
Name of Organization: SUNY Research Foundation

Type of Organization: College or University

Contact Information: Dr. James M. Haynes
Department of Biological Sciences, SUNY College at Brockport
350 New Campus Drive
Brockport NY 14420

Phone: (716) 395 - 5783 **Extension:**

Fax: (716) 395 - 2416

E-Mail: jhaynes@brockport.edu

Project Title: RAP Progress in the Rochester Embayment of Lake Ontario

Project Category: Pollution Prevention and Reduction - BNS

Rank by Organization (if applicable): 0

Total Funding Requested (\$): 134,260 **Project Duration:** 2 Years

Abstract:

In the 1980s, the International Joint Commission (IJC) initiated Remedial Action Plans (RAPs) in 43 Areas of Concern (AOCs) in the Great Lakes Basin. "Listing" as an AOC resulted from one or more of 14 "use impairments," the first of which is "fish and wildlife consumption advisories" due to the presence of bioaccumulative chemicals of concern (BCCs). Before the Rochester Embayment of Lake Ontario AOC can be "delisted" for its fish and wildlife consumption use impairment, several important questions must be answered. (1) Are snapping turtles and/or largemouth bass suitable sentinel species to monitor remedial progress in the AOC over time? (2) What are the absolute and proportional contributions of atmospheric deposition, watershed sources, and Lake Ontario sources to concentrations and loadings of BCCs in the AOC? (3) What are the spatial and temporal patterns of loadings and concentrations of BCCs in the AOC (i.e., are there higher levels of contaminants in some AOC sub-watersheds that can be identified and remediated)? (4) Can one BCC be a surrogate for all BCCs in future monitoring? Air, water, sediment and tissue samples will be collected above and below an impassable barrier in Salmon Creek, and at selected locations in the Genesee River and Irondequoit Creek watersheds. They will be analysed for dioxins/furans, PCBs and mirex/photomirex using standard analytical techniques and a cell-based assay with high sensitivity and low cost. We will adapt and calibrate an existing mass balance/bioenergetics/ bioaccumulation model for conditions in AOC sub-watersheds so that less intensive sampling in the future might predict concentrations in air, water, sediments and sentinel species. This project is designed to answer the questions above, to validate the cellular assay, and to assist creation of a monitoring plan that will allow government agencies to determine when the fish and wildlife consumption use impairment can be delisted in the Rochester Embayment AOC.

Geographic Areas Affected by the Project

States:

<input type="checkbox"/> Illinois	<input checked="" type="checkbox"/>	New York
<input type="checkbox"/> Indiana	<input type="checkbox"/>	Pennsylvania
<input type="checkbox"/> Michigan	<input type="checkbox"/>	Wisconsin
<input type="checkbox"/> Minnesota	<input type="checkbox"/>	Ohio

Lakes:

<input type="checkbox"/> Superior	<input type="checkbox"/>	Erie
<input type="checkbox"/> Huron	<input checked="" type="checkbox"/>	Ontario
<input type="checkbox"/> Michigan	<input type="checkbox"/>	All Lakes

Geographic Initiatives:

<input type="checkbox"/> Greater Chicago	<input type="checkbox"/> NE Ohio	<input type="checkbox"/> NW Indiana	<input type="checkbox"/> SE Michigan	<input type="checkbox"/> Lake St. Clair
--	----------------------------------	-------------------------------------	--------------------------------------	---

Primary Affected Area of Concern: Rochester Embayment, NY

Other Affected Areas of Concern: If the cell-based assay is validated, it will be of use in monitoring BCC remediation in all AOCs and other places where it is desired to know total levels of dioxin-like compounds in air, water, sediment or biota.

For Habitat Projects Only:

Primary Affected Biodiversity Investment Area:

Other Affected Biodiversity Investment Areas:

Problem Statement:

The Stage I&II Rochester RAPs identified fish and wildlife consumption advisories as a use impairment and recommended remedial actions, monitoring methods to track progress of remedial actions, and the development of "delisting criteria" to determine when the consumption use impairment no longer exists. Developing a procedure to monitor progress toward delisting the impairment is a high priority. However, separating contaminants remediable in the AOC from those transported into it from Lake Ontario or deposited by the atmosphere is hard. For example, many fishes on the advisories accumulate their body burdens of BCCs in Lake Ontario, particularly salmon that do not reside in the AOC until spawning at the end of their lives. Thus, the current fish consumption use impairment for the AOC appears to be more related to lake-wide than local conditions.

To sort out these issues, we will test two hypotheses, each with multiple possible outcomes. (1) There are no differences in BCC concentrations (standard analytical vs. cell-based assays) in sentinel species (snapping turtles, largemouth bass) and sediments above and below the impassable barrier in Salmon Creek, and the proportions of BCCs are the same between species and locations. (2) There are no differences in BCC levels and proportions in atmospheric deposition or in sub-watersheds (water, sediment) over space (Salmon Cr., Irondequoit Cr., Genesee R.) and time (four seasons) in the AOC watershed. Two outcomes are likely. (1) BCC levels are lower in biota and sediments above the barrier in Salmon Creek than in biota and sediments exposed to Lake Ontario below the barrier. Depending on how low BCC levels above the barrier are relative to the advisories, and considering results of air, water and sediment analyses, this result could lead to rapid delisting of the use impairment in the AOC and send this issue to the Lake Ontario LaMP for resolution. (2) There are no differences in BCC proportions and levels in air deposition or in sub-watershed waters and sediments over space and time, a result that would indicate a lack of BCC sources in the AOC and suggest that fewer sampling locations will be needed for future monitoring. If significant airborne BCCs are produced in the AOC watershed, we expect to see a gradient of low to high concentrations from the west to east and south to north in the sub-watersheds (based on prevailing winds and industrialization). If significant water or sediment sources of BCCs exist in one or more sub-watersheds, we will observe these differences and provide the information needed to identify and remediate those sources. (Note: There is no indication in the RAP that BCC point sources exist anywhere in the AOC watershed).

Testing hypothesis 1 will provide key information. (1) Subtracting weight-adjusted BCC concentrations in sentinel species above an impassable barrier from concentrations below it will estimate the contribution of Lake Ontario sources to BCC levels in resident biota below the barrier. (2) Will measuring one BCC in a sentinel species provide as much information on remedial progress as measuring more than one chemical? (If yes, and approved by regulatory agencies, this will save time and money in the future). (3) Do largemouth bass, which are generally low in lipids relative to turtles, accumulate sufficient levels of BCCs to be useful as biomonitors of RAP progress over time? (4) Are BCC levels so uniformly high in long-lived turtles across the AOC as to restrict their usefulness as a sentinel species? (5) Is the cell-based assay reliable?

Testing hypothesis 2 will provide key information. (1) Estimates of current atmospheric deposition of BCCs in the AOC watershed (RAP values are from the 1970s and 1980s). Subtracting BCC concentrations in the air from BCC concentrations

in the water and sediment will estimate current and potential BCC contributions to Lake Ontario from the Salmon Cr., Genesee R. and Irondequoit Cr. sub-watersheds. (2) Does measuring one BCC in one medium provide just as much information on remedial progress as measuring more than one BCC in multiple media (yes, if relative proportions in the media sampled are similar; such a finding will save time and money in the future)? (3) If we find temporal/seasonal differences in BCC concentrations in the air, water or sediments this will indicate that future sampling should take place at times when BCC levels are highest.

Proposed Work Outcome:

We will capture 20 snapping turtles using trap nets or baited hooks and 20 largemouth bass using trap nets, electrofishing or angling (10 of each species above and below a falls on Salmon Cr.). To analyze 40 specimens for three chemicals (total PCB, mirex, photomirex) will require 40 analyses. Body weight/body burden regression relationships will be established for turtles and bass above and below the barrier in order to compare tissue concentrations on a unit weight basis.

In each of four seasons, we will collect 24 h air, 200 L water and 2-4 L sediment samples at convenient locations above the first impassable barriers in Salmon Creek, the Genesee River and Irondequoit Creek, plus a sediment sample below the barrier in Salmon Cr. We will do the same at a relatively "unpolluted" site upstream in the Genesee River watershed. There will be a total of 20 sediment, 16 water and 16 air samples (4 months x 4 or 5 locations each) and one analysis (PCBs, mirex, photomirex) for each sample, or a total of 52 analyses.

Due to prohibitive expense (~ \$1,000 per sample), we will not analyze all samples for dioxins/furans. We will send composite samples of turtle and bass tissue (n=4), sediments (n=2), water (n=2) and air (n=2) from above and below the Salmon Cr. barrier to an approved contract laboratory. This limited analysis will allow us to (1) Determine AOC baseline levels of the 15 dioxins/furans known to be in Lake Ontario fish near the Rochester Embayment and (2) Compare levels of dioxins/furans in the sentinel biota, sediments, water and air above and below the barrier.

SUNY Brockport has all of the equipment needed to collect and analyze samples for this project. Turtles and bass will be placed on ice in the field, then processed and frozen in the lab in accordance with APHA standard methods. Frozen tissue samples will be stored in solvent-cleaned glassware. Skin-off fillets (bass) and right fore and hind quarters (turtles) will be analyzed after homogenization. Water will be sampled just below the surface and stored in glass bottles pre-rinsed in pesticide grade hexane with Teflon caps. After preservation to pH < 2 with nitric acid (Ultrex grade), water samples will be stored at 4C in glass containers and analyzed within 30 days. Sediment will be collected with Ekman or Ponar dredges in pool areas (high in organic debris that attracts BCCs). Samples will be stored at 4C in solvent-cleaned glass containers with Teflon caps. Air samples will be taken over a 24-hour period with Graseby High Volume air samplers equipped with PUF (polyurethane foam) cartridges to collect vapors from the air (EPA Method TO-4). Airflow rates will be calibrated with a Model G40 PUF calibration unit. Samples for dioxin and furan analysis will be collected, processed and shipped in accordance with instructions from the contractor, Columbia Analytical Services, a NYSDEC certified laboratory for chlorinated hydrocarbon analysis.

Mirex, photomirex and total PCBs in air, water, sediment and tissue samples will be analyzed using a Hewlett-Packard 5890 gas chromatograph in the splitless mode, equipped with an electron capture detector (ECD) and a DB-5 capillary column. Extractions and cleanups will be made with dedicated glassware using pesticide grade reagents. Analysis of water samples will be by liquid-liquid extraction (Method 6630B, APHA). Air samples will be collected on PUF and extracted for analysis. Sediment samples will be air-dried, weighed and extracted in a 1:1 acetone/hexane solution by sonification. Before analysis by GC-ECD, the extract will be cleaned up by passing it through a Florisil column. Confirmation for PCBs and mirex/photomirex will be with a mass spectrometer (H-P G1800C GCD Plus-Gas Chromatograph Electron Ionization Detector).

Analytical results will be compared to cell-based assays done by co-PI Victor McFarland (US Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS). P450RGS is a low-cost, rapid screening assay for total dioxins/furans/co-planar PCBs in environmental samples (response to dioxin is 10-10,000X > PCBs, etc.). Using an accelerated solvent extraction process with sulfuric acid/silica gel cleanup for sample preparation allows analysis of batches of 24 samples with 3 replicates per sample for a per sample cost of \$200.00. The assay conforms to APHA Standard Method 8070 and ASTM Standard E-1853. EPA has promulgated the P450RGS assay as EPA Method 4425 in update IVA of the EPA SW846 Methods Manual. The extraction procedure complies with EPA Method 3545. Limits of detection are typically 7 +/- 2 pg TCDD TEQ/g dry sediment.

The basic mechanism that responds to the presence of dioxins/furans/co-planar PCBs is the same in all vertebrate cells. The initial reaction is binding of the chemical to a cytosolic receptor protein known as the aryl hydrocarbon receptor (AhR). This association leads to the formation of a stable AhR-ligand complex which translocates to the cell nucleus and binds with dioxin recognition elements (DREs) on the DNA. As a result, certain genes (e.g., CYP1A1) are expressed and detoxifying enzymes are synthesized. The amount of enzyme produced is directly proportional to the concentration of

bound chemical. Hence, the quantitation of one of these enzymes serves as a measure of dioxin, etc. activity. Recently, advances in transgenic research have produced new ways of detecting dioxins and other chemicals that bind with the AhR. Recombinant cell lines have been developed in which non-mammalian reporter genes are inserted downstream from the DREs in the DNA of the cells. When the DREs are activated the reporter gene is switched on, producing a protein that can be detected instrumentally. For example, the 101L cell line used in the P450RGS assay is derived from human hepatoma HepG2 cells and is stably transfected with a plasmid containing the human CYP1A1 promoter sequence fused to the firefly luciferase gene as a reporter. The induction of CYP1A1 results in the production of luciferase. Light produced by the action of luciferase on a luciferin substrate is measured by a luminometer.

The SUNY Brockport (ELAP) and Waterways Experiment Station laboratories are certified and will extend routinely employed QA/QC procedures to this project.

T- or equivalent non-parametric tests will be used to determine if significant differences in BCC levels exist between bass/turtles and sediments above and below the barrier in Salmon Ck. For each of air, water and sediment samples, we will use a 3 (chemicals: PCBs, mirex, photomirex) X 4 (locations: Salmon Ck., lower Genesee R., upper Genesee watershed, Irondequoit Ck.) X 4 (months: May, August, November, February) multi-way ANOVA to explore differences among chemicals, locations and seasons and their interactions. For example, if there are no significant interaction effects among chemicals, this result would suggest that one chemical may be a good representative for all BCCs in the AOC. Results of initial statistical analyses will guide further exploration and analysis of the data by collaborator Dr. James N. McNamara, Emeritus Professor of Statistics, Department of Mathematics, SUNY Brockport.

Tissue and sediment samples will be split after extraction in order to compare analytical results with the cell-based assay using a paired comparison t-test. The cell-based assay generally has low variability, allowing for powerful statistical comparisons even when sample sizes are small. If one wished to detect a difference of 20% between treatments with 95% confidence, only 4 replicates per treatment would be required to conduct a t-test with 90% power. If analytical and cell-based results are similar, we will have validated a powerful new tool for future monitoring of remediation progress in Rochester and throughout the Great Lakes Basin.

The proposed project will quantify BCC concentrations in air, water, sediments and sentinel species at key locations in the AOC watershed, and it will estimate proportional contributions to BCC levels in sentinel biota coming from inside (air, water, sediments) and outside (air, Lake Ontario) the AOC watershed. Collaborator Dr. Thomas Young will calibrate existing models (mass balance, bioenergetics, bioaccumulation), created by Great Lakes Research Consortium faculty, by incorporating the physical characteristics of Salmon Creek and the food habits of the sentinel biota. We will use these models to predict BCC concentrations in sentinel species in the Genesee R. and Irondequoit Ck. and to test those predictions against samples collected in those sub-watersheds of the AOC.

If the models prove to be sufficiently robust, future monitoring may not have to measure all of the parameters we propose to measure. Most important is determining when the Rochester RAP can delist for the fish and wildlife consumption advisories (e.g., 0.1 mg/kg for mirex and 2.0 mg/kg for PCBs in fish/wildlife tissues). If we know the relative BCC contributions coming from the air and AOC sub-watersheds, modeling can tell us now and in the future how much, if any, remediation in the AOC will lead to BCC concentrations in sentinel species below those triggering advisories. For example, if background atmospheric deposition is the predominant source of BCC concentrations in sentinel biota, and the models show that no amount of remediation in the AOC watershed will lead to levels below consumption advisories for sentinel species, the RAP committee can recommend rapid delisting and addressing of the problem by the LaMP. Alternatively, if BCCs are coming mainly from AOC watershed sources, the committee must then recommend ways to identify and remediate locally generated sources.

We expect the following outcomes from the proposed project. (1) Baseline levels of BCCs in air, water, sediments and resident sentinel species in the AOC. (2) The extent to which the Rochester AOC contributes to Lake Ontario fish consumption advisories. (3) Which species (turtle or bass) and which chemical(s) (dioxin, PCBs, mirex) may be better for future biomonitoring of BCCs. (4) Enhance understanding of the spatial and temporal patterns of BCC loadings contributed by AOC sub-watersheds to Lake Ontario and loadings from the air to sub-watersheds. (5) Distinguish absolute and proportional contributions to contaminant levels in AOC sentinel species coming from air deposition, AOC sources (water and sediments), and Lake Ontario. (6) Validate the cell-based assay against standard analytical techniques. (7) Estimate the levels of BCCs that may be ingested by terrestrial animals that consume fish, such as mink and humans. (8) Provide critical information needed to help the RAP committee establish a monitoring plan and criteria that will lead to delisting of fish and wildlife consumption advisories as a use impairment in the Rochester AOC.

Project Milestones:	Dates:
Project Start	07/2000
Collect turtle and bass samples	09/2000
Begin collecting air, water, sediment	10/2000
End collecting air, water, sediment	07/2001
Begin analysis of samples	10/2000
End analysis of samples	10/2001
End modeling exercises	05/2002
Project End-Final Report	08/2002

Project Addresses Environmental Justice

If So, Description of How:

Indirectly, this project addresses environmental justice. Educated, middle class people in the AOC are well aware of current fish and wildlife consumption advisories and generally do not eat or greatly restrict their intake of Lake Ontario-exposed fish. This is not true of minority groups, particularly African- and Asian Americans, who for economic and cultural reasons eat much larger amounts of wild-caught fish and wildlife (this statement was documented in surveys done before the RAP committee prepared a fish consumption advisory pamphlet that was distributed to city hospitals, clinics and churches). To the extent that this project leads to identification and remediation of problems, or identifies relatively "safe" sources of fish and wildlife in the AOC, it will contribute to greater environmental justice.

Project Addresses Education/Outreach

If So, Description of How:

A major component of the entire Rochester RAP process since 1989 has been public education and outreach. Over the years, several major committees and numerous subcommittees and task forces have worked to develop the information and plans that have been incorporated into the RAP. This information has been shared with the public and suggestions from the public are incorporated throughout the recommendations of the RAP. The public asked for the RAP to address the fish and wildlife consumption advisories and it expects periodic reports on progress to delist the use impairment. Through collaboration with the Monroe County Department of Health and the RAP committee, the proposed project will provide the public with the information needed to evaluate progress and to understand what more needs to be done before we can delist the fish and wildlife consumption advisories use impairment.

Project Budget:

	Federal Share Requested (\$)	Applicant's Share (\$)
Personnel:	54,745	8,050
Fringe:	9,106	2,347
Travel:	1,925	0
Equipment:	0	0
Supplies:	6,925	0
Contracts:	20,500	0
Construction:	0	0
Other:	0	1,500
Total Direct Costs:	93,201	11,897
Indirect Costs:	41,059	6,038
Total:	134,260	17,935
Projected Income:	0	0

Funding by Other Organizations (Names, Amounts, Description of Commitments):

There is no current funding for this project, but a similar proposal to the NY Great Lakes Protection Fund is pending. If we receive funding from that agency, we will know of it before we may be asked to submit a final proposal to the GLNPO. If we are funded by the NYS GLPF and asked to submit a full proposal by the GLNPO, we would ask to modify this pre-proposal to avoid duplication, expand sampling and analysis of sentinel species to the Genesee River and Irondequoit Creek sub-watersheds of the AOC, and increase sample sizes (temporally and spatially) in the Salmon Creek sub-watershed so as to more fully evaluate the results of the traditional analytical and cell-based assay approaches to contaminant monitoring.

Description of Collaboration/Community Based Support:

The Stage 1 RAP identified fish and wildlife consumption advisories as a use impairments in the AOC. The Stage II RAP, developed by a committee with many citizen members, and after several public meetings, proposed remedial actions. Monitoring of levels of BCCs, particularly PCBs, mirex/photomirex and dioxins/furans, in resident biota was a high priority. The purpose of monitoring is to measure progress toward the goal of delisting the use impairment. Delisting can be achieved only when it can be shown that the Rochester Embayment watershed does not contribute significantly to lake-wide fish consumption advisories. If there are AOC watershed sources of BCCs contributing to the advisory, these sources must be identified so that remediation can take place. This project will provide the quantitative data needed to understand the AOC's contribution, if any, to BCC loadings into Lake Ontario. Without such data as a baseline to monitor remedial progress, we will not have the information needed in the future to delist the fish and wildlife consumption use impairment that currently exists in the AOC.

Collaborators

Dr. James M. Haynes (PD/PI, Analysis, Reports) and Dr. Joseph C. Makarewicz (PI, Analytical)

Department of Biological Sciences
SUNY College at Brockport
Brockport, NY 14420-2973

Dr. Victor McFarland (PI, Cell-Based Assays)

U.S. Army Corps of Engineers
Waterways Experiment Station
Vicksburg, MS 39180

Ms. Margaret Peet (Water Quality and RAP Coordinator)

Monroe County Department of Health
111 Westfall Road
Rochester, NY 14612

Dr. Thomas C. Young (Mass Balance/Bioenergetics/Bioaccumulation Modeling)

Department of Civil and Environmental Engineering

Clarkson University
Potsdam, NY 13699-5710
Dr. James N. McNamara (Experimental Design, Statistics)
Department of Mathematics (Retired)
SUNY College at Brockport
Brockport, NY 14420