costs*

The analytical cost associated with quantitative analysis of organochlorine (OC) pesticides and polychlorinated biphenyl compounds (PCBs) is currently $350 CDN per sample as charged by the contract laboratory at the Great Lakes Institute of Environmental Research at the University of Windsor; a sample may consist of individual or pooled eggs. The organochlorine pesticides and compounds that are typically measured by the CWS in snapping turtle eggs include: *p,p'*-dichlorodiphenyltrichloroethane (*p,p*'-DDT); 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene (*p,p*'-DDE); *p,p*'-DDD, *alpha*-, *beta*,- and *gamma*-hexachlorocyclohexane (HCH); hexachlorobenzene (HCB); octachlorostyrene (OCS); mirex; dieldrin; photomirex; *cis*- and *oxy*- chlordane; *cis*- and *trans*- nonachlor, and heptachlor epoxide (HC Epox). The PCB congeners that are currently measured in routine OC analyses by the CWS include the following 59 congeners: #16/32; 17;18; 22; 28; 31; 33/20; 42; 44; 47; 49; 52; 56/60; 64; 66; 70/76/ 74; 85; 87; 92; 95; 97; 99; 101/90; 105; 110; 118; 128; 130; 137; 138; 141; 146; 149; 151; 153; 156; 157; 158; 170/190; 171; 172; 174; 176; 177; 178; 179; 180; 183; 187; 194; 195; 196/203; 201; 200; 202; 206; 207, and 208. In addition, the Aroclor 1260 and Aroclor 1254:1260 (1:1) equivalents are provided with most analytical reports and provide important information that allows for historical comparisons when fewer congeners were measured.

Quantitative analysis of dibenzodioxins (PCDDs), dibenzofurans (PCDFs), and non-ortho PCBs (congeners # 37, 77, 81, 126, 189) is currently $1200 CDN per sample as charged by the contract laboratory of AXYS Analytical (Vancouver, British Columbia). The Great Lakes Institute of Environmental Research at the University of Windsor does not complete this type of chemical analysis (Dr. Ken Drouillard, University of Windsor, personal communication 2004). The following PCDD congeners are provided with most analytical reports: 2,3,7,8-TCDD; 1,2,3,7,8-PeCDD; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-HpCDD, and OCDD. The PCDF congeners most often reported are: 2,3,7,8-TCDF; 2,3,7,8-TCDF(C); 1,2,3,7,8-PeCDF; 2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF; 2,3,4,6,7,8-HxCDF; 1,2,3,4,6,7,8-HpCDF; 1,2,3,4,7,8,9-HpCDF, and OCDF. In addition, the following non-ortho PCB congerners are provided: PCB 37 (3,4,4' TriCB); PCB 77 (3,3',4,4' TetraCB); PCB 126 (3,3',4,4',5 PentaCB); PCB 169 (3,3',4,4',5,5' HexaCB), and PCB 189 (2,3,3',4,4',5,5' HeptaCB).

Of recent concern are brominated flame retardants (BFRs), especially polybrominated diphenyl ethers (PBDEs). At this time, nine brominated diphenyl ether congeners are measured: BDE-28; -47; -49; -99; -100; -138; 153; -154, and –183. The cost associated with analysis of PBDEs is estimated at approximately $350 CDN (Great Lakes Institute of Environmental Research) or $1200 CDN (AXYS Analytical) per individual or pooled sample, depending on the type of analytical method employed.

Total mercury is also routinely measured in snapping turtle eggs collected from sites where mercury concentrations are expected to be of concern. Methyl mercury is the biologically active form of mercury, but it is only measured when health effects from mercury exposure are suspected. Methyl mercury (approximately $100-150 CDN /sample) is much more expensive to analyze than total mercury ($30 CDN /sample).

4.0 Are Contaminants Measurable in Snapping Turtle Eggs?

The monitoring program should follow the approved protocol outlined in the Quality Assurance Project Plan (QAPP) (see Appendix). The methodology is divided into six specific components that will facilitate data collection and analysis: 1) wetland site selection; 2) sample collection and handling; 3) sampling frequency; 4) tissue storage, shipment, and preparation; 5) sample analysis and quality control; and 6) reporting and sharing of data.

Development and implementation of an integrated, bi-national monitoring program requires that participating researchers have the most current version of the approved QAPP (Appendix). It is important that the groups involved in monitoring activities should coordinate their efforts through the use of this protocol in sampling procedures, sample and data analysis, and reporting methods. This approach ensures a basin-wide (bi-lateral) consistency in data collection and methodologies among participating agencies in Canada and the United States, thereby enhancing the comparability and value of the data in identifying spatial and temporal trends in contaminant levels.

4.1 *Wetland Site Selection*

A selection of different types of lacustrine, riverine, and barrier-protected system wetlands with suitable habitat for snapping turtles may be included in this contaminants monitoring program to characterize contaminant levels in coastal wetland systems. Lacustrine system wetlands are controlled by waters of the Great Lakes and are strongly affected by lake-level fluctuations, near-shore currents, seiches, and ice scour. Riverine system wetlands occur in rivers and creeks that flow into or between the Great Lakes. Riverine wetlands within the Great Lakes also include those wetlands found along large connecting channels between the Great Lakes with different dynamics than smaller tributary rivers and streams. Barrier-protected system wetlands have originated from either coastal or fluvial processes. Under the influence of coastal processes, the wetlands have become separated from the Great Lakes by a barrier beach or other barrier feature.

Great Lakes coastal wetlands within these hydrologically based systems are further classified based on their geomorphic features and shoreline processes. For a complete summary of Great Lakes coastal wetlands classifications, refer to the classifications summary document on the Great Lakes Wetland Consortium web page (http://w.w.w.glc.org/wetlands/pdf/wetlands-class-scheme.pdf).

Snapping turtles throughout the Great Lakes region have a nesting season which generally overlaps during the middle two weeks of June; however, the laying of clutches may begin prior to this in the southern part of the Great Lakes range, or slightly later in the north (Ernst et al., 1994). Since persistent organic pollutants such as DDE have decreased during the first 10 days of incubation in loggerhead sea turtles (*Caretta caretta*) (Clark and Krynitsky, 1985), eggs for contaminant analysis should be collected during this two week laying period to ensure freshness and minimal embryonic development. The utilization of fresh or recently laid eggs (< 48 hours) removes the uncertainty of changes in contaminant concentrations by Phase I and Phase II metabolic enzymes (Bishop et al., 1995a).

Site monitoring should be conducted daily until all egg collections have been completed for the site because predation is extremely high (> 98%) within hours of the turtle eggs being laid. Nesting activity is greatest in the morning between 0500 and 0900, with less activity between 1700 and 2100. Mornings prove to be the most efficient and practical time to collect eggs, unless predation by raccoons is a serious concern. Nesting sites involve a variety of substrate types including sand, loam, clay, or vegetable debris.

However, we have also found turtle eggs buried in hard, pebbly areas such as along roadsides and ditch culverts. Sites tend to have substantial exposure to sunlight and generally have sparse vegetation at the time of oviposition. Natural sites, roadsides, railways, or dams are typically used. Shortly after laying, the nest is identifiable by two distinct mounds of excavated earth upon which the female may urinate giving the excavated material a granular appearance. The nest has been described as bowl shaped, with a narrow opening descending at an angle to a large chamber 7 to 18 cm below the surface (Ernst et al., 1994).

4.2 *Sample Collection and Handling*

Although Bishop et al. (1995) reported a non-significant intra-clutch variation in contaminant levels among freshly laid eggs, the first five eggs contained the highest mean concentration of all chemicals on a wet-weight basis and the highest mean lipid values relative to the last five eggs collected. To mitigate the potential for intra-clutch contaminant variation, the CWS collects a composite subsample of five eggs from throughout each clutch. The first eggs oviposited are considered to be the last eggs found at the bottom of the nest cavity, and the last eggs laid are likely the first eggs encountered when the nest is excavated from the soil surface. A composite sample is obtained in the field by the following method: eggs are removed from the nest and placed in a plastic Tupperware® container filled with moistened vermiculite to prevent desiccation and breakage; the eggs are placed in order from first egg laid to the last; each clutch is divided into five groups of approximately equal size, and from within each group, an egg is selected haphazardly (de Solla and Fernie, in press). The total number of eggs, the wetland site, latitude and longitude of the collection site, the collection date, as well as the collector's name and contact information, should be recorded on the top and sides of each shipping container for each clutch. The eggs not intended for contaminant analysis are immediately returned and reburied in the nest.

Since organochlorine concentrations among clutches can be highly variable within a snapping turtle population (Bishop et al., 1991; Struger et al., 1993, Bonin et al., 1995), it is preferable to use 5 to 10 clutches of eggs per site for biomonitoring in order to obtain

robust statistical comparisons among sites and among years within a site (Portelli and Bishop, 2000). Bishop et al. (1994) report that the coefficients of variation ranged from 38.6% to 55.9% among 15 clutches. This level of variation is comparable to studies using Great Lakes herring gulls, Atlantic puffins (*Fratercula arctica*), Leach's petrels (*Oceanodroma leucorhoa*) and spottail shiners (*Notropis hudsonius*) (Bishop et al., 1994).

4.3 *Sampling Frequency*

Data collected as part of long-term contaminant monitoring programs undoubtedly advance our understanding of the sources and fate of contaminants, as well as provide spatial and temporal trend information for assessing improvement in controlling contaminant outputs (Pekarik and Weseloh, 1998; Braune et al., 2001). However, the frequency of field sampling can affect the ability to detect temporal changes in persistent contaminant levels. Hebert and Weseloh (2003) examined the effect of different sampling frequencies (i.e., every year; every second year; every third year; every fourth year; and every fifth year) on the ability to identify significant temporal declines in persistent organic contaminant levels in the Great Lakes. The data used by these authors were taken from the analysis of 13 herring gull eggs collected annually from each of five Great Lakes colonies between 1980 and 2001. This study confirms that programs of shorter duration that sampled at widely spaced intervals produced data with a limited capability of detecting significant temporal changes in contaminant levels in the environment. This was attributed to the decreased statistical power associated with analyses of few data points. Sampling at every two, three or four year intervals was able to detect changes in contaminant levels, however, identifying a significant change in levels is delayed by years relative to results from annual monitoring efforts. Hebert and Weseloh (2003) indicate that frequent temporal trend data regarding the bioavailabilty of environmental contaminants is most important when timely information is needed; for example, in assessing the effectiveness of Remedial Action Plans. In addition, the design of a monitoring program must strike a balance among costs, logistics, quality of data, and program objectives.

4.4 *Measurement and Data Acquisition*

For information on Sample Handling, Analytical Methods, Quality Control Requirements and Data Management, please refer to the approved Quality Assurance Project Plan (Revision 3- QAPP Wetlands2-EPA-05) in the Appendix of this report.

5.0 Applicability and Reliability of Snapping Turtles to Measure Contaminants

Knowledge of the life history and patterns of movement of the snapping turtle is essential to understanding the potential for their exposure to environmental contaminants. Ernst et al. (1994) provides a comprehensive overview of the snapping turtle and its ecology.

According to Lower and Kendall (1990), the utility of a given species for biomonitoring is based upon its geographic distribution, home range, presence in a particular habitat, and availability and specificity of biological endpoints. Golden and Rattner (2003) rank the suitability of vertebrate species as sentinels of contaminant exposure based upon their geographic occurrence, exposure potential, ease of collection, and quantity of existing exposure and effects data. Here, we address each of these criteria in order to assess the utility of the snapping turtle as an indicator of contaminant exposure.

Snapping turtle populations are more sensitive to "crashing" (mortality) as a result of lethal sampling of adults than to mortality from sampling eggs (Struger et al., 1993; Bishop et al., 1996; Cunnington and Brooks 1996). Although blood sampling of adults is another viable method for monitoring contaminants in snapping turtles (de Solla et al. 1998), concentrations of PCBs increase with body size in adult males, whereas contaminants in eggs are independent of body size of the laying females (Bishop et al.,1994). Due to the relatively low lipid levels in blood plasma, concentrations of contaminants in blood plasma are much lower than those found in eggs. Furthermore, trapping adult snapping turtles is labor- and time-intensive relative to egg collection. Consequently, the collection of eggs is the most ecologically sound and practical approach to monitoring contaminant exposure. Given our focus on the utility of turtle eggs as an indicator of contaminant exposure, we also include information on the transfer of contaminants from female turtles to their eggs in section 4.6.

5.1 *Geographic Distribution*

The range of the common snapping turtle encompasses the St. Lawrence River and the shores of the Great Lakes, excluding most of the northern shore of Lake Superior in Ontario, Canada (Ernst et al., 1994). The range includes that area west of Thunder Bay, Ontario, on the northern shore of Lake Superior and south of the St. Marys River, a 112 km connecting channel between Lakes Superior and Huron. However, according to a report that identified species of reptiles native to 17 Ontario Areas of Concern (AOCs), no snapping turtles had been sighted in the above mentioned areas up until the time of the report's publication in 1996 (Shirose and Bishop, 1996). The most important factors affecting the northern distribution of snapping turtles are the lower temperatures during egg incubation, a shorter growing season, and differences in habitat quality (Bobyn and Brooks, 1993).

Snapping turtles are inhabitants of wetlands, and they may be found in a wide variety of habitats where there is abundant aquatic vegetation in slow moving, permanent water bodies such as swamps, marshes, ponds, lakes, streams and rivers. Sites with soft muddy bottoms are preferred. Snapping turtles are sedentary, full-time residents of wetlands, and over-wintering turtles hibernate beneath a covering of mud, logs or plant debris on pond bottoms, under riverbanks or in muskrat burrows. Depending on latitude, snapping turtles may enter hibernation as early as September and emerge in March or April when water temperatures are between 5 ^{o}C to 7.5 ^{o}C (Ernst et al., 1994).

5.2 *Home Range*

The small home range and short migration distances reported in the literature indicate that turtles nesting in wetlands live and feed within these systems, and so an adult snapping turtle and her eggs, reflect contaminant concentrations within that wetland system. Several studies (Table 1) indicate that the home range of snapping turtles is small. Ernst et al. (1968) estimated home range size of snapping turtles in Pennsylvania to be 1.84 ha (n = 9 live captured turtles). Using radio-telemetry, Murphy and Sharber (1973, cited in

Obbard and Brooks 1981) estimated the mean home range to be 0.65 ha in a Tennessee river. In small areas, home range size can be severely constrained; the mean home range size in a 0.8 ha pond was only 0.02 ha (Froese, 1974). While the home range of the common snapping turtle is thought to be determined by variation in food resources, body size, density and shelter (Galbraith et al., 1988; Brown, 1992), these parameters did not influence the home range of snapping turtles in a protected embayment in Hamilton Harbour, Lake Ontario (Pettit et al., 1995). These investigators determined that the mean home range did not vary significantly between 1990 (8.6 ha for females and 2.2 ha for males) and 1991 (9.7 ha for females and 3.4 ha for males) (Pettit et al., 1995). In a separate study, the mean home range of radio-tracked adults did not differ by habitat or wetland size, being similar among Lake Sasajewun (a 43.5 ha lake), Cootes Paradise (a 370 ha wetland exiting into Hamilton Harbour), and Lynde Creek Marsh (a 40 ha cattail marsh opening into Lake Ontario) (Brown et al., 1994).

In Algonquin Provincial Park, Lake Sasajewun is a small lake interconnected by the North Madawaska River to three other smaller lakes. In the late 1970s, nine radio-tracked snapping turtles remained within Lake Sasajewun, and the mean home range size was only 3.44 ha (min-max = 0.95 to 8.38; Obbard and Brooks 1981). A female caught in the North Madawaska River, immediately downstream of Lake Sasajewun, had a home range of 1.3 ha and was never observed to enter the lake (Obbard and Brooks 1981). In two separate studies in Algonguin Provincial Park, the mean home range size of snapping turtles was 8.14 ha in 1987-1990 (Brown, 1992) and 8.64 ha in 1991 (Brown et al., 1994). Movement of males, however, has occurred on rare occasions in this area. For instance, four male turtles made unusual but brief forays outside of their home ranges, traveling up to 1500 m away in May and early June but never after the nesting season (Obbard and Brooks, 1981). The maximum nesting migration distance for female snapping turtles in Cootes Paradise, Lake Ontario was 2020 m (Pettit et al., 1995), but this study indicated that no turtles moved from this site into the adjacent Hamilton Harbour. This is comparable to migration distances in South Dakota (Hammer, 1969) and in southeastern Michigan (Congdon et al., 1987.) These findings indicate that snapping turtles have a high site affinity.

5.3 *Exposure Potential*

Exposure potential is a measure of the likelihood of an individual's exposure to a contaminant by the oral, dermal, or inhalation route. Specific elements that affect exposure may include dietary and habitat preference, longevity, feeding habits, foraging strategy and use of agricultural, industrial, or urbanized areas with anthropogenic contaminant input. The extent of exposure of the snapping turtle to persistent, organic pollutants is related to the chemical availability, and the species' propensity to bio-accumulate these compounds. The principal route of exposure for the snapping turtle is from bioaccumulation through the diet. The snapping turtle is an omnivorous opportunist, basically consuming whatever is available. Food items include vegetation, insects, shellfish, earthworms, leaches, fish, amphibians, small turtles, snakes, birds, and small mammals (Ernst et al., 1994). The contents of 470 stomachs from snapping turtles studied in Michigan were composed of 36.5% plant matter and 54.1% animals by volume. Fish are known to constitute approximately one third of the turtle's diet (Alexander, 1943). Because of the snapping turtle's predatory nature, feeding on large fish, small ducklings and cygnets, as well as carrion (Ernst et al., 1994), it is further subject to food chain biomagnification, and thus is exposed to the greatest concentrations of persistent organic contaminants.

Most organic pollutants are highly lipophilic and thus can be retained by fatty tissues for long periods while the organism is continually exposed. Thus, the longevity of a species can also affect its accumulation of organic pollutants. In one Ontario population, adult females were thought to have an average life span of 40 years based on annual rings on carapace scutes (Galbraith and Brooks, 1989). However, this is probably an underestimate, as Brooks et al. (1997) have since attempted to validate annuli counts, and they were shown to greatly underestimate snapping turtle age.

5.4 *Ease of Collection*

Turtles are common in their range except where populations have been over harvested for human consumption. Factors that determine the ease of sample collection of an indicator species include social structure, abundance, accessibility of sampling unit,

ease of capture, and management status in the proposed study sites (Golden and Rattner, 2003).

Snapping turtles can be very abundant in areas with high primary productivity, and populations vary greatly in density and biomass density. In a pond with high nutrient levels and primary productivity in Hamilton, Ontario, the density was 66 turtles/ha (biomass, 340kg/ha) (Galbraith et al., 1988). This is similar to a study by Major (1975), which reported 60.5 turtles/ha in western West Virginia. In a more northern oligotrophic pond in Algonquin Provincial Park, Ontario the density was 2.4 turtles/ha (biomass, 16 kg/ha) (Galbraith et al., 1988), similar to a Wisconsin lake with a density of 1.9 turtles/ha (Petokas, 1981). The density appears to be negatively correlated with latitude and surface area of suitable habitat (Galbraith et al., 1988). The primary productivity of a habitat appears to be the most important parameter influencing the density of snapping turtle populations. High-density snapping turtle populations appear to be concentrated in marshes and other highly eutrophic bodies of water, whereas low-density populations occur in lakes and other mesotrophic or oligotrophic systems (Galbraith et al., 1988). Other factors such as predation by other turtles, trapping by humans, and predation by *Mustelids* during hibernation may affect population density (Ernst et al., 1994). Snapping turtles are quite tolerant of habitat disturbance, and thus can be found in highly modified wetlands within an urban landscape, even in areas that otherwise have low species diversity due to anthropogenic impacts. A summary of 17 reports on density or biomass of snapping turtle populations from the U.S. and Canada was published by Galbraith et al. (1988).

Collection of snapping turtle eggs for contaminant analysis is made easy because the nests are usually accessible and clutches contain sufficient eggs for analysis. Nesting sites can often be found much earlier than the beginning of oviposition, as the presence of egg shells from the previous year may be apparent. Only one clutch per female is laid in a given year and the number of eggs per clutch varies widely (12 to 72 in our own study). While egg collection may be hampered in locations with limited suitable nesting areas, eggs from as many as 10 clutches have been collected in a single morning in areas with high density populations (Shane de Solla, personal communication, 2003).

5.5 *Quantity of Existing Exposure and Effects Data*

This information will be covered in the section entitled "Availability of Complementary Existing Research Data" (Section 5.0).

5.6 *Maternal Transfer of Contaminants to Eggs*

There are two major periods during which egg development occurs in the one-year snapping turtle reproductive cycle. Between mid-summer and late fall, egg growth is most rapid and follicular development is dependent upon energy assimilated from recently harvested food sources. Egg development resumes following hibernation and consists of final follicular growth, embryo development, shelling, ovulation and oviposition (Ernst et al., 1994). The use of eggs to assess exposure of wildlife to persistent, organic contaminants illustrates the widely held belief that chemicals in eggs are derived from the adult female, and turtle eggs are useful indicators of localized geographic contamination (Stone et al., 1980; Helwig and Hora, 1983; Olaffson et al., 1983; Hebert et al., 1993; Struger et al., 1993; Bishop et al., 1996).

Research on a variety of vertebrate species indicates that concentrations of organic chemicals in eggs closely reflect the concentration in maternal tissues when the concentrations are expressed on a lipid-weight basis (Mineau, 1982; Pagano et al., 1999; Russell et al., 1999). During ovogenesis, chemical transport from maternal tissues to the eggs follows a set of passive transport processes resulting in a chemical equilibrium among maternal tissues and eggs (Russell et al., 1999). Organic chemicals are rapidly distributed because of their lipophilic nature and result in a homogeneous tissue distribution when concentrations are expressed on a lipid basis. The development of eggs in oviparous species involves the transfer of lipoproteins from maternal tissues to eggs, and there is very negligible biotransformation of organic chemicals in eggs because phase I and phase II enzymes are not yet active (Kleinow et al., 1999).

For the most part, available data are consistent with the model that chemical concentrations in eggs and maternal tissues achieve equilibrium. A wide-ranging collection of maternal transfer data was published by Russell et al. (1999), in which these investigators combined existing data with the results of field studies on Lake Erie to

determine maternal transfer, and in ovo bioaccumulation of 44 hydrophobic organic chemicals in nine species of fish, herring gulls, and the common snapping turtle. When chemical concentrations in the eggs and the females were adjusted for lipid content, the egg/female concentration ratios were normally distributed with a mean of 1.2. The mean egg/female concentration ratio for 24 chemicals in the snapping turtle, however, was 0.4. This suggests that snapping turtle eggs do not hold as much chemical as the equilibrium model predicted. These results, however, may have been due to a small sample size of only three snapping turtles. Nonetheless, when examining such a broad range of species, it is interesting to note that concentrations in eggs and maternal tissues were strikingly similar. The results of this study indicate that at the time of egg deposition, contaminant concentrations in the eggs and maternal tissues of fish, turtles, and birds are close to chemical equilibrium.

In a separate study, Pagano et al. (1999) analyzed tissues of six gravid snapping turtles within and outside of the Great Lakes Basin to determine if eggs can be used as indicators of maternal contaminant burdens. Based on the congener specific (mole percent) data, and average chlorine/biphenyl values (which allows assessment of the level of chlorination among maternal tissues and eggs), the results indicated that the primary source of energy for follicle growth was derived from recent food sources. In addition, a significant and positive correlation was found between concentrations of congener-specific PCBs, DDE, mirex and hexachlorobenzene (HCB) in maternal tissues (adipose tissue and liver) and eggs from highly (Massena, Industrial NY), moderately (Hudson River, Annandale NY), and low-level (Sodus Bay, Rice Creek NY) contaminated sites (r-values > 0.95). This indicated that *in ovo* exposure of developing embryos in various classes of oviparous organisms to persistent hydrophobic organic pollutants is similar to the exposure of the adults who deposit the eggs. Pagano et al. (1999) concluded that their findings support previous research that environmental contaminants are maternally transferred, and that snapping turtle eggs are useful indicators of localized geographic contamination.

5.7 *Limitations*

Because relationships have been found between body mass and PCB and organochlorine pesticide concentrations in the liver (Hebert et al., 1993), blood plasma (de Solla et al 1998), and PCBs in the fat of snapping turtles (Bishop, 1990), it would be advantageous to age females which oviposit the eggs used for contaminant analysis. Unfortunately, no such method exists and calculations of annual growth rate as a tool for ageing is problematical as growth annuli may not be formed each year. For instance, juveniles in Algonquin Provincial Park, Ontario, formed one growth annulus on scutes of the carapace each winter, however, approximately 50% of the adults did not add a growth annulus between captures one year apart (Galbraith and Brooks, 1987, 1989). Furthermore, in a validation study in which two casts were taken of the carapaces of adults and juveniles taken ten years apart, Brooks et al. (1997) determined that the number of annuli did not vary between the two age periods for adults. Consequently, age of females cannot be incorporated into models of contaminant exposure or fate. Body size is, however, correlated with the age of turtles, and thus body size of the laying females can be incorporated into models.

Body mass, clutch size, and clutch mass can vary among females within a population (Congdon et al., 1987). Bishop et al. (1994), however, found no significant correlation between body size (body mass, carapace length and width, and plastron length) and chlorinated hydrocarbon concentrations in eggs from 15 snapping turtle nests. These authors suggest that ecological parameters, individual food preferences, and/or foraging activities are more likely to cause variation in chemical concentrations among clutches of snapping turtle eggs in a population. Nonetheless, a larger sample size from other areas within the Great Lakes is needed to further examine and confirm the relationship between size of adult females and the contaminant concentrations in their eggs.

6.0 Availability of Complementary Research Data

The following section provides a chronological overview of published materials on PCBs, PCDDs, PCDFs and p, p'-DDE concentrations measured in snapping turtle eggs; supplementary data are provided in Table 2 with field sites used in these studies shown in Fig. 1.

Persistent organic compounds have a great affinity for tissues with high lipid content, with the highest concentrations measured in fat, followed by eggs, testes, liver, kidneys and muscle (Portelli and Bishop, 2000). The review here focuses on the results from contaminants analyzed in snapping turtle eggs. For information on contaminant data in other tissue types, and data on a suite of turtle species occurring in North America, the reader is referred to Hall (1980), Meyers-Schöne and Walton (1994), Bishop and Gendron (1998), and Portelli and Bishop (2000).

Snapping turtles have been used as a sentinel species of persistent organic contaminant exposure in wetland environments since the 1970s (Campbell, 1974; Stone et al., 1980), including by the CWS since 1984. In the early 1990s, work by the CWS focused on contaminant-related effects of contaminants on the snapping turtle (Bishop et al., 1991; 1998; de Solla et al., 1998). These efforts have increased the amount of information available on contaminant levels and their effects on snapping turtles inhabiting wetlands of the Great Lakes Basin. Since the organochlorine insecticide DDT, and especially its primary metabolite p, p'-DDE, continue to be found at high concentrations in wildlife, p,p'-DDE is included in this review. Other organochlorine insecticides such as aldrin, dieldrin, chlordane, endrin, heptachlor, mirex, and toxaphene are detected in egg samples and have been reviewed by Portelli and Bishop, (2000).

Improvements in instrument technology, methods and detection limits, has resulted in a substantial increase in the number and type of PCB congeners measured. Currently, as many as 71 PCB congeners may be included in routine organochlorine analytical reports. This is a significant improvement over early research which quantified PCBs as a mixture of Aroclors 1254:1260 (1:1) using PCB-128 (2,3,4,2',4',5'-hexachlorobiphenyl) as a surrogate. In order to compare older data with more recent analyses, Turle et al. (1991) developed conversion factors by using the analytical results of 41 PCB congeners and Aroclor 1254:1260 measured in herring gull eggs. This approach was used by Struger et al. (1993) to calculate total PCB concentrations in turtle eggs collected from 1981-1984. Using Aroclor equivalents also has the advantage of greater comparability when analyzing data from different sources, however, a possible disadvantage is misrepresenting the actual PCB levels in animal tissues. Although a combination of Aroclor 1254 and 1260 are the dominant mixtures in the lower Great Lakes, there are important local sources of these and

other Aroclors in the Great Lakes, such as 1248 and 1242 (Oliver and Bourbonniere, 1985; Sokol et al., 1994, Martin et al., 2003). Large deviations in exposure from the 1:1 Aroclor 1254:1260 may considerably affect the accuracy of the Aroclor equivalent estimates. For example, 1254:1260 (1:1) over-estimated sum PCBs in herring gulls compared to sum PCBs (71 congeners) in the Great Lakes by 1.7 times (Hebert et al., 2000). To this day, there are slight differences in the number and type of PCB congeners measured; therefore, it is advisable that the reader consult the primary literature for details relevant to contaminant values listed in Table 2.

Less information is available on PCDD and PCDF concentrations in turtle eggs compared to total PCBs. Typically egg samples from one site are not analyzed individually for PCDDs and PCDFs, but rather are pooled into one composite sample for the site for analysis; this is due to the cost of analyzing these chemicals. Most studies indicate that the congener 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD) is the predominant form of PCDDs in turtle eggs (Bishop et al., 1991; Struger et al; 1993; de Solla et al., 2001). However, the predominance of this dioxin congener depends on various factors such as differential metabolism of congeners and point sources of PCBs. With regards to PCDF compounds measured in turtle eggs, 2,3,4,7,8,-pentachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzofuran are generally dominant (Bishop et al., 1991; Struger et al., 1993; de Solla et al., 2001).

As previously stated, monitoring chemical parameters in water and sediment generally reflect the degree of pollution in a particular locality; however, the measurement of contaminant concentrations in tissues of snapping turtles provides a gauge of wildlife exposure and of toxicant bioavailability in wetland environments. Recently, the CWS has been working to determine the value of an effects monitoring program using snapping turtles as indicators of contaminant exposure and effects. Since 2001, the CWS (K. Fernie, project leader) has been systematically measuring contaminant exposure and effects in snapping turtles from wetlands in Canadian Areas of Concern (AOCs) in Lake Erie, Lake Ontario, and the St. Lawrence River.

6.1 *Polychlorinated Biphenyls, Dioxins, Furans, and p,p-DDE*

One of the first accounts of contaminated snapping turtle eggs was reported by the CWS in 1974. Snapping turtle eggs from Rondeau Provincial Park, located on the shores of northern Lake Erie, contained 0.21µg/g p,p'-DDE. Unfortunately, no data are available for total PCBs, dioxins or furans (Campbell, 1974, from Bishop and Gendron, 1998).

Outside of Canada, Stone et al. (1980) reported that unlaid eggs from six gravid snapping turtles collected from the highly contaminated Hudson River in New York State contained 28.9 µg/g total PCBs (lipid weight). A second report also comes from the Hudson River, in which Bryan and colleagues (1987) compared the difference in partitioning of organochlorine compounds between the yolk versus the albumen (egg white) and egg shell. The lipid rich yolk had between 6.0 and 9.4 times more total PCB concentration than the albumen or egg shells.

Struger and colleagues (1993) later tested the hypothesis that snapping turtle eggs would be useful indicators of geographic variation in contaminant levels among Great Lakes wetlands. In 1981, eggs were collected from a site with low background contamination located in Algonquin Provincial Park, Ontario, and Big Creek Marsh National Wildlife Area, Ontario (Lake Erie). Since Algonquin Provincial Park has no known local PCB sources, the PCB concentrations in the turtle eggs were assumed to represent background airborne exposure to PCBs. Eggs from eight other wetland locations on Lake Ontario, Lake Erie and along the St. Lawrence River in Canada were collected in 1984. The mean summed concentration of PCB congeners at the reference site was 1.87 µg/g, while the Great Lakes and St. Lawrence River sites ranged from 1.00 to 4.76 µg/g. Eggs from Grindstone Creek in Hamilton Harbour contained the highest mean total PCB and p,p'-DDE concentrations from among the ten sample locations (Struger et al., 1993). When compared to other data, turtle eggs were generally more contaminated than spottail shiners collected at the mouth of the Hamilton Harbour, but less contaminated with chlorinated hydrocarbons than herring gull eggs. Unexpectedly, a combined extract of sub-samples from three clutches collected at the mouth of Grindstone Creek in Hamilton Harbour contained similar or higher concentrations of certain dioxin congeners than eggs of herring gulls. This suggests that there are inter-specific differences in the metabolism of organic contaminants among species. The results of this study clearly revealed widespread

and geographically variable contamination among Great Lakes wetland ecosystems, particularly those associated with areas of high contaminant loads.

Between 1986 and 1989, Bishop et al. (1991) measured concentrations of PCBs, PCDDs, PCDFs, and organochlorine pesticides in eggs collected from the reference site in Algonquin Provincial Park, and four wetlands located on the shores of Lake Ontario and Lake Erie. Eggs collected from each site were also incubated to determine hatching success, and the incidence of deformities in embryos and hatchlings. Significant geographic variation in persistent organic chemical concentrations was similar to the results of Struger et al. (1993). Results of the four-year study showed that eggs from Cootes Paradise and Lynde Creek contained the highest concentrations of summed PCBs and p,p-DDE among the five sites. Eggs from Cranberry Marsh and Big Creek Marsh were comparable and moderately contaminated, while reference eggs from Lake Sasajewun (0.028 μg/g w.w.) were the least contaminated. Lynde Creek also had the highest concentrations and the highest number of dioxin and furan congeners. The developmental study revealed that eggs from Cootes Paradise, Lynde Creek and Cranberry Marsh had higher incidences of poor hatching success and deformities relative to eggs collected from Big Creek Marsh and the reference site in Algonquin Provincial Park in all years of study.

Bonin et al. (1995) compared organochlorine and PCB levels in 39 snapping turtle clutches collected from 10 sites along a highly polluted stretch of the St. Lawrence River in Canada (Cornwall, Ontario through to the east end of Montreal Island) and the much less polluted Ottawa River in Canada. Similar to Bishop et al. (1991) and Struger et al. (1993), sites demonstrated a high inter-site variability in contaminant levels. Eggs collected from Raquette River near Masena NY, were highly contaminated with PCBs reaching concentrations of 10.97 μg/g (w.w.). The total PCB concentrations were much lower at the Ottawa River site where the maximum PCB level did not exceed 0.173 μg/g. Among the organochlorine pesticides, p,p'-DDE was generally detected at the highest level. Ingleside, a site upstream from Cornwall, had the highest p,p'-DDE concentration, while turtle eggs from the Ottawa River site had the lowest.

Bishop et al. (1996) compared the geographic contaminant patterns in eggs collected from five sites in Ontario, Canada. Collections were made by Struger et al. (1993) in 1981 and 1984, and later collections were made by Bishop and colleagues

between 1988 and 1991. The pattern of significant geographic variation in organochlorine concentrations in snapping turtle eggs among locations was consistent from 1988 – 1991, and consistent with patterns reported by Struger et al. (1993). In 1989, when eggs were collected from all six sites, the general contaminant pattern for sum PCBs was as follows: Lynde Creek > Cootes Paradise > Rondeau Park > Cranberry Marsh > Big Creek Marsh > Algonquin Provincial Park. A similar pattern however, was not detected for p,p'-DDE: Cootes Paradise > Lynde Creek > Cranberry Marsh >Big Creek Marsh = Rondeau Park > Algonquin Provincial Park. The PCDD and PCDF concentrations in eggs consistently indicated that Lynde Creek was the most contaminated site, while eggs from Cootes Paradise and Cranberry Marsh were the next most contaminated. Eggs from Algonquin Provincial Park had the lowest PCB concentrations, non-detectable levels of PCDFs and PCDD congeners with the exception of octachlorodibenzo-p-dioxin.

Similar to a previous study by Bishop et al. (1991), snapping turtle eggs were again collected for contaminant analysis and incubation to assess developmental abnormalities (Bishop et al., 1998). Eggs were collected from eight sites in Ontario, Canada and Akwesasne, New York, USA during the reproductive seasons from 1989 to 1991. Several of the sample sites such as Lake Sasajewun, Lynde Creek, Cootes Paradise, Cranberry Marsh, Big Creek Marsh, and Rondeau Provincial Park, were common to the 1991 study. Eggs were also collected from the Trent River, a site which drains into the Bay of Quinte, Ontario. In the St. Lawrence River area, samples were collected from Raquette River, St. Regis River, and the Snye marshlands, all within the boundaries of Akwesasne, New York. Eggs from the reference site at Algonquin Provincial Park, Ontario contained the lowest PCB concentrations. Egg samples collected from shoreline sites of Lake Erie and Lake Ontario, ranged from 0.24 μg/g (w.w.) at Cranberry Marsh, to 3.57 μg/g (w.w.) at Cootes Paradise. Eggs from the Trent River contained 0.83 μg/g (w.w.) and Akwesasne samples were the most contaminated with 3.95 μg/g (w.w.) of PCBs. Rankings for PCDDs and PCDFs were somewhat different with low or non-detectable concentrations in eggs from Algonquin Provincial Park, Big Creek, and Rondeau, and higher concentrations and a greater number of detectable congeners in eggs from Cranberry Marsh, Akwesasne, Trent River, Hamilton Harbour, and Lynde Creek. Variations in egg concentrations of p,p'-DDE showed similar trends to those of total PCBs, except that Cootes Paradise had the highest

levels. The authors report a significant increase in abnormal development with increasing concentrations of 10 PCBs, 7 PCDDs, and 11 PCDFs. Cytochrome P-4501A and 7-ethoxyresorufin O-deethylase (EROD), both indicators of exposure to dioxin-like compounds and Ah-receptor mediated response (Safe, 1994), were also measured in livers of hatchling turtles. The mean EROD activity was 8 times higher and the mean CYP1A was 50 times higher in hatchlings from Lynde Creek compared to Algonquin hatchlings.

In 1998, de Solla et al. (2001) collected eggs from the shorelines of marshes within the Mohawk Nation in Akwesasne, New York. The sites were located two to 13 km downstream from PCB-contaminated landfill sites, and included St. Regis River, Raquette River, Snye Marsh, and Turtle Creek. Total PCB concentrations ranged from 2.37 μg/g at Snye Marsh, to 737.68 μg/g at Turtle Creek. The total PCB concentrations measured in eggs at Turtle Creek are among the highest recorded in any tissue of a free-ranging animal. In a pooled sample of eggs from all four sites, the summed concentrations of non-ortho PCBs were 54.54 ng/g, and the summed dioxin and furan concentrations was 85.8 ng/g. Concentrations of p,p'-DDE followed the same pattern and ranged from 9.80 ng/g to 852 ng/g.

Ashpole et al. (2003) collected turtle eggs in 1999 and 2000 from Algonquin Provincial Park, four sites on the St. Lawrence River, Cootes Paradise in Hamilton Harbour, and Walpole Island in the St. Clair River AOC. Total mean PCB concentrations from Walpole Island were 0.239 μg/g (w.w.). Total mean PCBs were 1.93 μg/g in eggs from Hamilton Harbour, while they ranged from 0.17 μg/g to 60.96 in the St Lawrence River at the Cornwall/ Massena AOC. From lowest to highest, egg p,p'-DDE concentrations were as follows: Cooper Marsh < Algonquin Provincial Park , Walpole Island < Greys Creek = Snye Marsh < Raquette Rive < Cootes Paradise. From the one-pooled sample (five clutches with 5 eggs/clutch or 25 eggs/pool), the concentrations of PCDDs (2.44 ng/kg w.w.) and PCDFs (1.18 ng/kg w.w.) measured in eggs were low compared to the results from Hamilton Harbour (total PCDDs 7.81 ng/kg w.w.; total PCDFs 5.19 ng/kg w.w.) and the Cornwall-Massena AOC area (total PCDDs ranging from 11.64 to 26.9 ng/kg w.w.; total PCDFs ranging from 1.58 to 57.94 ng/kg w.w.).

7.0 The Sensitivity of Snapping Turtles to Changes in Contaminant Levels in Wetlands

The major goal of this White Paper was to validate the use of snapping turtle eggs as indicators of wetland health relative to contamination, as well as geographic and temporal trends in environmental contaminant levels. The collective body of literature on the common snapping turtle reveals that they are excellent indicators of the geographic variation in persistent organic contaminants. In a review by Golden and Rattner (2003), the snapping turtle is ranked seventh out of 25 contaminant indicator species evaluated. With regards to temporal trends in contaminant concentrations in a given wetland, it is important to realize that detection of changes over time is facilitated by frequent data collection. Limited information is available on concentrations of persistent, organic contaminants in snapping turtle eggs collected from sites over a long time span. Here, the spatial analysis of organochlorine and pesticide concentrations in snapping turtle eggs involves multiple sites across the Lake Erie, Lake Ontario, and St. Lawrence River basins (described below), while the temporal analysis has been completed for Algonquin Provincial Park, Cootes Paradise in the Hamilton Harbour AOC, Akwesasne in the St. Lawrence River AOC, and Walpole Island in the St. Clair River AOC.

7.1 *Sample Collection*

Currently, data for geographic contaminant patterns in snapping turtle eggs are being gathered since 2001 as part of a Fish and Wildlife Health Effects and Exposure Study (K. Fernie, CWS, unpublished data). Turtle eggs were collected from Canadian AOCs on the lower Great Lakes, as designated by International Joint Commission (IJC). An AOC is defined as a geographic area that has experienced environmental degradation due to an excess of nutrients in the water (eutrophication), bacteria or chemical contaminants in the environment, or loss of fish and wildlife habitat. For temporal trends in contaminants in turtle eggs, eggs were analyzed from the CWS Tissue Bank to supplement existing data from Struger et al. (1993), Bishop et al. (1996), and Fernie (CWS, unpublished data). Turtle eggs were collected with the greatest frequency from Algonquin Provincial Park and

Cootes Paradise in Hamilton Harbour. Other sites were sampled less often, but still provide sufficient data for temporal trend analysis. Data are also presented from the recent analysis of polybrominated diphenyl ether (PBDE) concentrations in turtle eggs, the first time that this chemical has been analyzed in snapping turtle tissues.

The reference sites and AOCs surveyed were divided into geographical regions as follows (Fig. 1):

1. Reference Sites: from 2001 to 2003, a traditional reference site located near Lake Sasajewun in Algonquin Provincial Park, remote from any industrial or agricultural contaminant sources, was used by Canadian Wildlife Service researchers. Tiny Marsh was chosen as a second reference site and is situated south of Georgian Bay near Midland, Ontario.
2. Lake Erie basin: in 2001, three AOCs within the Lake Erie basin were used: the Detroit River AOC (Turkey Creek drains both the city of LaSalle, and an industrial zone in Windsor, Ontario); the St. Clair River AOC (the St. Clair National Wildlife Area (NWA) and Big Point Hunt Club); the Wheatley Harbour AOC (Muddy Creek, located within the Wheatley Harbour AOC, and Wheatley Provincial Park, and Hillman Marsh Conservation Area, both located approximately 2-3 km of the Wheatley Harbour AOC boundaries).
3. Lake Ontario: in 2002, snapping turtle eggs were collected from two Lake Ontario AOCs: the Hamilton Harbour AOC (Grindstone Creek, Cootes Paradise) and the Niagara River AOC (Lyons Creek near Welland, Ontario). In addition, eggs from Wheatley Provincial Park on Lake Erie and Turkey Creek were collected to increase the sample size from the previous year, and to measure dioxin and furan concentrations. In 2003, two sites from the Toronto AOC located on the Humber River were sampled.
4. St. Lawrence River: in 2003, eggs were collected from both the Canadian and Akwesasne/American sides of the St. Lawrence River AOC: one site upstream from Cornwall at the Upper Canada Bird Sanctuary at Ingleside, and east of Cornwall along the Raisin River; sites also included the Snye Marsh in Akwesasne.

Industrial facilities located in Cornwall, Ontario and Massena, New York historically discharged significant quantities of contaminants, including mercury, zinc, polychlorinated biphenyls (PCBs) and lead to the St. Lawrence River.

7.2 *Chemical Analysis*

Both p,p'-DDE and PCB congener concentrations in turtle eggs collected from 1981 to 1991 were measured using analytical procedures outlined by Bishop et al. (1996). The limit of detection for PCBs was 0.005 mg/kg wet weight (w.w.), and 0.0025 mg/kg w.w. for p,p'-DDE. The value of total PCBs reported is the sum concentration of the following congeners which were measured individually in 1989-1990 samples: 28, 31, 42, 44, 47, 49, 52, 60, 64, 66, 70, 87, 97, 99, 101, 105, 110, 118, 128, 129, 137, 138, 141, 146, 151, 153, 158, 170, 171,172, 174, 180, 182, 183,185, 194, 200, 201, 203, 206; in 1991: all congeners measured in 1989-1990 except #47 and PCBs #74, 149; in 1988, only 16 congeners were measured: PCB #28, 31, 52, 99, 101, 105, 110, 118, 138, 153, 174, 180, 170/190, 194, 66/95, 182/187 (IUPAC number; Ballschmiter and Zell, 1980).

Egg samples were analyzed by Dr. Ken Drouillard and Dr. Robert Letcher of the Great Lakes Institute of Environmental Research (GLIER, University of Windsor, Windsor, ON). The egg samples were thawed to room temperature and extracted with dichloromethane (DCM):hexane (1:1 v/v) after the samples were dehydrated with anhydrous Na_2SO_4. The lipids and biogenic material were removed using gel permeation chromatography and cleaned by florisil column chromatography. All of the 2002 samples were analysed using capillary gas chromatography coupled with an electron capture detector (GC/ECD), whereas the samples analyzed in 2003 used a mass selective detector (GC/MSD). Each cleaned sample was injected to determine organochlorine compounds by using twenty-one organochlorine standards. The method quantification limits (10 x the detection limits) ranged between 0.01 to 0.09 ng/g for the eggs samples analysed at GLIER. Non detectable concentrations were treated as 0.05 ng/g. The PCB congeners measured in 2002 were #42, 44, 49, 52, 60, 64, 70, 74, 87, 97, 99, 101, 105, 110, 118, 128, 138, 141, 146, 151, 153, 171,172, 174, 177, 178, 179, 180,183, 194, 195, 200, 201, 203,

206, 31/28, 66/95, 170/190, 182/187. In all samples, Aroclor 1260 was estimated as (PCB 180/10.96)×100, and Aroclor 1254:1260 (1:1) was estimated as (PCB 138 /14.6)×200.

For some contaminant analyses, certain congeners would co-elute, and the individual congeners could not be distinguished from one another, although the quantity of the total co-eluting congeners could be determined. In order to increase the comparability of the data, congeners that sometimes co-eluted were pooled for all analyses. Occasionally, the second co-eluting congener was not reported. Generally, one of the two (or three) co-eluting congeners have much lower concentrations than the other, so the failure to include the less common congener would have a negligible effect on the final concentrations. For example, PCB 132 co-eluted with PCB 153 using GC-MSD in 2003 samples; in 2001 samples they were reported separately, but PCB 132 contributed only on average 0.34% to the sum of PCB 132 and 153. Similarly, PCBs 56 and 60, and PCBs 70 and 76 were pooled. Non-detection limits varied among methods and laboratories, thus we treated non-detection levels as 0.

7.3 *Statistics*

Since the number of congeners varied among years, we report the sum of only those PCB congeners (n = 34) which were common to all analyses for spatial and temporal comparisons. The thirty-four congeners common among all analyses and so used for measuring sum PCBs, included: 42, 44, 49, 52, 56/60, 64, 70/76, 87, 97, 99, 101, 105, 110, 118, 128, 138, 141, 146, 151, 153/132, 158, 170, 171, 172, 174, 180, 182/187, 183, 194, 195, 196/203, 200, 201, and 206. The sum PCBs using these 34 common congeners was only 7.4% lower than the sum of all 71 congeners used in various studies. Since Aroclor equivalents are not dependent upon the number of congeners measured, Aroclor 1254:1260 (1:1) was also used for statistical comparisons.

For the geographic pattern of contaminant concentrations, a non-parametric procedure, Kruskal-Wallis one-way analysis of ranks, was used to compare PCB and p,p-DDE levels among sites. A simple regression was used to analyze the temporal trend in contaminants within each site. A general linear model (GLM) was used to compare mean PBDE levels among sites. Contaminant data were log transformed prior to analysis, unless

otherwise stated; however, graphical data were presented as untransformed values. GLM was also used to determine if the relative contribution of each BDE congener to sum PBDE varied with relative exposure. Tukey HSD tests were used for post-hoc comparisons.

Identifying PCB mixtures based on congener patterns is important as it helps to determine point sources and because there are toxicity differences among Aroclor mixtures. Therefore, the geographic PCB congener patterns characteristic of different mixtures were examined using ANOVA and Principal Components Analysis (PCA). Contaminants were not transformed, and were expressed on a wet weight basis for comparisons. Patterns of PCB congeners in eggs were examined using ANOVA and Principal Components Analysis (PCA) using varimax normalized rotation on untransformed contaminant concentrations. The 15 most prevalent PCB congeners were included, and were expressed as a proportion of the sum PCBs. Fishers LSD test was used for multiple comparisons of the factor scores among sites. Due to the large number of sites, only a select number of sites from 2001 – 2003 were included for illustrative purposes.

7.4.0 Results and Discussion

7.4.1 *Geographic Contaminant Patterns in Areas of Concern*

Contaminant concentrations in snapping turtle eggs varied among the St. Clair River, Detroit River and Lake Erie AOCs. de Solla and Fernie (in press) also differentiated study sites based upon the profile of the PCB congener profiles in eggs. Concentrations of sum PCBs were highest in the snapping turtle eggs from Turkey Creek (0.327-1.902 μg/g), followed by Wheatley Provincial Park (0.249-0.950 μg/g) and Canard River (0.067-0.896 μg/g). When the PCB congener profile among the Lake Erie and St. Clair River sites was examined, results indicated that snapping turtle eggs from Canard River had a similar profile to eggs from both Turkey Creek and the St. Clair River AOC. The PCB congener profile in the turtle eggs from Turkey Creek reflects the historical Aroclor 1260 sources. The largest single source of the majority of organics, including PCBs and organochlorine pesticides in Lake Erie, is thought to originate from contaminant input from the Detroit River (Kelly et al., 1991). However, the main source of PCB contamination occurring in

Wheatley Harbour is thought to be derived from the local discharge of fish offal from Lake Erie fish processing plants (Bedard, 1985, cited in de Solla and Fernie, in press). Although the PCB source at Wheatley Harbour is unknown, the PCB congener profile in these turtle eggs suggests an Aroclor 1260 source. Concentrations of *p,p*'-DDE in turtle eggs were highest in the areas near the Wheatley Harbour AOC (0.017-0.038 μg/g). This is not surprising given the intensive agricultural activity of this area. Turkey Creek had the next highest *p,p*'-DDE concentration (0.011-0.036 μg/g), while eggs from Tiny Marsh, St. Clair NWA AOC and Canard River had similar and low concentrations (0.0047 μg/g to 0.0059 μg/g). The lowest *p,p'*-DDE concentrations were found in the eggs from Algonquin Provincial Park (0.0008-0.0016 μg/g).

Within the Lake Ontario AOCs, turtle eggs collected from Grindstone Creek (0.715-3.275 μg/g) and Cootes Paradise (0.361-2.058 μg/g) in Hamilton Harbour contained the highest sum PCB concentrations followed by Lyons Creek (0.220-2.793 μg/g) in the Niagara River AOC (near Welland ON), then the Humber River (0.278-1.165 μg/g) draining into Lake Ontario at Toronto. Egg p,p'-DDE concentrations were lowest in the industrialized Niagara River-Welland region (0.001-0.018 μg/g) and highest in eggs collected from Grindstone Creek in Hamilton Harbour (0.130-0.182 μg/g). Sources in Hamilton Harbour most likely originated from local historical agricultural use of DDT, and were deposited to the Harbour by fluvial processes via the Grindstone and Spencer Creeks.

Within the St. Lawrence River, turtle eggs from the Snye Marsh contained the highest total PCB concentrations (0.013 to 1.339 μg/g), followed by eggs from Ingleside (0.010 to 0.197 μg/g) and the Raisin River (0.005 to 0.336 μg/g). In contrast, eggs from Ingleside contained the highest p,p'-DDE concentrations (0.0005 to 0.048 μg/g) relative to the Raisin River (0.0005 to 0.022 μg/g) and the Snye Marsh (0.0003 to 0.031 μg/g).

Basin-wide comparisons of contaminant concentrations in turtle eggs revealed significant differences (Kruskal-Wallis test, $P < 0.01$) among the Great Lakes basin AOC sites sampled. Turtle eggs from Hamilton Harbour contained the highest sum PCB congener concentration, and exceeded reference values by approximately 50 times (Fig. 2). The geographic contaminant patterns reported here are similar to trends observed by Struger et al. (1993) and Bishop et al. (1996). Eggs collected from Turkey Creek and Lyons Creek were next highest in total PCB concentration, and were approximately 40

times greater than reference turtle eggs. Significant differences were also found between these sites and the less contaminated sites at Lake St. Clair and the St. Lawrence River sites at Ingleside and the Raisin River. Eggs from the Canard River, Wheatley Harbour, Humber River, and the Snye Marsh were moderate in PCB concentrations and fell between these two groups (Fig. 2). When the Aroclor equivalent (1254:1260) was compared among sites, a similar contaminant pattern emerged (Kruskal-Wallis test, $P < 0.01$), however, eggs from Hamilton Harbour had the highest Aroclor equivalent, and differed significantly from all other sites except Turkey Creek . Significant differences were also found between Turkey Creek and eggs from the reference sites, Lake St. Clair and Ingleside and the Raisin River on the St. Lawrence River (Fig. 3).

Concentrations of p,p'-DDE also differed significantly among AOC and reference sites (Kruskal-Wallis test, $P < 0.01$). Turtle eggs from Hamilton Harbour contained p,p'-DDE concentrations that were approximately 40 times above reference values, and were significantly higher than all other sites monitored within the Great Lakes- St. Lawrence basin. There was a high variation within each site, such that no differences were detected among the remaining AOC sites. Egg p,p-DDE concentrations were comparable among these sites and ranged from 0.003 to 0.058 µg/g wet weight. These results are similar to that reported for p,p'-DDE by Struger et al. (1993) and Bishop et al. (1996).

7.4.2 *Principal Component Analysis*

Four principal components were extracted, accounting for 83.0% of the total variance, and the first component explained 47.8% of the variance. The factor scores for the first component varied among sites (PC1, $F_{[5,50]} = 13.7$, $P < 0.0001$). Snye Marsh had significantly lower scores than any other site, Lyons Creek and UCBS had the next lowest scores, although Algonquin Provincial Park and UCBS were not significantly different from each other. There were no differences among Algonquin Provincial Park, Turkey Creek, and Cootes Paradise. PC1 was positively correlated (> 0.6) with PCBs 180 and 170, which are characteristic of Aroclor 1260, and negatively correlated with PCBs 118 and 105, which are characteristic of Aroclor 1254 (Fig 4a, Frame, 1997). The factor scores for the second component varied among sites (PC2, $F_{[5,50]} = 15.1$, $P < 0.0001$). Lyons and

Algonquin Provincial Park had lower scores than any other site, whereas Cootes Paradise and Turkey Creek had higher scores than any other site except UCBS. Snye Marsh and UCBS had intermediate scores. PC2 was highly positively correlated with PCBs 138 and 128, which are characteristic of Aroclor 1260, and negatively correlated with PCBs 66/95, which are characteristic of Aroclor 1254 (Fig 4a, Frame, 1997). Using the PCA analysis, the different sites were grouped according to the PCB burden in the eggs (Fig. 4b): in general, turtles from Snye Marsh, Lyons Creek and UCBS had the largest relative exposure to Aroclor 1254, whereas Cootes Paradise and Turkey Creek had the largest relative exposure to Aroclor 1260.

The PCA analyses demonstrated that the congener profile in snapping turtles varies geographically, and these differences were associated with different Aroclor sources. It is unlikely that differences in volatilization or trophic transfer could account for differences in congener composition among these sites. Previous studies have demonstrated the difference in Aroclor use throughout the lower Great Lakes region (Oliver and Bourbonniere, 1985; Sokol et al., 1994, Martin et al., 2003). Examining the PCB source is not just pertinent if there is a point source, but is also important in areas in which there are no local sources of PCBs. Algonquin Provincial Park has no known local sources of PCBs (Bishop et al. 1991), and consequently the PCB burdens likely reflect background PCB contamination via airborne deposition. Aroclor 1260 is dominated by hexa and hepta biphenyls, which may explain the similarity of the congener profile in Algonquin Provincial Park to Aroclor 1260.

Biota used for monitoring purposes should be able to discriminate among PCB sources, particularly in situations where there is a prominent point source. Sedentary snapping turtles typically have very small home ranges. Consequently, the maternal burden would reflect the environment throughout their home range. Although Russell et al, (1999) found that the ratio of contaminants between eggs and muscle in snapping turtles deviated from the equilibrium partitioning model, there was good agreement in relative concentrations between maternal and egg burdens (Pagano et al. 1999), and the partitioning of contaminants among tissues was independent of the octanol:water partition coefficients (Russell et al. 1999). Consequently, snapping turtle eggs adequately reflect local contamination. Certainly, the data show the utility of snapping turtle eggs to monitor local

contamination adequately, and can be used to discriminate among different locations within the lower Great Lakes Basin.

7.4.3 *Temporal Variation Within Sites*

Algonquin Provincial Park

Concentrations of PCB 1260 in from turtle eggs Algonquin Provincial Park decreased by 86% ($R^2 = 0.3102$; $P < 0.01$) (Fig. 5) and the Aroclor equivalent (1260:1254) decreased by 65% ($R^2 = 0.3187$; $P < 0.01$) from 1981 to 2003. No temporal change was observed in the total PCB concentrations when the common sum of PCB congeners was examined between the years 1989 and 2003. However, a significant decreasing trend in PCB concentrations was found between 1993 and 2003 ($R^2 = 0.3111$; $P < 0.01$). These results generally correspond to those reported by Bishop et al. (1996), who observed a significant decrease in total PCB concentration in Algonquin Provincial Park eggs examined between 1981 and 1991. Our results also revealed that egg p,p'-DDE concentrations show little change over time, suggesting that p,p'-DDE levels possibly have reached a steady state in wetlands of this region.

Cootes Paradise/ Hamilton Harbour

Turtle eggs from Cootes Paradise collected from 1984 to 2002 revealed a 54% reduction in PCB 1260 concentrations ($R^2 = 0.1194$; $P < 0.01$) (Fig. 6). a decrease of 65% in the Aroclor equivalent (1260:1254) ($R^2 = 0.1818$; $p < 0.01$) and a decrease of 60% in p,p'-DDE concentrations ($R^2 = 0.2778$; $P < 0.01$). Similarly, the common total PCB congener concentration decreased significantly ($R^2 = 0.1750$; $P < 0.01$) in eggs from Cootes Paradise between 1986 and 2003. These results contrast with Bishop et al. (1996), who reported a significant increase in total PCB concentrations in turtle eggs from Cootes Paradise between 1984 and 1991. The discrepancy in contaminant trends between our results and the results of Bishop and colleagues (1996) may be due to the construction of a barrier between Cootes Paradise and Hamilton Harbour in 1996. This barrier prevents large migratory carp (*Cyprinus carpio*), which possibly contain high contaminant burdens, from

entering Cootes Paradise. The decrease in carp in the diet of turtles may therefore explain the steady decrease in PCB and p,p'-DDE residue concentrations in snapping turtle eggs.

Snye Marsh/ St. Lawrence River

Turtle eggs, collected from the Snye Marsh located on the St. Lawrence River near Akwesasne, New York between 1990 and 2003, showed a significant decrease of 89% in PCB 1260 concentrations ($R^2 = 0.4153$; $P < 0.01$). Similarly, the concentrations of Aroclor equivalent (1260:1254) decreased by 83% ($R^2 = 0.3277$; $P < 0.01$), with a 81% decline in the sum of the common PCB congeners ($R^2 = 0.2949$; $P < 0.01$), and a 76% decline in p,p'-DDE concentrations ($R^2 = 0.2951$; $p < 0.01$).

Walpole Island/ St. Claire River

Turtle eggs from Walpole Island on the St. Clair River, were collected in 1992, 1995 and 1999. Discharge of chlorinated organic compounds, heavy metals, oils and greases, phenols, suspended solids from petroleum and chemical industries, spills, as well as historically contaminated sediments, are found in the St. Clair River and pose major community concerns (International Joint Commission 1999). No significant change in PCB or p,p'-DDE concentrations in eggs from Walpole Island were detected over this time span (1992, 1995, 1999).

7.4.4 *Temporal Variation in Chemical Concentrations at Hamilton Harbour: A Comparison of Contaminant Trends in Suspended Sediments, Herring Gull Eggs, and Snapping Turtle Eggs.*

An effective means to test the utility of the snapping turtle as an indicator of the bioavailability of persistent organic contaminants, is to compare contaminant concentrations in turtle eggs with other environmental media from the same site over time. Comparing contaminant trends in turtles with other indicator species is also useful, although caution should be exercised when interpreting the results since there are behavioral and dietary differences that will affect the exposure to chemicals.

Results from Marvin (2003) for sum PCB concentrations in suspended sediment collected from Hamilton Harbour were compared with sum PCB concentrations measured in snapping turtle and herring gull eggs collected from 1986 to 2002 (Canadian Wildlife Service Contaminants Database, 2003). The same number of PCB congeners (# 18, 44, 49, 52, 101, 105, 151, 138, 180, 183, 199, 149 and 118) were chosen based on the analytical results for suspended sediments (Fig. 7). Hamilton Harbour was chosen because of the extensive contaminants data collected for snapping turtles since 1996, and because of the high contaminant concentrations found at this location.

Declines in PCB concentrations in suspended sediment from Hamilton Harbour are most apparent from the mid-1980s to the early 1990s, with little change in concentrations in recent years. This pattern corresponds with the temporal variation of declining PCB concentrations in snapping turtle eggs collected from Cootes Paradise, a wetland contiguous with the Harbour. For the selected congener types, results from analysis of herring gull eggs also demonstrate decreasing contaminant burdens occurring mostly from the mid 1990s to present. This indicates that changes in contaminant concentrations in turtle eggs reflect changes measured in other environmental matrices from the same site, further substantiating the usefulness of the snapping turtle as an indicator of contaminant bioavailability.

7.4.5 *A New and Emerging Chemical of Concern: Polybrominated Diphenyl Ether (PBDE) Concentrations in Turtle Eggs*

Polybrominated diphenyl ethers enter the environment by leaching from plastics, textiles, and foams in which they are incorporated, and have generated substantial environmental concern. Once in the environment, penta-PBDEs are persistent, lipophilic, and readily bioaccumulate through the food chain (Hickey et al., 2002). Research and monitoring programs indicate that there is a global occurrence of PBDEs in wildlife, particularly the lower brominated congeners (BDE-47, - 99, -100, -153,- 154) (Sellstrom et al., 1993; Law et al., 2002; Luross et al., 2002). Retrospective analyses of wildlife tissues illustrate an exponential increase in total PBDEs (BDE-47, -99, -100) in herring gull (*Larus argentatus*) eggs collected from the Great Lakes between 1981 and 2000 (Norstrom

et al., 2002). This review provides insight into the occurrence of PBDEs in turtle eggs found in wetland systems in the Great Lakes basin.

The mean log transformed sum of PBDE congeners varied among sites ($F[8,43] = 12.07$, $P < 0.0001$). Sum PBDEs varied from a mean of 6.1 (Algonquin Provincial Park) to 107.0 (Humber River; Fig. 8). Although there were a number of differences among sites, generally levels were lowest at Algonquin Provincial Park, where airborne deposition is assumed to be the main contaminant source. Similar concentrations were found in eggs from Lyon's Creek, and the Upper Canada Bird Sanctuary. Consistent with reports that urban areas contain the highest PBDE concentrations, turtle eggs from Cootes Paradise in Hamilton Harbour and Humber River in Toronto, were the most contaminated among all sites.

We grouped each site into categories in relation to relative sum PBDE concentrations (low, medium, and high), and expressed each congener as a proportion of sum PBDEs, and log transformed Fig. 9). The congeners BDE - 47 and BDE - 99 contributed the most to the sum PBDEs, BDE – 28, -138, and -209 were detected in a limited number of samples. The proportion of BDE 47 increased with sum BDE concentrations ($F_{[2,49]} = 8.56$, $P < 0.0006$), whereas the proportion of BDE 153 and 183 decreased with sum BDE ($F_{[2,49]} = 6.03$, $P < 0.0046$; $F_{[2,49]} = 17.76$, $P < 0.0001$, respectively). Presently, the sum of PBDE concentrations in herring gull eggs are the third highest among groups of organohalogen compounds in the Great Lakes. PBDE concentrations in gull eggs are expected to reach current environmental concentrations of PCBs and DDE in as little as 10 years (Norstrom et al., 2002). Thus, we can expect similar trends in snapping turtle eggs, thereby warranting further use of this indicator to monitor trends in chemicals of recent concern.

8.0 The Usefulness of Snapping Turtles in a Monitoring Plan in Terms of Being Able to Set Endpoint(s) or Attainment Levels Relative to Contaminant Levels in Wetlands of the Great Lakes Basin.

The cumulative data that emerged from this literature review and validation study indicate that the common snapping turtle is indeed sensitive to contaminant exposure.

Furthermore, the geographic patterns and temporal changes in contaminant residues in eggs prove beyond doubt, its usefulness as an indicator model for environmental contaminant studies. A long-term, basin-wide contaminants monitoring program will be much stronger when it involves a large number of sites, greater frequency of sampling, and a longer time frame for data collection (years). Given the above conditions, the objective of providing information to policy makers on environmental contamination in coastal wetland systems in the Great Lakes basin can be met. Given the wide geographic distribution of the snapping turtle and its ability to live in close proximity to urban centers, chemicals of scientific and public concern, including newly emerging ones like polybrominated diphenyl ethers, can be characterized in wetland systems occurring in highly developed settings. Temporal and geographic trend information for these compounds could provide the necessary evidence needed to bring about the curtailment of their use.

The 1978 Great Lakes Water Quality Agreement has committed both Canada and the United States to the "virtual elimination of persistent toxic substances and restoring and maintaining the chemical, physical and biological integrity of the Great Lakes Basin Ecosystem" (International Joint Commission United States and Canada, 1988). With these objectives in mind, the data from this monitoring program are suitable measurement endpoints that could be used to verify attainment of this goal. In the short term, information can be used by policy makers and resource managers to substantiate progress on remediation measures at the local level in Areas of Concern via Remedial Action Plans (RAPs), or at the lake-level via lake wide management plans (LaMPs). The data generated as part of the program can also be used to increase public awareness of environmental contaminant levels in biota, report progress on reaching attainment levels, and facilitate assessment programs in both Canada and the United States.

According to Servos et al. (1999), defining virtual elimination is problematic, and the traditional approach using chemically defined detection limits or levels of quantification in routine sampling and analytical methods may be unrealistic. One problem inherent to this approach is improvements in analytical techniques resulting in an ever-diminishing chemical detection limits. Biological responses may also result from chemical concentrations currently not detectable when using current analytical techniques. It is suggested that an effects-based approach to establish biologically relevant endpoints in

sentinel species would be more useful to determine exposure and set targets for virtual elimination of substances of concern. In this regard, not only can the snapping turtle provide information on the types, levels, and bioavailability of pollutants, but also information on the biological effects of exposure to environmental pollutants. The results of studies on developmental toxicity, alterations in endocrine function, and functional immune response in snapping turtles could be used as a basis for decisions and policies regarding the effects of chemical exposures on wildlife inhabiting wetland ecosystems, as well as human populations.

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Table 1. Mean home ranges (ha) of snapping turtles from wetland sites within Canada and the United States.

Mean (SD) Home range (ha)	Method	Reference
1.84	Pennsylvania	Ernst 1968
0.65	Tennessee	Murphy and Sharber 1973
0.29 (0.27)	Tennessee	Froese 1974
3.44 (2.2)	Lake Sasajewun, Ontario	Obbard and Brooks 1981
0.71 (0.29)	Broadwing Lake, Ontario	Galbraith *et al*. 1987
8.14 (3.00)	Lake Sasajewun, Ontario	Brown 1992
8.64 (2.92)	Lake Sasajewun, Ontario	Brown *et al*. 1994
6.53 (6.15)	Cootes Paradise, Ontario	Brown *et al*. 1994
Male: 2.2-3.0, Female: 8.9-9.7	Cootes Paradise, Ontario	Pettit *et al*. 1995
5.13 (1.86)	Lynde Creek, Ontario	Brown *et al*. 1994

Table 2. Polychlorinated biphenyls (PCBs), dioxins (PCDDs), furans (PCDFs) and dichlorodiphenyl ethylene (p,p'-DDE) concentrations measured in snapping turtle eggs.

Location	# Clutches sampled	Total PCB µg/g [a]	Total PCDD ng/kg [a]	Total PCDF ng/kg [a]	p,p'-DDE µg/g [a]	Reference/Agency	Comments
Rondeau Provincial Park, Lake Erie	NM [b]	NM	NM	NM	0.21	Campbell, 1974, Canadian Wildlife Service	PCB Aroclor 1254:1260 1.92 µg/g w.w.
Hudson R, NY	6	28.9	NM	NM	NM	Stone et al. (1980)	Egg contents (w.w.)
Hudson R, NY	2	1.11-2.86	NM	NM	NM	Bryan et al. (1987), State University of New York at Albany	Egg yolks
Hudson R, NY	2	0.12-0.48	NM	NM	NM		Egg while and shells
Algonquin Provincial Park	7-14	0.025-0.076	ND	ND	0.0080	Bishop et al. (1991), Canadian Wildlife Service, Environment Canada	Data collected 1986-1989; 5 eggs pooled/ clutch
Cootes Paradise	8-21	0.947-2.854	46	17	0.877		
Lynde Creek	4-10	1.360-2.709	124	39	0.472		
Big Creek Marsh	7-18	0.223-0.690	8	5	0.044		
Cranberry Marsh	5-12	0.257-0.605	16	4	0.081		
Algonquin Provincial Park	6	0.187	NM	NM	0.027	Struger et al. (1993), Environmental Quality Branch, Environment Canada	Data collected 1981-1984; 5 – 10 eggs pooled /clutch
St. Lawrence River	5	0.537-0.914	NM	NM	0.010-0.180		Loon Island, Ingleside, Morrisburg
Bay of Quinte	5	0.271-2.751	NM	NM	0.020-0.350		South of Moira River, Sawguin Cr. Big Island
Murray Canal	5	1.324	NM	NM	0.090		
Lynde Shores C.A.	5	1.017	NM	NM	0.090		
Hamilton Harbour, Cootes Paradise	3	1.315	NM	NM	0.200		
Hamilton Harbour, Grindstone Creek	3	4.706	80	14	0.340		PCDDs and PCDFs; subsample from three clutches; 10 eggs pooled/clutch
Big Creek	4	1.006	NM	NM	0.097		

National Wildlife Area							
Rondeau Provincial Park	5	1.093	NM	NM	0.042		
Lake St. Clair	5	0.344-1.392	NM	NM	0.115-0.140		Thames River, St. Clair National Wildlife Area, Mitchell Bay
Port Franks	4	1.166-1.542	NM	NM	0.116		Pinery Provincial Park, Thedford
Hamilton Harbour, Cootes Paradise, Ontario	15	54.3	NM	NM	NM	Bishop et al. (1994), Canadian Wildlife Service, Environment Canada	5 eggs pooled/clutch; all data are % lipid basis
Hamilton Harbour, Cootes Paradise, Ontario	4	23.952	NM	NM	0.049	Bishop et al. (1995), Canadian Wildlife Service, Environment Canada	first 5 eggs oviposited in nest; data collected 1986
		28.574	NM	NM	NM		composite of 5 eggs per nest
		20.138	NM	NM	NM		last 5 eggs oviposited in nest)
Hoople Creek, Cornwall	4	0.678	NM	NM	0.055	Bonin et al. (1995), St. Lawrence Valley Natural Historic Society, Quebec	Data collected 1989-1990; egg contents; 5 eggs pooled /clutch
Ingleside, Cornwall	3	2.834	NM	NM	0.372		
Grays Creek, Cornwall	5	0.873	NM	NM	0.023		
Raquette R. Massena NY	5	5.094	NM	NM	0.075		
St. Regis R. Massena NY	1	0.942	NM	NM	0.0435		
Akwasasne Massena NY	2	1.575-5.073	NM	NM	0.035-0.047		
Dundee, St. Lawrence R.	7	1.862	NM	NM	0.219		
Beauharnois, St. Lawrence R.	3	1.837	NM	NM	0.068		
Boucherville, S. Lawrence R.	2	0.181-3.343	NM	NM	0.003-0.078		
Thurso, Ottawa R. Ontario	7	0.106	NM	NM	0.007		
Algonquin Provincial Park	15	0.32-3.38	16.8	ND	0.04-0.49	Bishop et al. (1996), Canadian Wildlife Service, Environment	Data collected 1981-1991; all data are %

						Canada	lipid basis
Cranberry Marsh	15	5.27-9.36	287.1	75.8	1.09-1.39		
Big Creek Marsh	12	6.23-14.25	58.1	54.4	0.74-1.44		
Rondeau Park	12	10.95-22.13	35.0	50.7	0.66-0.83		
Lynde Creek	26	20.50-37.64	4499.9-1898	732.1-1534.8	1.72-5.93		
Cootes Paradise	31	21.78-54.36	282.3-1230	206.5-273.7	4.52-10.65		
Algonquin Provincial Park	7	0.018	0.90	ND	0.0018	Bishop et al. (1998), Canadian Wildlife Service, Environment Canada	all data are on a wet weight basis
Cranberry Marsh	3	0.241	14.5	3.6	0.032		
Big Creek Marsh	5	0.388	3.1	2.9	0.0547		
Rondeau Park	6	0.617	2.0	2.9	0.0369		
Lynde Creek	8	1.430	107.8	81.5	0.232		
Cootes Paradise	7-12	2.082-3.574	19.7-39.7	14.4-16.7	0.312-0.389		
Trent River	4	0.835	68.0	6	0.071		
Akwesasne/USA	7	3.946	22.9	9.1	0.068		
Algonquin Provincial Park	9	0.024	NM	NM	0.006	Canadian Wildlife Service Database, 1999	
St. Lawrence River, Cooper Marsh	9	0.1604	NM	NM	0.003		
St. Lawrence River, Snye Marsh	9	1.9425	2.945-5.36	2.00-3.66	0.015		
Hamilton Harbour, Cootes Paradise	9	1.9287	3.61	2.26	0.069		
Raquette R., Akwesasne/ USA	5	4.9564	5.74 – 15.46	3.925-6.61	0.0436		
Cornwal, Grey's Creek	4	0.6197	8.385	6.09	0.0157		
Lake St. Clair, Walpole Island	5	0.187	2.44	1.18	0.00875		
Hosaic Creek	3	0.01166	1.67	0.94	0.00066	Canadian Wildlife Service Database, 2000	
Grey's Creek	2	0.5745	NM	NM	0.018		
Raquette R., Akwesasne/ USA	3	2.4736	14.56	5.083	0.024		
Akwesasne/St.	1	6.785	9.65	76.15	0.011	De Solla et al.	PCDD and

Regis River, New York						(2001), Department of Zoology, University of Guelph, Ontario	PCDF concentrations were measured in a pool of 5 eggs/clutch
Akwesasne/Raquette River, New York	1	5.960	NM	NM	0.029		
AkwesasneSnye Marsh, New York	5	2.378	NM	NM	0.009		
Akwesasne/Turtle Creek, New York	1	737.683	NM	NM	0.852		
Algonquin Provincial Park	6	0.0157	3.165	0.744	0.0013	De Solla and Fernie, (2003, in prep), Canadian Wildlife Service, Environment Canada	
Tiny Marsh	9	0.0411	2.166	0.762	0.0049		
St. Clair National Wildlife Area	6	0.0742	NM	NM	0.0059		
Turkey Creek	8	0.9286	22.96	3.585	0.0244		
Canard River	4	0.2005	NM	NM	0.0047		
Wheatley Provincial Park	8	0.491	17.40	3.453	0.0579		
Algonquin Provincial Park	2	0.0175	NM	NM	0.0014	Canadian Wildlife Contaminant database/2002	
Tiny Marsh	4	0.030	NM	NM	0.0048		GLIER
Hamilton Harbour/ Cootes Paradise	5	1.306	8.593	6.487	0.0879		GLIER
Hamilton Harbour/ Grindstone Creek	5	1.706	15.599	9.908	0.1477		GLIER
Turkey Creek	4	1.074	NM	NM	0.0311		GLIER
Niagara River/ Lyons Creek		1.234	7.149	3.592	0.009		
Wheatley Park	5	0.589			0.0218		GLIER
Algonquin Provincial Park	4	0.0130	NM	NM	0.0032	Canadian Wildlife Contaminant database/2003	
Tiny Marsh	3	0.0075	NM	NM	0.0027		
Humber River, Toronto, Clairville	2	0.6211	NM	NM	0.0092		
Humber River, Toronto, Etobikoke	5	0.5635	NM	NM	0.0360		

Ingleside, Cornwall	11	0.119	NM	NM	0.016
Raisin River, Cornwall	10	0.1348	NM	NM	0.0079
Lyons Creek, Niagara River AOC	4	1.054	NM	NM	0.0029
Snye/ Akwesasne	4	0.2364	NM	NM	0.0051
Snye/ Quebec	4	0.8659	NM	NM	0.0193

[a] NM = not measured, ND = non-detectable
[b] wet weight unless otherwise stated

Figure 1. Study sites used to determine the levels of persistent, organic environmental contaminants in snapping turtle eggs.

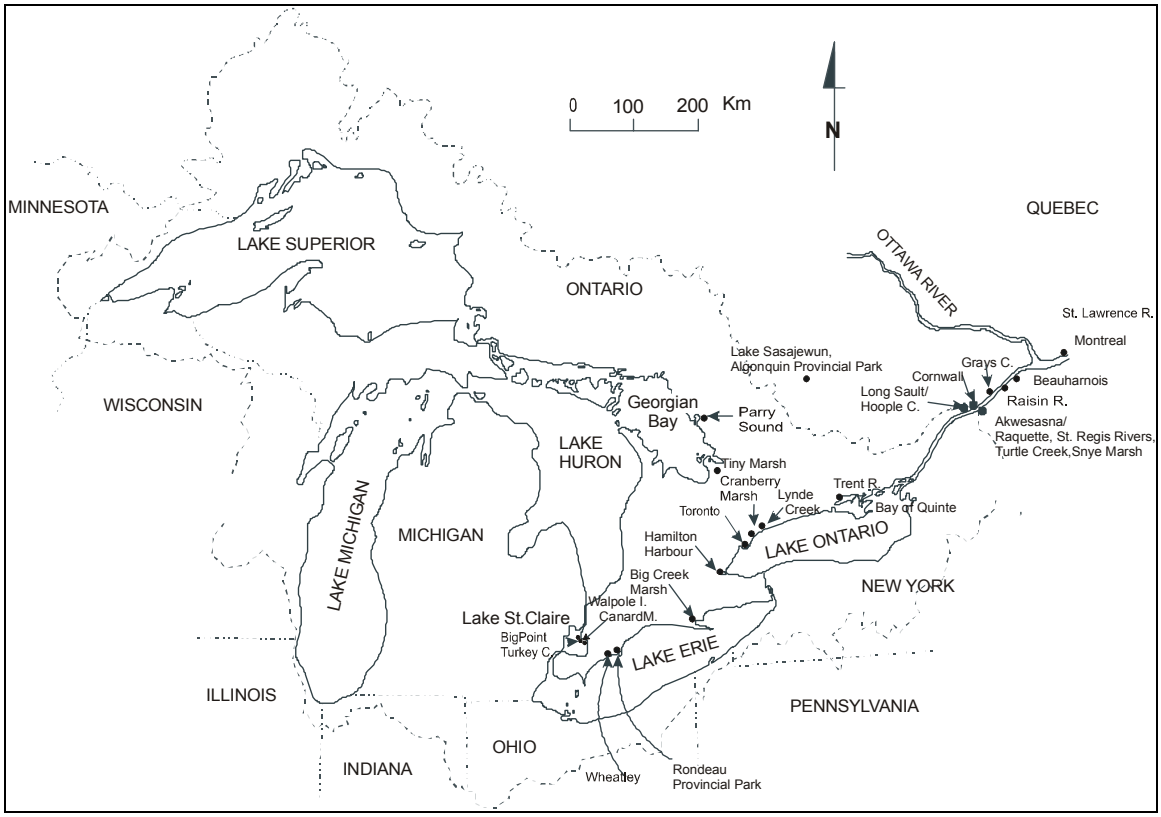
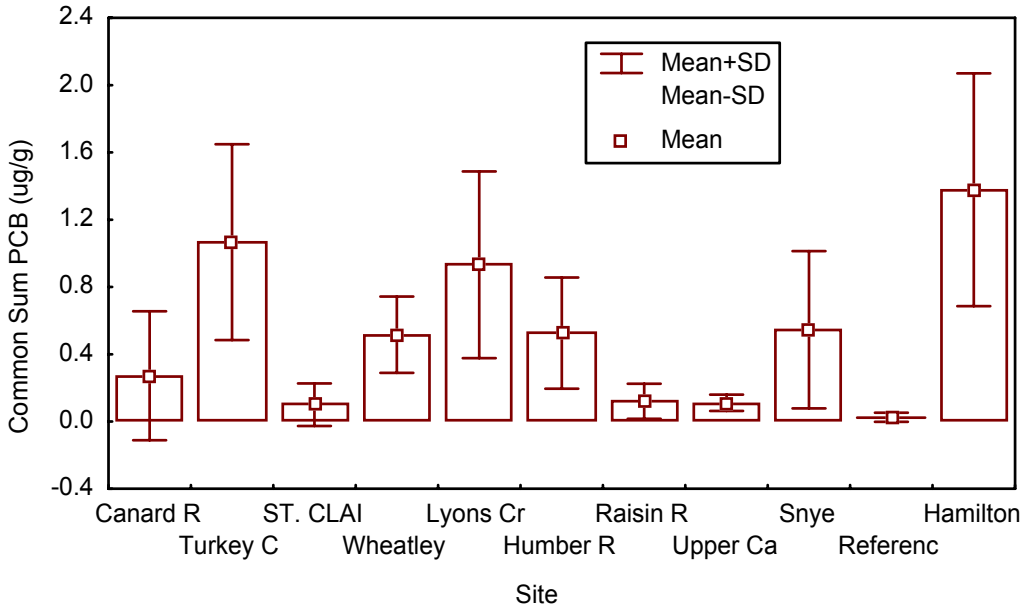


Figure 2. The spatial (geographic) pattern of total PCB concentrations in snapping turtle eggs collected from reference sites and Canadian Areas of Concern in the Great Lakes – St. Lawrence River Basin (2001-2003).



Canard R: The Canard River is located downstream of Windsor ON.

Turkey C: Turkey Creek is located within Windsor ON and runs into the Detroit River.

St. Clai: The St. Clair sites are located within one kilometer (by water) of the St. Clair Area of Concern (AOC) and Walpole Island. Both the St. Clair National Wildlife Area and one private property were sampled.

Wheatley: Clutches were collected from Wheatley Provincial Park and adjacent to the Hillman Marsh Conservation Area (2001 only).

Lyons Cr: Lyons Creek is located adjacent to the Welland Canal and is within the Niagara River AOC.

Humber R: This site is located at the Humber River Marshes at the mouth of the Humber River, Lake Ontario in Toronto ON.

Raisin R: Raisin River runs between Cornwall and Lancaster ON, exiting into the St. Lawrence River.

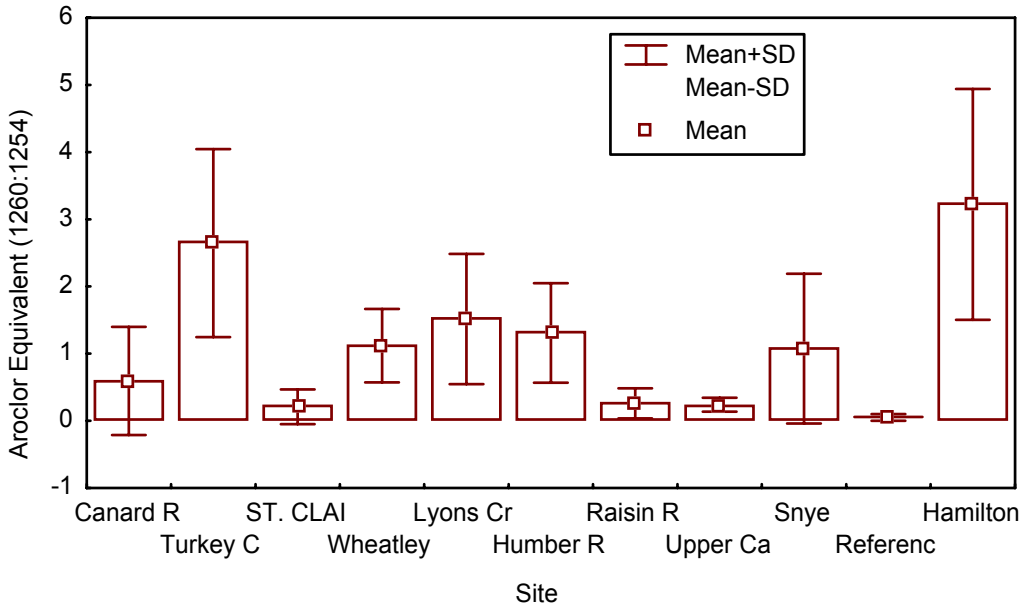
Upper Ca: The Upper Canada Bird Sanctuary is located within the St. Lawrence River upstream of that AOC near Ingleside ON.

Snye: Snye Marsh is located in Akwesasne and enters into the St. Lawrence River.

Referenc: The reference site is Algonquin Park.

Hamilton: This site is Cootes Paradise near Hamilton ON and located within the Hamilton Harbour AOC.

Figure 3. The spatial (geographic) pattern of the Aroclor equivalent (1260:1254) in snapping turtle eggs collected from wetlands at reference sites and Canadian Areas of Concern in the Great Lakes – St. Lawrence Basin (2001-2003).



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Referenc: The reference site is Algonquin Park.

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Figure 4a. Principal component loadings of PCB congeners in snapping turtle eggs from Great Lakes study sites used in 2001-2003. PC1 is dominated by higher chlorinated biphenyls associated with Aroclor 1260.

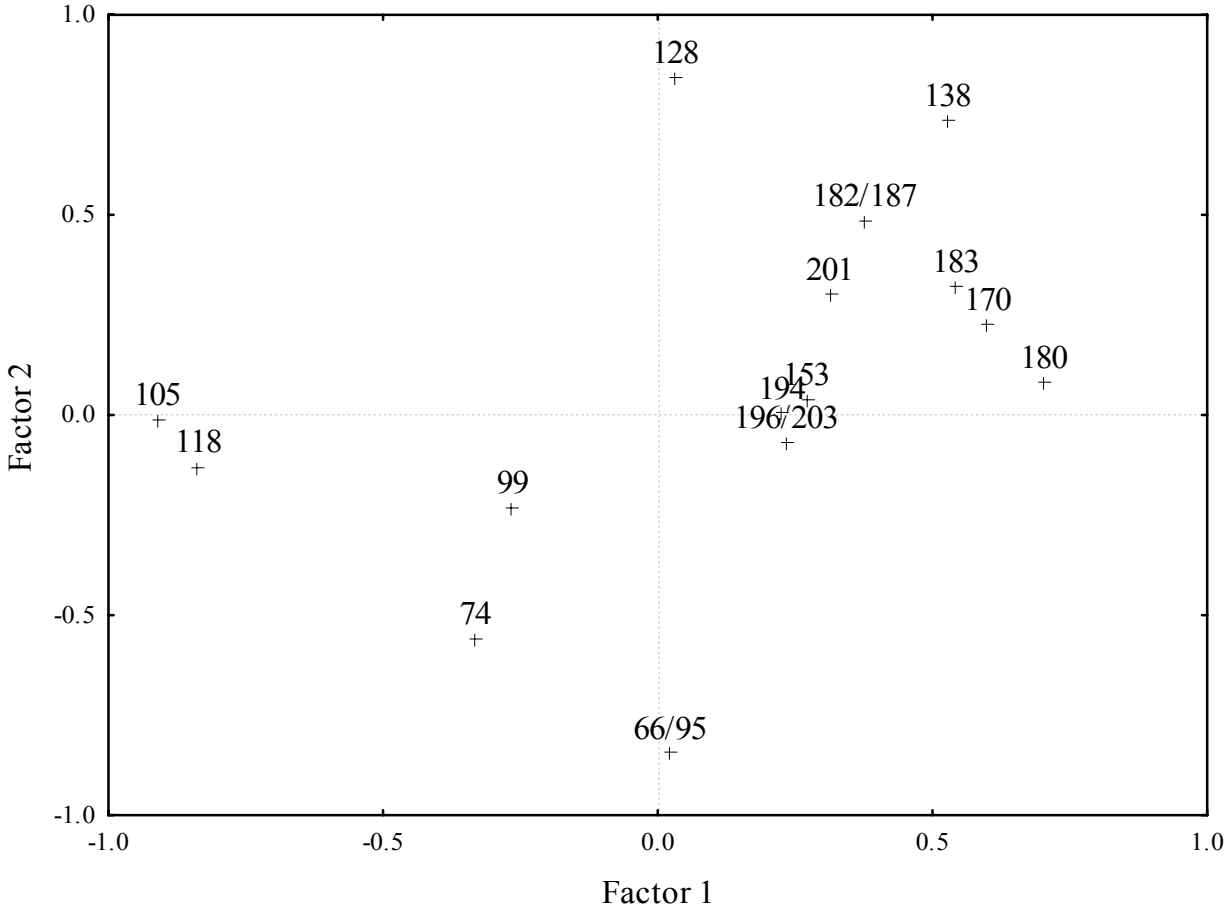


Figure 4b. Factor scores from egg samples for each location during 2001-2003 of the CWS Wildlife Health Effects Study. The boundary illustrates the clustering of different sites based upon the PCB burden in eggs.

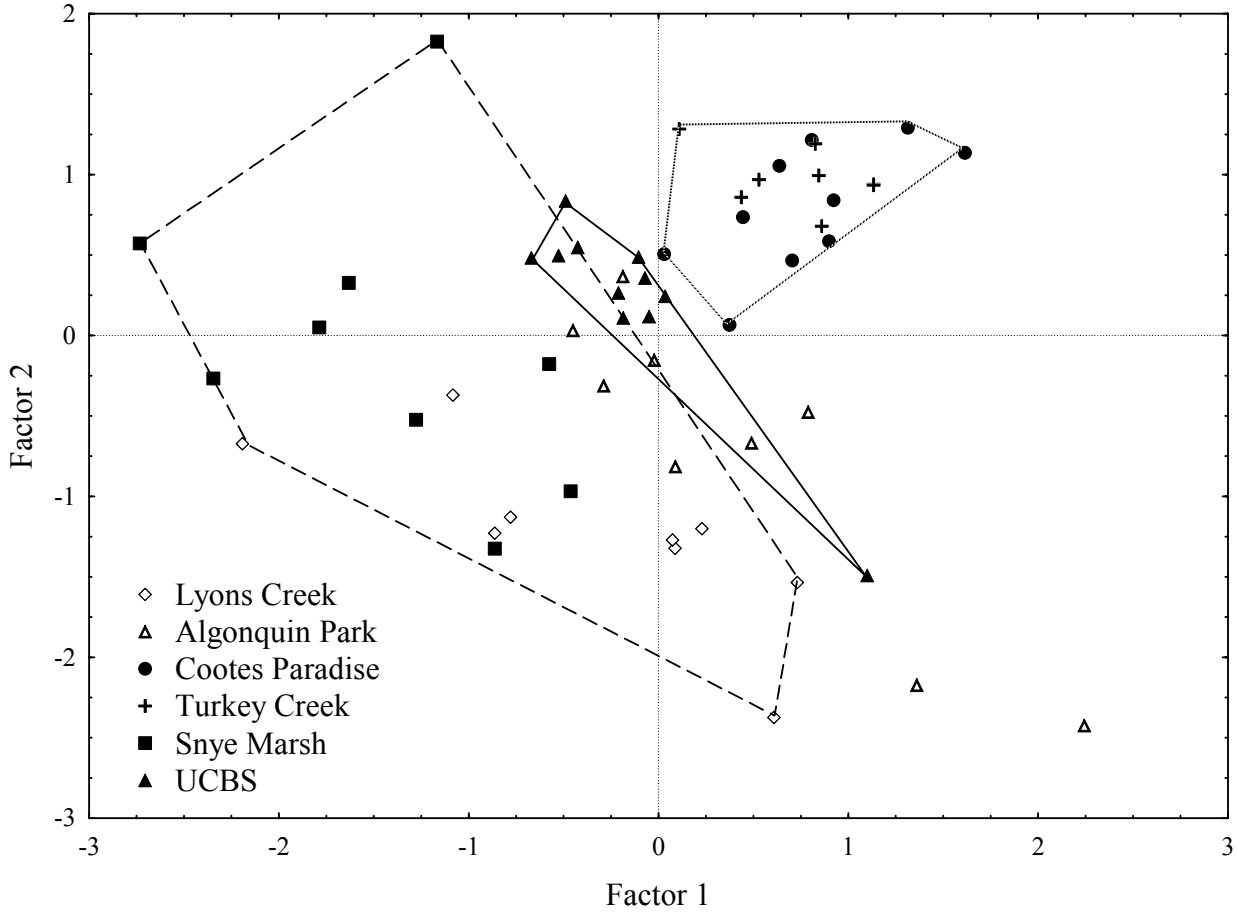


Figure 5. Temporal trends in PCB 1260 concentrations in snapping turtle eggs from a non-contaminated reference site in Algonquin Provincial Park, Ontario.

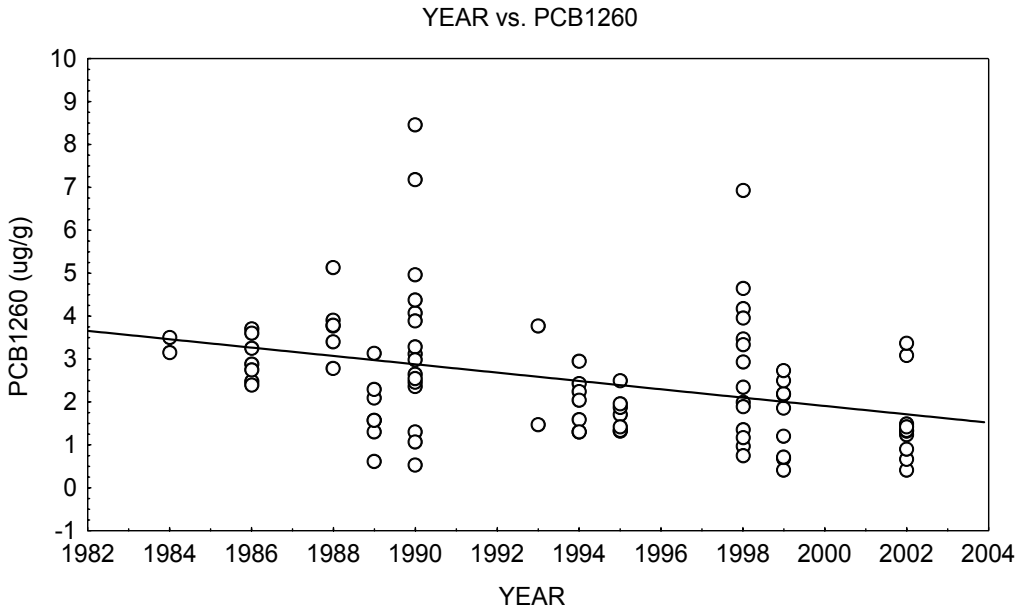


Figure 6. Temporal trends in PCB 1260 concentrations in snapping turtle eggs from Cootes Paradise, Hamilton Harbour AOC, Lake Ontario.

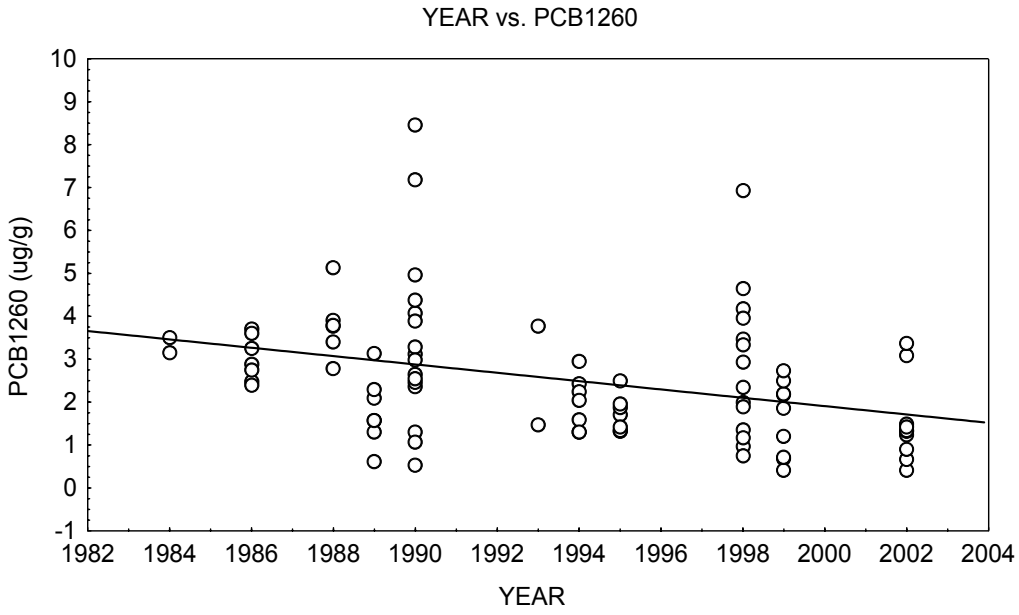


Figure 7. A comparison of mean sum polychlorinated biphenyl concentrations in suspended sediment, and eggs of herring gulls and snapping turtles collected from Hamilton Harbour from 1986 to 2002.

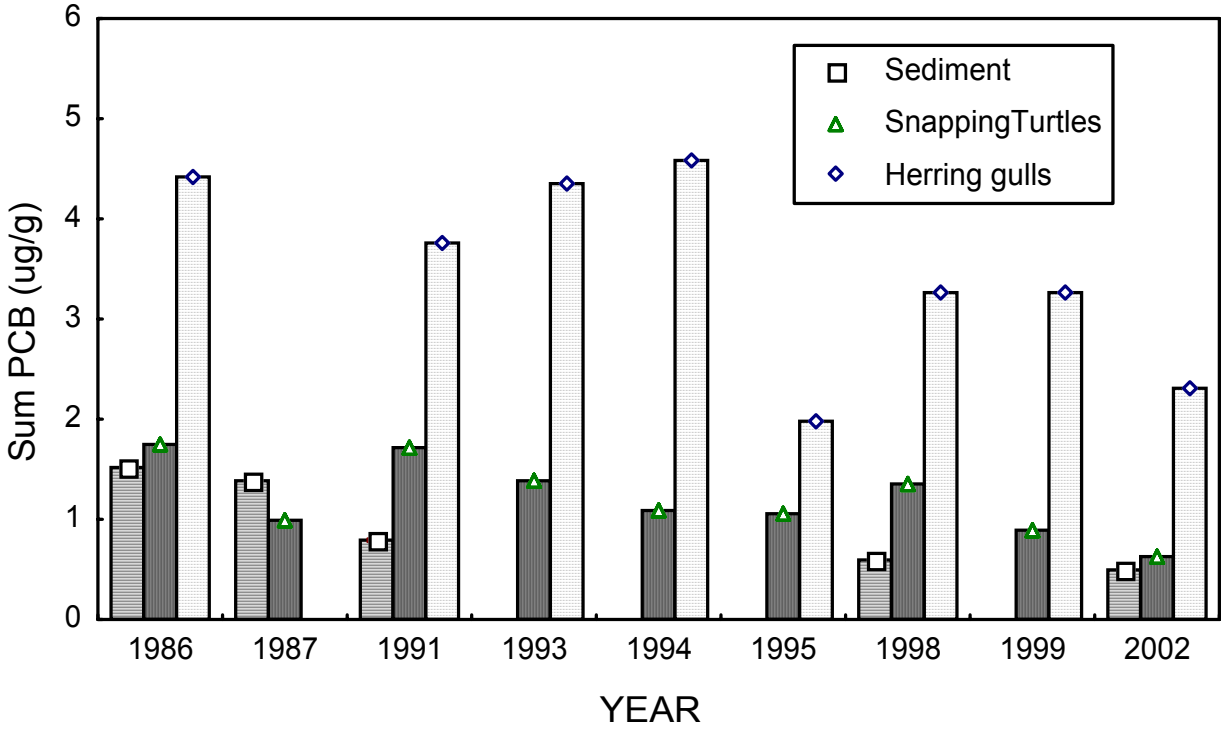
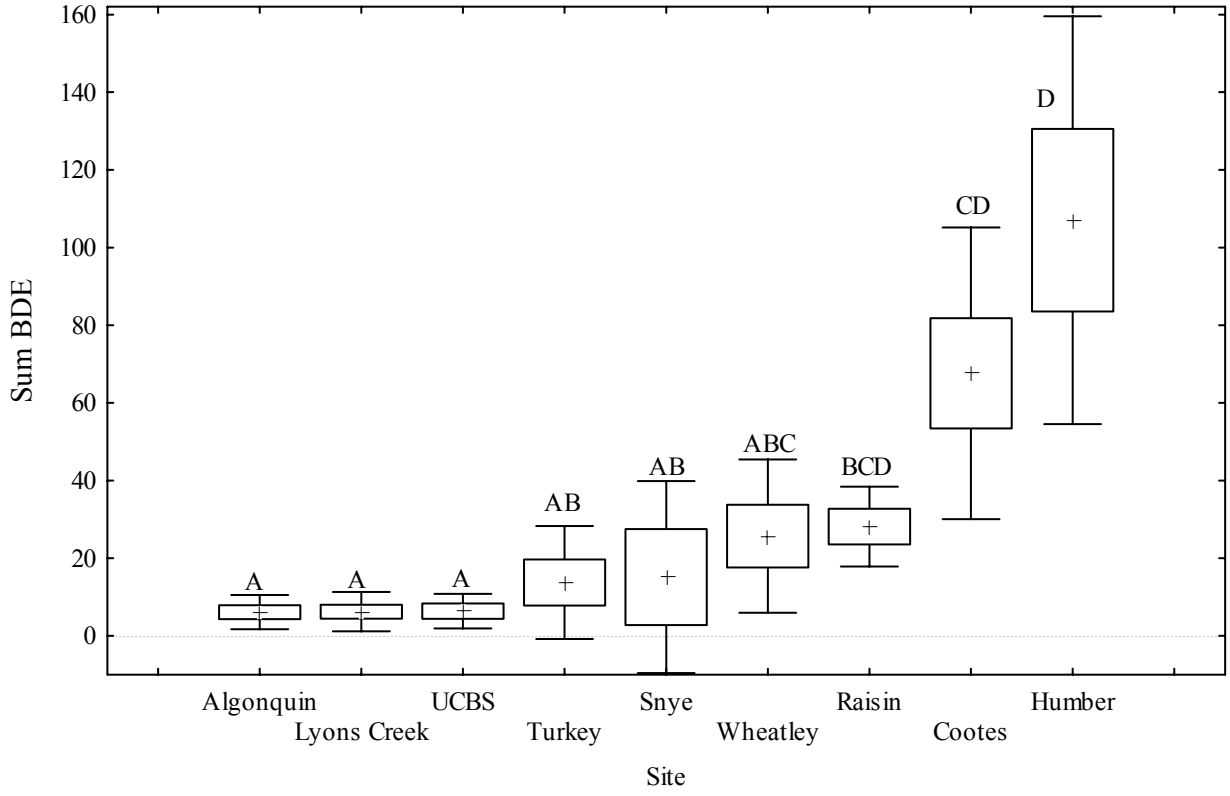


Figure 8. The spatial (geographic) pattern of polybrominated diphenyl ether (PBDE) concentrations in snapping turtle eggs collected from reference sites and Canadian Areas of Concern in the Great Lakes – St. Lawrence Basin (2001-2003).



Algonquin Park is the reference site.

Turkey: Turkey Creek is located within Windsor ON and runs into the Detroit River.

Wheatley: Clutches were collected from Wheatley Provincial Park and adjacent to the Hillman Marsh Conservation Area (2001 only).

Lyons Creek: Lyons Creek is located adjacent to the Welland Canal and is within the Niagara River AOC.

Humber: This site is located at the Humber River Marshes at the mouth of the Humber River, Lake Ontario in Toronto ON.

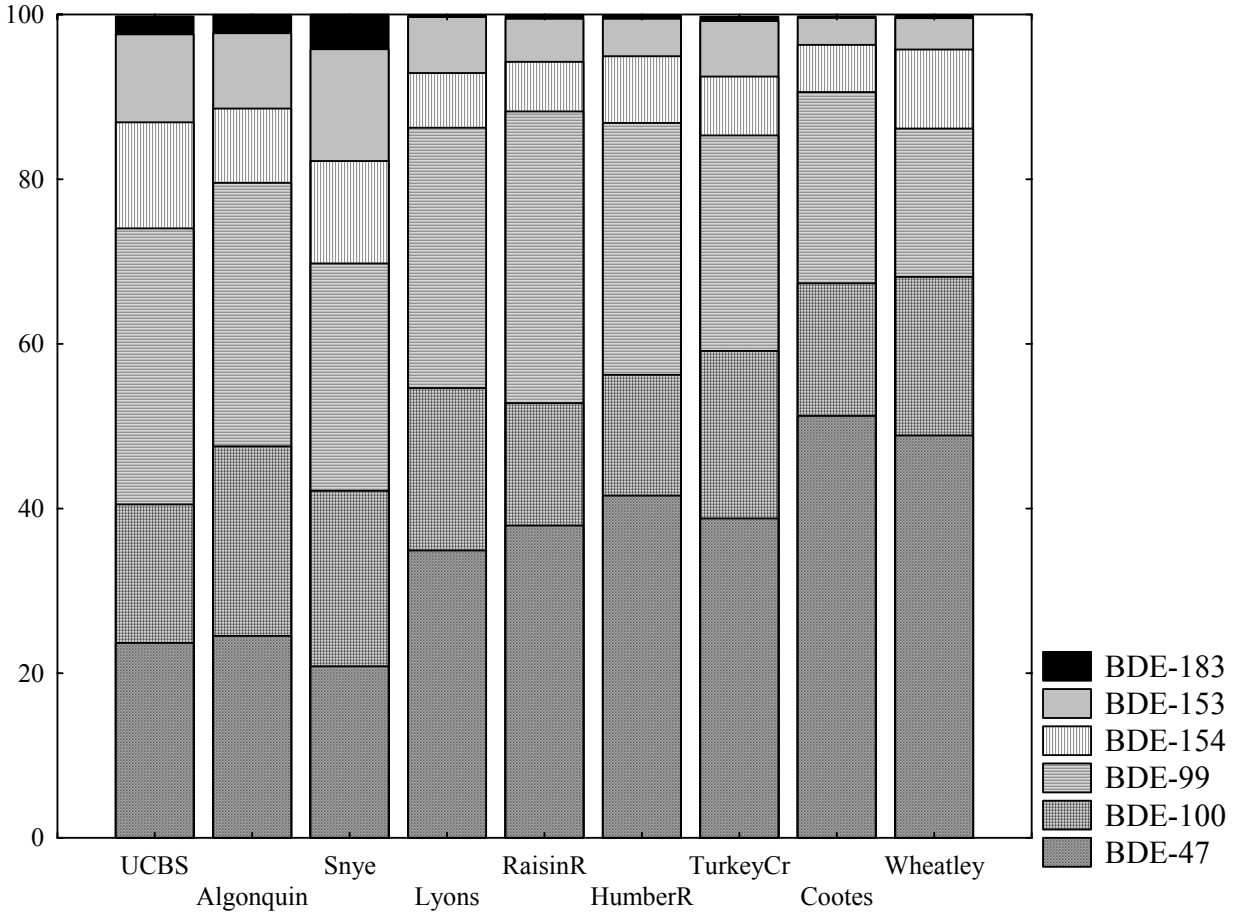
Raisin: Raisin River runs between Cornwall and Lancaster ON, exiting into the St. Lawrence River.

UCBS: The Upper Canada Bird Sanctuary is located within the St. Lawrence River upstream of that AOC near Ingleside ON.

Snye: Snye Marsh is located in Akwesasne and enters into the St. Lawrence River.

Cootes: This site is Cootes Paradise near Hamilton ON and located within the Hamilton Harbour AOC.

Figure 9. The contribution of individual polybrominated diphenyl ether (PBDE) congener concentrations (log transformed) relative to the total PBDE concentration measured in snapping turtle eggs collected from reference sites and Canadian Areas of Concern in the Great Lakes – St. Lawrence Basin (2001-2003).



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Appendix

A1 TITLE AND APPROVAL SHEET

QUALITY ASSURANCE PROJECT PLAN FOR

Measuring of Contaminants in Snapping Turtle Eggs
in Great Lakes Coastal Wetlands

Kim Fernie, Project Manager
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In Partnership with

Chip Weseloh, Quality Assurance Manager,
Canadian Wildlife Service - Environment Canada, Ontario Region
Tel. (416) 739-5846 Fax (416) 739-5845 chip.weseloh@ec.gc.ca

Ric Lawson
GLCWC Project Manager, Great Lakes Commission, rlawson@glc.org

Greg Mayne, Canadian Wildlife Service (Contractor) 125 Longwood Rd. N. Hamilton, Ontario. L8S 3V8 Tel. (905) 522-8534 greg.mayne@sympatico.ca

Signature		Date
……..	Kim Fernie	…….
……..	Chip Weseloh	…….
……..	Ric Lawson	…….
……..	Greg Mayne	…….

Project # WETLANDS2-EPA-05
Revision #3
December 22, 2003

A2 TABLE OF CONTENTS

D DATA VALIDATION AND USABILITY

A3 DISTRIBUTION LIST

Ric Lawson, Great Lakes Wetlands Consortium, Great Lakes Commission
John Hummer, Great Lakes Wetlands Consortium, Great Lakes Commission
Kim Fernie, Canadian Wildlife Service, Ontario Region, Environment Canada
Chip Weseloh, Canadian Wildlife Service, Ontario Region, Environment Canada
Greg Mayne, Canadian Wildlife Service-contractor

A4 PROJECT/TASK ORGANIZATION

Kim Fernie, of the Canadian Wildlife Service (CWS-Ontario) is the Project Manager and is responsible for project development and implementation, data transfer and coordination issues between other collaborating investigators within the overall Great Lakes Coastal Wetlands Consortium. Kim Fernie will also maintain the official, and approved Quality Assurance Project Plan. Chip Weseloh (CWS-Ontario), will act as the Quality Assurance Manager. Kim Fernie and Chip Weseloh, will develop a detailed methodological framework that incorporates the use of snapping turtle eggs as an indicator of contaminant levels in coastal wetlands of the Great Lakes basin. When followed, this plan will yield information that can detect change and eventually establish basin-wide comparisons and temporal trends of contaminant levels in snapping turtle eggs collected from various wetland sites. As part of another Environment Canada project, Kim Fernie will oversee snapping turtle egg sample collection for contaminant analysis from the Toronto and St. Lawrence River Areas of Concern (AOCs). In addition, archived snapping turtle eggs collected from previously monitored coastal wetland sites will be analyzed by the National Wildlife Research Centre with the aim of detecting temporal and spatial differences in contaminant levels across multiple coastal wetland sites. Greg Mayne, a CWS-Ontario-contractor, will assist in writing the methodological framework. He will also write a “White Paper” that reviews the scientific and government literature relevant to

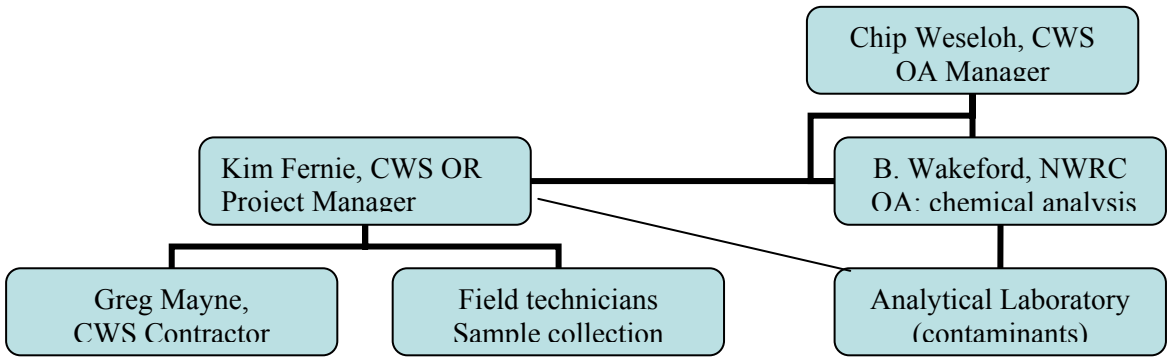
snapping turtles and contaminant levels in their eggs. As part of a sustainable monitoring program, Greg Mayne will contact the appropriate state and provincial agencies to determine the cooperation and willingness of these groups to collect snapping turtle eggs for contaminant monitoring purposes.

Collaborating Project Teams

To be decided following contact of appropriate individuals and agencies.

Project Organization

Dr. Chip Weseloh will provide the quality assurance for this project. Dr. Kim Fernie will report to Dr. Weseloh, providing him with final copies of all reports and seeking his advice when necessary; she reports to him as a wildlife biologist for the Canadian Wildlife Service. Greg Mayne as a contractor, will report to Kim Fernie; his services are contracted for other projects directed by her for the CWS. Dr. Fernie will coordinate the chemical analysis with appropriate labs and the QA manager (Bryan Wakeford). The chart below outlines the reporting structure of this group.



A5 PROBLEM DEFINITION/BACKGROUND

While progress has been made toward developing indicators that will lead to effective monitoring of coastal wetland quality, the consensus formulated at the State of the Lakes Ecosystem Conference (SOLEC) indicated a need for a system that would consistently measure or monitor the status of coastal wetlands loss or degradation.

Subsequent to this, wetland scientists identified indicators that would facilitate evaluation of wetland integrity. The Great Lakes Coastal Wetlands Consortium (GLCWC) was established to develop and implement a sustainable, long term basin-wide monitoring plan that would facilitate assessment programs and reporting capabilities of Canada and the U.S. under the Great Lakes Water Quality Agreement. As part of this long-term goal, the GLCWC has specified a set of metrics relevant to contaminant levels in wildlife that need to be validated for implementation within a long-term monitoring strategy. The ultimate goal of the present study is to validate the snapping turtle as a bioindicator of contaminant levels in Great Lakes coastal wetlands.

Floral and faunal assemblages have been used for centuries by humans as indicators of water quality or general environmental integrity (Landres et al., 1988). A particularly useful biosentinel of contaminant exposure is the common snapping turtle (*Chelydra serpentina serpentina*) (Bishop et al., 1994, 1995; 1996; Struger et al., 1993). The utility of the snapping turtle for biomonitoring purposes is based upon various life history traits. This ubiquitous species inhabits wetlands throughout eastern North America including the Great Lakes-St. Lawrence River basin (Weller and Oldham, 1988). They have a sedentary nature and a small home range and thus reflect local changes occurring in wetlands exposed to contaminants (Hammer, 1969; Congdon et al., 1987; Pettit et al. 1995). The snapping turtle is an omnivorous opportunist, basically consuming whatever is available. Because the snapping turtle occupies a high trophic position, it is subject to food chain biomagnification, and are consequently exposed to high concentrations of persistent organic contaminants (Ernst et al., 1994; Bishop and Gendron, 1998). In addition, there is evidence indicating that concentrations of hydrophobic organic chemicals in eggs reflect the concentration in maternal tissues of snapping turtles (Pagano et al., 1999; Russell et al., 1999). Female snapping turtles lay a single clutch of eggs each year and chemical analysis of a subsample of eggs provides a means to measure contaminant burdens in the body of the female turtle at the time and place of egg-laying (Bishop et al., 1994).

Canadian Wildlife Service researchers have been collecting snapping turtle eggs and measuring chlorinated hydrocarbon contaminant levels in wetland environments since the early 1980s (Struger et al., 1993; Bishop et al., 1994; 1995; 1996). To date, twenty organochlorine pesticides, total mercury, 59 polychlorinated biphenyl (PCBs) congeners, six non-*ortho* PCBs, approximately 10 polychlorinated dibenzodioxins (PCDDs), and 14 polychlorinated dibenzofurans (PCDFs) have been measured in snapping turtle eggs from the Great Lakes-St. Lawrence River basin (Bishop and Gendron, 1998; de Solla et al., 2001). This biomonitoring program has provided important spatial patterns of contaminant levels in the Great Lakes basin (Struger et al., 1993; Bishop et al., 1996). Monitoring efforts using the snapping turtle as a sentinel of wetland integrity continues to provide valuable information on contaminant levels of Great Lakes-St. Lawrence River wetlands (de Solla et al., 2001; K. Fernie, manuscripts in preparation, Environment Canada "Fish and Wildlife Health and Contaminant Concentrations in Selected, Canadian Areas of Concern").

The results from measurement of organic hydrocarbon contaminants in snapping turtles eggs collected from Great Lakes wetlands will eventually establish basin-wide temporal and spatial trends in contaminant levels in Great Lakes coastal wetlands. These data will provide important contaminants trend data useful to resource managers and policy makers to facilitate the evaluation and effectiveness of clean-up actions. Participation from

both U.S. and Canadian wildlife management agencies is important in evaluating the status of Great Lakes coastal wetlands. As such, development and implementation of a systematic, long-term, contaminants monitoring program with a binational focus will ensure that basin-wide information are available for regulatory purposes.

A6 PROJECT/TASK DESCRIPTION

This project is part of a three-year GLCWC initiative to develop a monitoring plan and data support system for Great Lakes coastal wetlands. The objective of this study is to create a methodological framework for the use of snapping turtle eggs as an indicator of contaminant exposure and levels within coastal wetlands of the Great Lakes basin. The use of snapping turtle eggs as a viable means to assess wetland contaminant status will be tested for incorporation within a long-term monitoring strategy. Snapping turtle eggs collected from the Toronto and St. Lawrence River Areas of Concern (AOCs) in 2003, and archived snapping turtle eggs collected from coastal wetland sites in previous years, will be analyzed to measure hydrophobic organic chemicals. Provincial and State wildlife agencies will be contacted to determine the cooperation and willingness of these groups to collect snapping turtle eggs for future monitoring purposes.

The framework includes a "White Paper" that provides a detailed methodological plan that utilizes snapping turtle eggs to measure and monitor contaminant levels in lacustrine, riverine and barrier-protected wetland systems of both upper and lower Great Lakes wetlands. This monitoring program, which when followed, will produce information that can detect change and eventually establish temporal trends and basin-wide comparisons for contaminant levels. The "White Paper" will review the scientific and government literature relevant to snapping turtles and their eggs, and how they may be used to measure contaminant exposure. In future years, investigators involved in the monitoring program will collect snapping turtle eggs from wetland sites within the Great Lakes basin and measure contaminant levels using standardized protocols.

Sampling Locations

Snapping turtles lay one clutch per season, typically in June in Southern Ontario. Archived egg samples will be chosen to maximize sample sizes so as to best represent wetland types and provide spatial and temporal data, while addressing financial constraints. To this end, the following 2003 study site locations are being considered:

Site Name	**Site Type**	**Hydrogeomorphic Type**	Provincial R.M./County	**Longitude**	**Latitude**
Humber River/ Toronto	AOC	Open, Drowned River-Mouth	Metropolitan Toronto, Ontario	43,38'10.21"	79,28'37.94"
Raisin River/ St. Lawrence	AOC	Open, Drowned River-Mouth	Stormont, Dundas & Glengarry; Ontario	45,07'44.21"	74,29'36.04"
Upper Canada Bird Sanctuary/ Cornwall	Up-stream AOC	Protected Embayment	Stormont, Dundas & Glengarry; Ontario	44,57'10.55"	75,02'40.69"

Snye River/ St. Lawrence	AOC	Open, Drowned River-Mouth	St. Regis, Quebec	45,00'10.55"	74,31'45"

Criteria of the Great Lakes Coastal Wetlands Consortium

Six criteria that originate from the Request for Proposals (RFP) distributed by the Great Lakes Commission on behalf of the Great Lakes Coastal Wetlands Consortium will be addressed. These criteria fall under "Scope of Work" in the RFP as one of the goals "to test the feasibility of applying indicators in a monitoring plan."

The criteria are as follows:

1. Cost – The cost of implementing a program using snapping turtle eggs to measure routine organochlorine contamination and pesticides will be assessed. The cost and availability of analytical methods to measure other chemicals of concern (e.g., polyaromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs)), will be addressed;

2. Measurability – This section will provide detailed information regarding specific project design and methodology, including the selection of wetland sites that will provide necessary spatial and temporal data to assess contaminant trends in a Great Lakes coastal wetlands monitoring plan;

3. Applicability – Basin-wide applicability and reliability of snapping turtles to measure contaminants in various wetland types across the Great Lakes basin, including both the lower and upper basin, will be determined. The "White Paper" will identify other part(s) of the suite of indicator species for contaminants in tandem with the snapping turtle and provide advantages and disadvantages for this approach;

4. Complementary data – Availability of complementary existing research and data relevant to the use of snapping turtles to determine contaminant levels will be identified. A review of published materials will be used to identify previous researchers and organizations involved in the historical and current snapping turtle work of the CWS, what methodology was used to identify contaminant concentrations and the compounds targeted. Information relevant to contaminant levels in snapping turtle eggs and contaminant-induced health effects at possible sites for the monitoring plan will also be reviewed. In addition, the CWS currently has archived snapping turtle egg samples which would be analyzed to further establish contaminant levels and trends at possible monitoring sites;

5. Sensitivity – The sensitivity of snapping turtles will be assessed in terms of detecting changes in the contaminant conditions of wetlands over time as well as space. This task will be accomplished through a review of the published literature as well as analyses of archived and to-be-collected snapping turtle egg samples;

6. Endpoints – The "White Paper" will address the usefulness of snapping turtles for a monitoring plan in terms of being able to set endpoint(s) or attainment levels relative to contaminant levels and health effects in wetlands of the Great Lakes basin.

Work Schedule

June -August, 2003: Collect snapping turtle eggs at all 2003 study sites (see previous table). Transfer eggs to analytical laboratory at the National Wildlife Research Centre (NWRC, Ottawa, Ontario) or to certified, quality-controlled laboratories under contract to the NWRC for contaminant analysis.

September 2003 – April, 2004: Measurement of organochlorine levels in snapping turtle eggs collected from the Toronto and St. Lawrence River Areas of Concern (AOCs) in 2003. Measurement of organochlorine levels in archived snapping turtle egg samples collected from various coastal wetland types within the Great Lakes basin, including the Toronto and St. Lawrence River AOCs.

September 2003 – May, 2004: Write a comprehensive literature review of published materials relevant to snapping turtles and their eggs, and how this species will serve as a useful bioindicator model for contaminant exposure and effects. Identify researchers and organizations involved in the historical and current snapping turtle work of the CWS and elsewhere, what methodologies were used to identify contaminant concentrations and the compounds targeted.

A7 QUALITY OBJECTIVES AND CRITERIA

The primary quality objective of this study is to create a methodological framework for a long-term, basin-wide study which incorporates the use of snapping turtle eggs to detect temporal and spatial patterns of contaminants in specific types of Great Lakes coastal wetlands. A complete review of published materials will be conducted and this information will be provided along with the framework. As secondary materials are derived from numerous sources, primary importance will be placed on works published in peer-reviewed scientific journals and reports from scientific government sources. Snapping turtle eggs collected in the first year of study (2003) and archived egg samples will be analyzed in an effort to confirm the usefulness of the snapping turtle as an indicator of contaminant exposure. The contaminants targeted in routine chemical analysis include organochlorine pesticides, PCB congeners including non-*ortho* PCBs, PCDDs, and PCDFs. "Data acceptability" for chemical results will be contingent upon analytical methods following the Standard Operating Procedures established by Canadian Wildlife Service chemists and scientists. This approach will ensure that results of this project are comparable to (past and) future projects occurring around the Great Lakes and that data collected as part of this project can be integrated into centralized databases for to determine long-term trends in contaminant levels in snapping turtle eggs.

As part of the Quality Control criteria for chemical analysis of organochlorines and PCBs, a five-point initial standard curve is made with the organochlorines and PCBs standard mixtures to cover the range of interest. This established calibration curve is

verified daily by analyzing a calibration verification standard having a mid-point concentration.

Reports from chemical analysis will include detection limits, which indicate the lowest quantifiable concentration using the associated method. A minimum detectable concentration is described as the concentration of analyte which produces a signal in an instrument three times the average noise level. In multi-residue analysis, such is the case of this project, it is not always practical to list the detection limits for each compound of interest. As a general rule, a detection limit of at least 0.0001 PPM is achievable for all compounds. In reporting the data, results having less than 0.0001 PPM are reported as NS (not detected) in the Laboratory Services analytical test report, and one half the detection limit is used in the statistical analysis. If a computed result falls in the range of 0.0001 and 0.0009 PPM, the compound is listed as TR (trace) and the median value of the trace range is used for statistical analysis.

Precision and recovery will be addressed by running an aliquot of the standard NWRC QA Reference Material (Herring gull eggs) along with each batch of samples. Concentrations of the major compounds (PCB-52, PCB-66, PCB-101, PCB-110, PCB-149, PCB-118, PCB-146, PCB-153, PCB-138, PCB-187, PCB-180, PCB-170, PCB-201, PCB-203, HCB, p,p'-DDE, photo-mirex, mirex, oxychlordane, cis-nonachlor, hetachlor epoxide and dieldrin) are determined and the results are compared to the previously established acceptance limits (i.e., ±2 SD of the long-term mean plotted in a Shewart chart). To determine the degree of analyte loss during sample cleanup, each sample is spiked with 13C-labelled chlorobenzenes/PCBs internal standard mixture.

Systematic biases in contaminant analysis are avoided through the proper preparation and analysis of method blanks. Method blanks ensure contamination of glassware or other equipment in the laboratory is accounted for. On each sampling date, one type of blank is prepared and analyzed. All three types of blanks should be below the prescribed method detection limit.

In the event of sample contamination or equipment failure, the data will be flagged accordingly. The use of these data will be restricted until an investigation resolves the issue of contamination or inaccurate results. Only values that meet the data quality objectives for accuracy, precision and bias will be used without caution. This ensures that the data reported are reliable, reproducible and accurate.

Representativeness of the entire snapping turtle clutch will be ensured by selecting and pooling five eggs collected from each clutch of eggs, and homogenizing this composite sample prior to chemical analysis. In an attempt to ensure that contaminant levels are representative of a particular wetland site, field biologist will attempt to collect eggs from approximately 10 clutches per site. This approach should provide the necessary means to represent contamination of each site, and then compare contaminant levels among various wetland study sites situated in the Great Lakes.

A8 SPECIAL TRAINING REQUIRMENTS

Kim Fernie and Greg Mayne will identify wetland study sites, and Kim will supervise collection, handling, labelling and storage protocols of snapping turtle eggs. Experienced chemists at the National Wildlife Research Centre in Ottawa, Ontario, will conduct the contaminant analysis of the snapping turtle eggs.

A9 DOCUMENTATION AND RECORD

Development and implementation of an integrated binational Great Lakes coastal wetland monitoring program using snapping turtle eggs as an indicator of contaminant exposure will require that participating researchers and organizations have the most current version of an approved Quality Assurance Project Plan (QAPP). If any changes in the QAPP occur, a new, updated version will be submitted to the Great Lakes Commission by Kim Fernie. The transfer of this QAPP would occur in the next stage rather than this current stage that only involves initial contacting of people and agencies.

Data obtained during field operations will be entered into field logs. Data will be reviewed for completeness each day by the field crew lead. All field logs will be stored at CWS-Ontario office and entered into the snapping turtle database. Contaminant analysis data will be provided by National Wildlife Research Centre chemists in hard copy and electronic file format. Original copies will be stored at the National Wildlife Research Centre in Ottawa, Ontario, Canada. Electronic data back ups will be completed regularly and copies of the data stored at the CWS- Burlington office. All records and reports generated from this study will be stored by CWS-Burlington and CWS-Downsview following study completion.

A "White Paper", detailing the methodological plan and including a review of the scientific and government literature significant to snapping turtles and their eggs will be produced as both hard copies and electronic files. Copies will be available to both the CWS and the Great Lakes Coastal Wetlands Consortium.

DATA GENERATION AND ACQUISITION

B1 SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)

Study and Design Rationale

Canadian Wildlife Service biologists and contractors will collect snapping turtle eggs from wetland sites in the Toronto and St. Lawrence Areas of Concern (AOCs), as well as traditional reference study sites located inland of the Great Lakes in Ontario in 2003. Eggs will be analyzed for contaminant levels along with archived egg samples (locations, dates, sample size to be determined) from various wetland types including the Toronto and St. Lawrence River AOCs. In addition, the CWS currently has historical contaminant data for snapping turtle eggs collected from inland reference sites, the Hamilton Harbour AOC and other St. Lawrence River AOC sites. All data being collected as part of this project are considered critical to meeting GLCWC and project objectives. Taken together, our analytical results, combined with existing contaminant databases, will be beneficial in testing and validating the snapping turtle as an indicator of Great Lakes coastal wetland contamination by:

1. confirming the usefulness of the snapping turtle as an indicator of temporal and spatial contaminant trends in different hydrogeomorphical wetland types;

2. determining how well contaminants in snapping turtle eggs reflect environmental contaminants in sediment and/or water samples taken at these sites.

Wetland sites used in this study are known to have high contaminant levels; some of these sites are within IJC-designated Areas of Concern (AOCs). Other sites will be chosen because historical contaminant data already exists, they represent a specific type of wetland, and/or they are upstream of the AOCs for comparative purposes, or because they contain low contaminant levels and are useful as reference sites. Wetland sites for future monitoring efforts will be chosen based on their respective hydrogeomorphical characteristics, contaminant levels, and/or geographic location within the Great Lakes basin. The latter will be chosen based on information provided by wildlife managers with offices in the Great Lakes – St. Lawrence River basin. In the event that sampling sites become inaccessible, eggs will be collected from other, representative sites within the same wetland complex. This will be done by looking for evidence of previous nest sites, sites that offer optimal nesting habitat, or by actively searching for nesting females.

Although Bishop et al. (1995), reported a non-significant intra-clutch variation in contaminant levels among freshly laid eggs, the first five eggs contained the highest mean concentration of all chemicals on a wet-weight basis and the highest mean lipid values relative to the last five eggs collected. In order to estimate the "average" contaminant concentration of a nest, five eggs are typically selected from the clutch. The method suggested by Bishop et al. (1995), was to select one of the first few eggs laid, one of the last few eggs laid, and three eggs from the rest of the clutch. This pooled sample is assumed to approximate the median concentration of that clutch. More recently, we have selected eggs in a pseudo-random but stratified manner; eggs were ordered from first to the last egg laid, and each clutch was divided into five groups of approximately equal size. Within each group, an egg was selected haphazardly (de Solla and Fernie, submitted). Normally, five eggs were selected from each clutch for contaminant analysis, but if the clutch is to be used for other purposes, as few as one egg may be used.

There appears to be no literature reporting congener-specific PCB pattern/chlorination changes during embryonic turtle development. Nonetheless, the utilization of fresh eggs (< 48hours) removes the uncertainty of changes in contaminant concentrations by Phase I and Phase II metabolic enzymes (Bishop et al., 1995a). When possible, 10-15 clutches will be collected from each wetland study site in order to obtain a measure of variance of contaminant levels associated with each wetland. For further details, see Sampling Methods below.

The measurement parameters of interest include organochlorine pesticides and approximately 59 PCB congeners; oxy-, trans-, and cis-chlordanes; trans- and cis-nonachlor; p,p'-DDE, DDD, and DDT; octachlorostyrene; mirex; dieldrin; hexachlorobenzene and heptachlor epoxide. Pending cost constraints, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and non-ortho PCBs may also be measured.

B2 SAMPLING METHODS

A composite sample of five eggs will be collected from each clutch as outlined above. The remaining eggs in the clutch are immediately reburied without excessive rough handling. The five eggs selected for contaminant purposes are placed in a plastic container (e.g., sandwich container) and surrounded with moist vermiculite or sand to prevent breakage en route to the field base. If possible, 10-15 clutches per wetland site will be sampled. Egg samples will be identified by the site name, sample number, latitude and longitude of the collection site, the collection date, and the total number of eggs will be recorded for each clutch. Eggs will be cleaned of particulate matter, placed in foam-lined containers to prevent breakage and kept in coolers to prevent over-heating while in the field. Eggs will then be temporarily stored in a 5 ^{o}C walk-in refrigerator, or frozen in a – 20 ^{o}C chest freezer until the day of shipment to the Laboratory Service Section of the National Wildlife Research Centre in Ottawa, Ontario. The contents of five eggs will be pooled and stored in hexane rinsed jars at – 20 ^{o}C at the National Wildlife Research Centre, Ottawa, Ontario, Canada until the date of analysis following the Tissue Preparation Unit's standard operating procedure SOP-TP-PROC-07.

If problems are encountered during sample collection, transport, or storage, Kim Fernie will take the necessary corrective actions by reviewing each phase of sample handling with field personnel. If necessary (and/or possible), new samples will be collected from the same sites and reworked for analysis. Any problems, changes, or otherwise, will be reported to the GLCWC by Kim Fernie in quarterly reports or via email correspondence.

B3 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

Canadian Wildlife Service biologists and contractors will collect snapping turtle eggs from the designated wetland sites in 2003, as well as selecting archived egg samples collected in previous years. Snapping turtle egg samples may be archived for extended periods of time (e.g., years) prior to contaminant analysis if they are stored under appropriate conditions (i.e., contents of eggs placed in solvent-rinsed glassware at –80^{o}C freezer). The NWRC currently manages a "tissue bank" that allows wildlife tissues to be stored until contaminant analysis occurs. This allows for historical contaminant analysis as well as the analysis of contaminants once suitable methodologies are developed (e.g., PBDE).

Personnel at the National Wildlife Research Centre responsible for registry of biological samples will be given at least one week advance notice of the date of arrival of samples at NWRC to ensure that appropriate materials are in place upon arrival of the shipment. If individuals other than CWS staff (i.e., air or courier) deliver samples to the National Wildlife Research Centre, a weighbill number is required so that the shipment can be traced. Examples of data collection sheets and custody forms are provided below.

PROJECT / PROJET :	CONTACT AND PH0NE NUMBER / PERSONNE RESSOURCE ET NO. DE TÉL										
This form is used to complement the collection data sheet (FORM-TP-11). Please send one sheet for every shipment to NWRC											
Ce formulaire sert à compléter les données de collecte (FORM-TP-11). S.V.P. faire parvenir une lettre d'accompagnement pour chaque envoi au CNRF											
Part A - Collection data related to specimens / Données concernant les spécimens											
Source / Origine :	wild / sauvage										
	other / autre										
Collecting technique of whole specimen (e.g. shot, netted, picked up by hand, trapped, gaffed) / Technique de prélèvement (e.g. tiré, ramassé (oeuf), pêché, attrappé au filet):											
Condition when collected (e.g. fresh, dead-no info, dead-with info., sick) / État lors du prélèvement (ex. frais, mort/avec information, mort/pas d'info., malade) :											
Sacrifice :											
Part B - Data related to specimen preparation and preservation prior to shipment to NWRC /											
Tissue type / Type de tissu	Collecting technique, condition of tissues and remarks / Technique de collecte, condition des tissus et autres	Storage / Entreposage	Container and cap liner / Contenant et couvercle	Container treatment / Traitement							
Tissu type: e.g. egg content, liver-left lobe, head, plasma …											
Collecting technique: e.g. biopsy, heparinized seringe, dissection with chemically cleaned instruments, homogenization (give details											
Container: e.g. glass jar, polyethylene (PE) bag, polypropylene (PP) scintillation vial, cryovial, egg carton, Teflon vial, etc											
Cap liner: metal foil, rubber, PE, Teflon											
Container treatment : rinsed with nitric acid (A); rinsed with organic solvents (S); not rinsed (N); unknown (U)											

Part C - Other comments / Autres commentaires
List exceptions, contamination problems, etc. / Énumérer les exceptions, les problèmes de contamination, etc.

Environment Canada / Environnement Canada
Canadian Wildlife Service / Service canadien de la faune
National Wildlife Research Centre / Centre national de la recherche faunique
Refer to SOP-TP-DOC-03 for explanatory notes / *Consulter la procédure SOP-TP-DOC-03 pour not*
PROJECT / *PROJET*
PROJECT LEADER / *AGENT DE PROJET*
List of abbreviations used / *Abbréviations utilisées* (e.g. K = kidney, LLL = liver left lobe, SNTU= snapping turtle)

USOX	**Specimen no. / *No. d'échantillon***	**Type of tissue and number of containers/ *de tissu et nombre de contenants***	**Species / *Espèce* (common name) /*(nom commun)***	**Age**	**Sex /Sexe**	**Collection date / *Date de collecte (yyyy/mm/dd)***	**Collection site / *Emplacement du prélèvement***		**Location / *Enfroit***
							Latitude deg/min	**Longitude deg/min**	**Province**

B4 ANALYTICAL METHODS

Snapping turtle egg samples provided to the Trace Organic Chemistry Laboratory at the NWRC, Ottawa, are prepared as described in the Tissue Preparation Unit's standard operating procedure SOP-TP-PROC-07. The analytical method used for contaminant analysis of snapping turtle eggs is outlined in Technical Report Series Number 335 "Multiresidue Methods for the Determination of Chlorinated Pesticides and Polychlorinated Biphenyls (PCBs) in Wildlife Tissues by Gas Chromatography/ Mass Spectrometry" (Won et al., 2001).

EXTRACTION OF CONTAMINANTS FROM EGGS

Egg samples are homogenized and between 1.5 g to 3.0 g of the homogenate is treated with 25 g anhydrous Na_2SO_4 in a glass mortar and pestle until a free-flowing mixture is obtained. This mixture is then poured into a 2.1 cm x 35 cm glass column packed with treated glass wool and 1 cm Na_2SO_4. The mortar and pestle is rinsed three times with a dichloromethane/hexane (1:1) solution and transferred to the column and allowed to soak for 30 minutes. An additional 200ml of dichloromethane/hexane is added to the column and allowed to elute at 5-10 ml/min into a 500 ml flask. The eluate is evaporated to less than 5 ml on a rotary evaporator with a water bath (30^oC) then quantitatively transferred into a graduated centrifuge tube. Dichloromethane/hexane (1:1) is then added to obtain a final concentration of 0.2 g/ml (i.e., 3 g of tissue in 15 ml of Dichloromethane/hexane. An aliquot equivalent to 1.0 g of egg is transferred into a gel permeation chromatography (GPC) tube. The extract is spiked with 50 µl of ^{13}C-chlorobenzene internal standard spiking solution and diluted to 10 mL with dichloromethane/hexane. The GPC flow-rate is set at 5 ml/min of (1:1) dichloromethane/hexane. The eluate is evaporated to 3 ml on a rotary evaporator.

SAMPLE CLEANUP BY FLORISIL COLUMN

The florisil column is designed to isolate compounds of interest from any residual lipid. A Florisil column is packed with treated glass wool, saturated in 40 ml hexane, and 8g de-activated Florisil added, followed by approximately 1 cm Na_2SO_4. The solution is allowed to flow through the column until the solvent level is slightly above the Na_2SO_4 layer. The extract is loaded to the top of the Florisil column using a Pasteur pipet. A 150 ml flat-bottomed evaporating flask is rinsed with 3-4 small portions of dichloromethane/hexane and then added to the column and 95 ml of dichloromethane/hexane (1:1) is added and then eluted at 5 mL/min. The eluate is concentrated to less than 3 ml with rotary evaporator and quantitatively transferred to a 10 mL flask and further concentrated to 400 µl with rotary evaporator. The eluate is quantitatively transferred to autosampler vials, spiked with 20 µl of normalization standard and diluted to 570 µl. The autosampler vials are capped and thoroughly agitated.

CONTAMINANT ANALYSIS

Contaminant levels are determined by high-resolution gas chromatography coupled to a mass selective detector (GC/MSD) operated in selected ion monitoring mode for use in the analysis. Identification of contaminants is accomplished by comparing gas chromatography retention times and specific mass fragments known to be present in the spectra of authentic compounds. Quantification is accomplished by comparing the intensity of mass fragments of contaminants of interest in egg specimen extracts to the same compounds in a standard mixture, injected separately on the GC/MSD system.

In the event of problems occurring within the above mentioned methodologies, such as an instrumentation failure, the laboratory chemist will review all aspects of the analytical procedure and samples will be re-worked for analysis. All remaining samples from pooled extracts are archived in the Canadian Wildlife Service Specimen Bank, National Wildlife Research Centre, Ottawa, Ontario, Canada. Problems encountered during analysis of egg samples will be relayed to Kim Fernie by analytical chemists. These problems and the corrective actions taken will then be reported to the GLCWC by Kim Fernie in quarterly reports or via email correspondence.

B5 QUALITY CONTROL REQUIREMENTS

Compliance with the QA/QC program will be coordinated and monitored by the quality assurance manager and appropriate personnel at NWRC. The objectives of the QA/QC program are as follows: to ensure that all analytical procedures are documented, including any changes in administrative and/or technical procedures; to ensure that all field procedures are conducted according to sound scientific principles and have been validated; to ensure that all equipment is clean, calibrated and properly functioning; to monitor the performance of the sample collection procedures and provide for corrective action as necessary; and to ensure that all data are properly recorded and archived. Internal quality control procedures will be conducted by audits.

Quality control activities for contaminant analysis of tissues are outline in Technical Report Series Number 335 “Multiresidue Methods for the Determination of Chlorinated Pesticides and Polychlorinated Biphenyls (PCBs) in Wildlife Tissues by Gas Chromatography/ Mass Spectrometry” (Won et al., 2001).

Biweekly checks using certified reference standards will be performed to determine laboratory accuracy and equipment performance. A five-point calibration standard curve is made with the organochlorines and PCBs standard mixtures to cover the appropriate concentration range for the test. The calculated concentration of each compound must be within 20% of its actual known value. The final concentration of any reportable compounds must be within the demonstrated linearity of the detector. If necessary, samples are diluted with iso-octane to meet the calibration range. Laboratory accuracy should be within 80%-120% for all parameters tested (Won et al., 2001).

Detection Limits and Reporting Limits

A nominal or minimum detectable concentration is usually described as the concentration of analyte which produces a signal in an instrument three times the average

noise level. In this multi-residue method, it is not practical to list the detection limits for each compound of interest. Variability between compounds arises due to varying background noise and response factors for each compound due to the different mass ions being monitored. As a general rule, a detection limit of at least 0.001 PPM is achievable for all compounds.

Ongoing Precision and Recovery

An aliquot of the QA Reference Material (Herring gull eggs) is analyzed along with each batch of samples. The concentration of the major compounds (PCB-52, PCB-66, PCB-101, PCB-110, PCB-149, PCB-118, PCB-146, PCB-153, PCB-138, PCB-187, PCB-180, PCB-170, PCB-201, PCB-203, HCB, p,p'-DDE, photo-mirex, mirex, oxychlordane, cis-nonachlor, hetachlor epoxide and dieldrin) is determined and the results are compared to the previously established acceptance limits (i.e., ±2 SD of the long-term mean plotted in a Shewart chart).

To determine the degree of analyte loss during sample cleanup, each sample is spiked with 13C-labelled chlorobenzenes/PCBs internal standard mixture. Analysis is accepted when the % internal standard recoveries for most PCBs and OCs are between 80% and 110%, and for the highly volatile compounds are over 60%.

Accuracy

The accuracy of the quantitation standards is verified annually with a second source standard (containing most of the congeners of interest) as described in SOP-CHEM-PROC-13.

Method Blank

A method blank is run with each batch of samples to determine the levels of contamination associated with the processing and analysis of samples. If problems with the blank exist, associated data are carefully evaluated and appropriate corrective actions are applied. Blank values are not subtracted from reportable values. A compound found in a blank and also in an associated sample is flagged in the analytical test report when present at a ratio of at least 5/1, sample to blank.

Data Validation

Data validation is ensured by an internal quality assurance audit done by an independent reviewer (Head of the Laboratory Services Section), before the release of the analytical test report. If large discrepancies are noted in the analytical data between the specimens from close geographical areas, the raw data are examined and re-analysis of the sample aliquot may be indicated.

Systematic biases

Systematic biases in contaminant analysis are avoided through the proper preparation and analysis of method blanks. Method blanks ensure contamination of glassware or other equipment in the laboratory is accounted for. On each sampling date, one type of blank is prepared and analyzed. All three types of blanks should be below the prescribed method detection limit. The method detection limits indicate the lowest quantifiable concentration using the associated method. For the purpose of reporting data,

no results less than this concentration are reported and a result of NS (not detected) appears in the Laboratory Services Section analytical test report. If a computed result falls in the range of 0.0001 and 0.0009 PPM, the compound is defined as being detected but the result would be too variable to be reliable so a designation of TR (trace) is listed beside the compound in the final report. In the event of sample contamination or equipment failure, the data will be flagged accordingly. The use of these data will be restricted until an investigation resolves the issue of contamination or inaccurate results. Only values that meet the data quality objectives for accuracy, precision and bias will be used without caution. This ensures that the data reported are reliable, reproducible and accurate.

B6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

No specialized equipment is required for collecting eggs samples in the field. Instrumentation required for chemical analysis consists of a GC/MSD, Hewlett-Packard gas chromatograph (GC) 5890 Series II equipped with an autosampler (7673A), a Galileo Channeltron electron multiplier (5778) and linked to a Hewlett-Packard 5970 (or 5971A) mass selective detector (MSD) with MS ChemStation,.

The Mass Selective Detector (MSD) is tuned weekly with the perfluorotributylamine (PFTBA) calibration standard using the Auto Tune program, and daily with the Quick Tune Program. The tuning of the instrument must meet the criteria for conformance outlined in SOP-CHEM-PROC-12 before sample analysis. Tune files are archived in a logbook at NWRC.

Laboratory technicians supervised by the chemist are responsible for testing, inspection and maintenance of laboratory instrumentation. Standard operating procedures for the maintenance of the GC/MSD are found in SOP-CHEM-MAIN-04 located in the trace organic analytical laboratory. The tuning of the mass selective detector (MSD) must meet the criteria for conformance outlined in SOP-CHEM-PROC-12 before sample analysis; certified technicians will be used to make the necessary repairs. Tune files are archived in the laboratory logbook.

B7 INSTRUMENT CALIBRATION AND FREQUENCY

A four-point initial calibration curve is generated every six months for the major compounds (e.g., oxychlordane, PCB-153, etc.) found in the control material to cover the range of interest. This established calibration curve is verified daily, by analyzing a calibration verification standard (quantitation standard) having a mid-point concentration. The calculated concentration of each compound must be within 20% of its actual known value. The final concentration of any reportable compounds must be within the demonstrated linearity of the detector. Calibration is documented daily in a laboratory log book by the technician or chemist performing the calibration. If problems are encountered, such as final concentrations of a reported compound falling outside the demonstrated linearity of the detector, the sample will be diluted with iso-octane to meet the calibration range.

B8 INSPECTION/ACCEPTANCE for SUPPLIES

The working standard solutions can be found in Table 1 – Supplier, catalogue number and concentration of PCBs and organochlorine standards of the Technical Report Series Number 335 "Multiresidue Methods for the Determination of Chlorinated Pesticides and Polychlorinated Biphenyls (PCBs) in Wildlife Tissues by Gas Chromatography/ Mass Spectrometry" (Won et al., 2001).

Chemists and technicians at the National Wildlife Research Centre in Ottawa, Ontario are responsible for inspection and acceptance of supplies. Acceptable supplies are those items that do not have any visual sign of defects/flaws and regents/chemicals that are not past expiry dates. Tracking records for supplies and consumables are kept in the trace organic analytical laboratory at the National Wildlife Research Centre, Ottawa, Ontario, Canada.

B9 NON-DIRECT MEASUREMENTS

Background information files will be accessed for all existing contaminant data in snapping turtle eggs, sediment samples and water samples, and this information will be incorporated into the project literature review. Background information will include researcher(s), organization(s), study locations, methodologies and contaminant data. As interest has been expressed in contaminant levels in tissues other than eggs, the results from chemical analysis of other liver, skeletal muscle and other tissues will be discussed. Sources of information will include published papers from peer-reviewed scientific journals as well as government reports and databases. These existing contaminant databases will be beneficial in testing and validating the snapping turtle as an indicator of temporal and spatial contaminant trends in different Great Lake coastal wetlands.

B10 DATA MANAGEMENT

All field data will be recorded in field logs and inspected at the end of each field day. All data will then be transferred to a central file at CWS-Ontario office in Burlington, Ontario where photocopies and electronic files will be made and stored. Original field logs and electronic files will be under the care of Kim Fernie. Contaminant data generated from snapping turtle egg analysis at the National Wildlife Research centre in Ottawa, Ontario, will be forwarded to Kim Fernie at the CWS- Burlington office in Burlington, Ontario, where it will be entered into a contaminants database by CWS technicians or contractors. The data are recorded electronically using Excel files on IBM-compatible computers. CWS technicians confirm and correct data entry to insure accuracy. CWS computers are back-up nightly using the Veritas program.

ASSESSMENT/OVERSIGHT

C1 ASSESSMENTS AND RESPONSE ACTIONS

As field operations are simple basic procedures, there are no expected sources of error in field sampling procedures. Similarly, chemists analyzing snapping turtle eggs at NWRC laboratories adhere to strict Good Laboratory Practice (GLP) principles. Kim Fernie will be responsible for supervising field staff with respect to appropriate and correct field sampling methods and oversight in data collection and review of field data logs for missing data daily while on site. Before the release of analytical reports, data validation is completed by the head of the Laboratory Services Section at the National Wildlife Research Centre in Ottawa, Ontario. Results of data verification are recorded on the "Data Validation Form for OC/PCBs Reports". The raw data is examined prior to release to CWS biologists and decisions are made by the head of the Laboratory Services Section regarding re-analysis of samples.

C2 REPORTS TO MANAGEMENT

Reports to the GLC will occur on a semi-annual basis and occur in December of 2003 and June of 2004 with a final report in June, 2004. These reports will include a brief narrative of progress to date and must detail any problems encountered as well as any changes to the project including personnel, schedule, and deliverable contents. The final report including all items as identified in the Project/Task Description and Data Quality Objectives sections of this project plan, and a financial report, will be submitted by Greg Mayne before June 30, 2004. All data collected as part of the project will be submitted in electronic format via electronic mail, or on CD or other compatible storage medium to Ric Lawson, Coordinator of Great Lakes Coastal Wetlands Consortium.

DATA VALIDATION AND USABILITY

D1 DATA REVIEW, VALIDATION, AND VERIFICATION REQUREMENTS

The project manager will review all documented field and laboratory operations including sample collection, handling, storage and analysis to ensure that methods conform to the specified QA/QC criteria. Analytical data will be examined for discrepancies (i.e., contaminant concentrations that fall far below or above the mean contaminant level for each site) upon delivery from testing laboratories.

D2 VALIDATION AND VERIFICATION METHODS

Data validation is ensured by an internal quality assurance audit done by an independent reviewer before the release of analytical reports. Results of this verification are recorded on a "Data Validation Form for OC/PCBs Reports". Analytical data on snapping turtle egg contaminant results will be examined for discrepancies by Laboratory Service technicians at NWRC. If large discrepancies are found in contaminant data for egg samples collected from the same site, analytical results will be re-examined. In instances where data validity comes into question and cannot be resolved, the specimen will be re-

analyzed by NWRC chemists. In the event that there is an omission of data, such omissions will be reported the project manager and conveyed to the GLCWC project manager and other collaborators identified in this QAPP. All analytical procedures and results will be fully documented; such documentation will reside in a file with the project manager.

D3 RECONCILIATION WITH DATA QUALITY OBJECTIVES

The framework for a sustainable basin-wide monitoring program using snapping turtles eggs as an indicator of contaminant exposure will be reviewed for completeness by the quality assurance manager and senior wildlife scientists within the Canadian Wildlife Service. Communication between field biologists and the quality assurance manager will be maintained on a daily basis throughout the data collection phase in the field to ensure a sufficient sample size for inter- and intra-site comparisons. Chemical reports will be provided by Canadian Wildlife Service chemists from the Laboratory Services Section in Ottawa, Ontario to the project biologist (Kim Fernie). Reports contain general information, methods, results, comments and detection limits on contaminants specific to snapping turtle eggs. Proper statistical methods will be used to analyze data for inter- and intra-site variation in contaminant levels in snapping turtle eggs. In the event that data quality objectives could not be attained for specific aspects of the sampling (i.e., insufficient sample size), the reason for not meeting the data quality objectives will be documented and reported in semi-annual progress reports and in the final report to the GLC.

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