2004 Progress Report: Development and Evaluation of Chemical Indicators for Monitoring Ecological Risk

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Center: Great Lakes Environmental Indicators Project
Center Director: Gerald J. Niemi
Title: Development and Evaluation of Chemical Indicators for Monitoring Ecological Risk
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RFA: Environmental Indicators in the Estuarine Environment Research Program (2002)
Research Category: Ecological Indicators/Assessment/Restoration

Description:

Objective: Our **overall goal** is to identify and validate effective contaminant indicators of adverse impacts on estuarine ecosystem health. Indicators will be developed in the Great Lakes, but will also be applicable to both marine and freshwater ecosystems. These contaminant indicators will be used to evaluate ecological condition. Specifically we will focus on the evaluation of two indicators:

1. indicator PAHs of photo-induced toxicity to fish and benthic organisms; and
2. organic chemical indicators of xenoestrogenic exposure to fishes.

The assessment of ecological condition in an effective manner is best accomplished using integrative indicators of condition. These indicators should be cost-effective, be applicable across multiple scales, and provide useful information for environmental managers. Within the omnibus project, this contaminants subproject focuses on contaminant indicators that will provide a measure of condition of the estuarine ecosystem. These indicators will also serve as diagnostic indicators that will identify the primary stressors affecting the specific ecological endpoint of concern. We have focused on PAH compounds and environmental estrogens since they are widespread in the environment and have existing sources, and thus are of current concern.

The **specific hypotheses** we are testing are:

1. Specific PAHs in combination with UV penetration are indicators of potential loss of vulnerable species within coastal fish and or benthic communities; and
2. Specific chemicals are indicators of endocrine disruption in fish via the estrogen receptor. Data collected to test these hypotheses will be used to demonstrate the degree of usefulness of these two groups of indicator compounds as diagnostic indicators for estuarine ecosystems.

Our **overall approach** to this project is as follows. For both indicators, we will compare contaminant concentrations to a biological endpoint or condition across a gradient of non-degraded to highly degraded sites in approximately 20 locations being studied by the other indicator project groups in the program. For the PAH photo-induced toxicity indicator, we will collect the necessary field data to test the model developed in the lab by the collaborators at EPA-MED (Diamond, Mount, Erickson). These data include the concentrations of PAHs in sediment, larval fish, and oligochaetes (to determine the BAFs and to provide the doses for the model); sediment photo-induced toxicity potential (assayed in the lab using the aquatic annelid *Lumbriculus* [lab test organism] and field sediments); and UV dose (obtained from field measurements). The toxicity that is predicted from the model will be compared to that measured in the lab assay. Results will be used to calibrate the model, and independent field data will be used to validate the model. While photo-induced toxicity has been extensively studied and its acute toxicity demonstrated in the laboratory, this will be the first field test of such an indicator.

The xenoestrogen indicators will be identified in an analogous manner. A suite of potential xenoestrogens will be measured in fish tissue, sediment, and/or water and compared to vitellogenin induction in wild and caged male fish (a bioindicator of individual estrogen exposure) at the same gradient of sites. Using correlative statistical techniques, we will identify specific indicator xenoestrogens that are associated with vitellogenin induction. This would represent the first link of vitellogenin induction and chemical exposure in field sites other than near sewage treatment plants.

Progress Summary:

PAH Phototoxicity
Photo-induced toxicity of PAHs to larval fish is a function of exposure to both PAHs and ultraviolet-A (UV-A) light. Over the past year we have performed work on both determining PAH exposure at our field sites, on developing a model for UV-A exposure, and refining the indicator model through controlled lab experiments.

UV-A exposure depends on factors such as light intensity, dissolved organic carbon and total suspended solids. We have developed a model for measuring UV-A attenuation in the water column of the coastal Great Lakes. This model involves the measurement of spectral attenuation using a spectrophotometer, a simple piece of equipment common to most laboratories, and suspended particulate matter. Due to the ease of the measurements and incorporation of the influence of suspended particulate matter on attenuation we have created a useful tool for managers of the coastal Great Lakes. Our method can be used to evaluate the UV-A exposure setting at other sites around the Great Lakes and more importantly, predict how changes in suspended particulate matter might affect UV-A attenuation. For instance, the introduction of zebra mussels have dramatically reduced the amount of suspended particulate matter in the coastal areas and, therefore, may have a commensurate increase in UV-A exposure to larval fish in those areas. A manuscript of our UV-A model results has been prepared and is currently undergoing internal review for submission to the *Journal of Great Lakes Research*. This paper is the basis for Andy Adams' MS thesis which he will defend in the coming months. Andy will continue on for his PhD working on the PAH exposure and phototoxicity modeling portions of the project.

PAH exposure is a function of partitioning from the sediments to the water column and into larval fish. This process is described as a bio-sediment accumulation factor (BSAF). In order to describe BSAFs at our sites we have analyzed sediments, oligochaete worms placed in the sediments and larval fish collected in the field for a suite of PAHs. These sample analyses are completed, and the QA/QC review of the data is underway, to be followed by determination and interpretation of the BSAFs.

Once PAH and UV-A exposure is elucidated, they will be incorporated into a paper describing the phototoxic potential at each site. Results of our UV-A model have been presented at two scientific conferences this past year, and the bio-sediment accumulation work will be presented at this spring's International Association for Great Lakes Research Conference.

During the past year, EPA-MED staff completed additional field experiments examining the response of the zooplankter *Daphnia magna* to ambient exposures of PAHs and sunlight in a contaminated embayment in the St. Louis River estuary. Photo-activated toxicity was measurable but less dramatic that in the previous field season, which corresponded to lower ambient PAH concentrations in the water column. These data provide important linkages between field exposures and expected biological response, as well as between responses measured in the laboratory and field, and assist in finalizing the indicator.

In addition, controlled exposures to a single PAH and laboratory-generated UV continued. The list of test species was expanded to include yellow perch, an important species in all of the Great Lakes. Yellow perch embryos were exposed to UV and pyrene in the laboratory to develop exposure response relationships that can be extrapolated to field conditions. Field measurements of ambient UV were also made in the field at locations where yellow perch were spawning in local, inland lakes. Also in the field, collections were made of zooplankters inhabiting the upper water column in local lakes during mid-day, in an effort to identify species at highest risk for UV/PAH exposure. Finally, laboratory exposures using *D. magna* were expanded to include longer exposure durations (21-d) and reproduction as an endpoint to determine the extent to which chronic effects of UV/PAH exposure may be important.

Environmental Estrogens (EEs)
The efforts on this indicator over the last year were spent on an assessment of why this indicator failed to be transferred from the lab to the field, and also on conducting some additional experiments to further bound the utility of some of the tools available for assessing estrogenicity. Finally, a series of three manuscripts were written that addressed the above issues as part of the PhD dissertation of Randy Lehr, who has worked on this project since its inception.

The first manuscript provided a comprehensive review and critical assessment of the tools that have been developed to assess estrogenic exposures and response in fish, from the measurement of chemical concentrations in fish tissue all the way to the proteomic and genomic measurements that indicate a response at the cellular or molecular level. To establish exposure-effect relationships, researchers have identified a number of measurement endpoints that characterize signal transduction at a number of intermediary steps throughout the estrogen response pathway. However, development of these assays has not followed a standardized approach and different measurement endpoints have been quantified using different analytical techniques and exposure

scenarios. As a result, the sensitivity and diagnostic and predictive potential for these assay systems is different. In general, assays that characterize estrogen signal transduction at lower levels of biological organization are the most amenable to high throughput and diagnostic analysis, but the poorest predictors of potential effects at individual and population levels. Conversely, assays that characterize estrogen signal transduction at higher levels of biological organization are the best predictors of potential effects, but the least amenable to high throughput, diagnostic analysis. This complicates the linkage of exposure and effect using a single endpoint and requires the analysis of multiple endpoints to mechanistically link exposure and effect. This approach is recommended, but is not amenable to adopt as a monitoring approach for the end-users of this project. At the beginning of this project, the complexity of the estrogen response pathway was not fully appreciated, nor were these tools fully developed.

One of the more promising assessment tools we had hoped to utilize was measuring response of vitellogenin mRNA induction in fathead minnows placed in cages at sites around the Great Lakes. However, there are different versions of this assay developed by different investigators, and the sensitivity of the assay varies considerably, influencing the utility and interpretation. So we conducted an experiment to determine some of the factors that might influence the differences observed in the assays. Research has demonstrated that the sensitivity of Vtg endpoints is affected by a variety of different assay parameters, but one parameter that has not yet been thoroughly evaluated is the use of different carrier solvents. To examine the effects of carrier solvent selection on the Vtg mRNA endpoint, we exposed male fathead minnows (*Pimephales promelas*) to two different doses (10 and 100 ng/L) of 17α–ethinylestradiol (EE2) across four different carrier solvent types, water (H_2O), dimethyl sulfoxide (DMSO), ethanol (EtOH) and triethylene glycol (TEG) using a 48-hr static exposure. Among the carrier solvent treatments, the Vtg mRNA response was highest in the H_2O and EtOH and lowest in the DMSO and TEG treatment groups. Results suggest that use of the DMSO and TEG carrier solvents (at a concentration of 50 uL/L) creates a more uniform EE2 exposure, but suppresses the magnitude of the Vtg mRNA response. In addition, the data suggest that use of a saturation-based solution to generate carrier solvent-free aqueous exposures may be confounded by estrogenic impurities in analytical standards.

The final manuscript in the dissertation is directed at providing advice to environmental managers who wish to monitor for environmental estrogens (EEs). Management of chemical contaminants is highly dependent upon the establishment of exposure-effect relationships. Establishment of exposure-effect relationships for EEs is complicated by a variety of factors and as such, the management of EEs presents a variety of challenges. To aid the management process, researchers have developed a variety of assays to establish exposure-effect relationships and each of these assays is likely to be best suited for different aspects of the management process. Assays that quantify exposure and effect at higher levels of biological organization integrate EE exposure and are likely to be more appropriate for assessment of ecosystem condition and long-term monitoring. Assays that assess exposure and effect at lower levels of biological organization are more mechanistically diagnostic and thus, likely to be more appropriate for the identification of specific chemicals of concern and design of management interventions. The unique physical-chemical and toxicological properties of EEs also affects the design of management plans and the ability to communicate management results.
In summary, the complexity of the estrogen response pathway necessitates having indicators that can both assess exposure, and assess an integrated measure of the response elicited as a result of

that exposure. The tools available do not do both of these well, and a monitoring program requires the use of multiple tools to assess exposure as well as assess specific and integrated responses to provide the link of exposure and effect. Furthermore, tools are needed to bridge the assessment of individuals to populations and communities. These tools are largely still in the research and development phase, and few have been used effectively to assess effects of EEs to fish populations in the field.

Abstracts and Publications:

Journal	Adams, A. D.; Lehr, R. A.; Swackhamer D. L.; Diamond, S. A.; Mount D. R.; Simcik, M. F. Measuring attenuation of ultraviolet-a radiation in Great Lakes Coastal ecosystems: implications for photo activation of polycyclic aromatic hydrocarbons. In preparation for *Journal of Great Lakes Research.* To be submitted by 4/1/05.
Journal	Lehr, R.A., Adams, A., Simcik, M., Ankley, G., Swackhamer, D.L. Effects of carrier solvent selection on the Vitellogenin (Vtg) mRNA response in male fathead minnows (*Pimephales promelas*). Draft completed, in preparation.
Journal	Lehr, R.A., Adams, A., Simcik, M., Ankley, G., Swackhamer, D.L. Linking exposure and effect for environmental estrogens: the exposure-effect paradox. Draft completed, in preparation.
Journal	Lehr, R.A., Adams, A., Simcik, M., Ankley, G., Swackhamer, D.L. Managing environmental estrogens in aquatic ecosystems. Draft completed, in preparation.
Presentation	Andy D. Adams, Randy A. Lehr, Matt F. Simcik, Deborah L. Swackhamer, Stephen Diamond and David Mount. Photo-Induced Toxicity in Larval Fish from the Coastal Great Lakes. International Association of Great Lakes Research Conference, Waterloo, ON, May 24-28, 2004.
Presentation	Andy D. Adams, Randy A. Lehr, Matt F. Simcik, Deborah L. Swackhamer, Stephen Diamond and David R. Mount. Photo-induced Toxicity of Polycyclic Aromatic Hydrocaborns and Indicator Development. Joint Meeting of the Midwest and Ozark Prairie Chapters of the Society of Environmental Toxicology and Chemistry, Lacrosse, WI, March 4-5, 2004.
Presentation	Lehr, R.A., A. Adams , J. Erb , G. Ankley , M. Simcik and D. Swackhamer. Developing Indicators of Environmental Estrogen Exposure in the Great Lakes. Poster, All-EaGLe Meeting, Duluth MN, September 30-October 1, 2004.
Presentation	Andy D. Adams, Randy A. Lehr, Matt F. Simcik, Deborah L. Swackhamer, Stephen Diamond and David R. Mount. Photo-induced Toxicity of Polycyclic Aromatic Hydrocaborns and Indicator Development. Poster, All-EaGLe Meeting, Duluth MN, September 30-October 1, 2004.

Presentation Swackhamer, D.L. Contaminant Indicators. Presentation at All-EaGLe Meeting, Duluth MN, September 30-October 1, 2004.

Presentation Lehr, R.A., Adams, A.D., Erb, J.L., Denslow, N.D., Ankley, G.T., Simcik, M.F., and Swackhamer, D.L. Integrating and Interpreting Integrators of Sublethal Exposures to Environmental Estrogens. Society of Environmental Toxicology and Chemistry, Portland, OR, November 14-18, 2004.

Thesis Lehr, Randy A. 2005. Assessment and Management of Environmental Estrogens. PhD thesis, Water Resources Science, University of Minnesota. 175 pp.

Supplemental Keywords: *PAHs, photo-induced toxicity, xenoestrogenic compound, fishes, endocrine disruption, larval fish, vitellogenin induction, Lumbriculus, environmental estrogen, environmental indicators. coastal wetlands, Great Lakes*

Relevant Websites: http://glei.nrri.umn.edu