Example: Study Proposal

Title: ASSESSMENT OF TOXICITY IN AMPHIBIAN HABITATS

Date of Proposal: Summer 2000

Investigators:
Edward Little, Ph.D. (PI)
USGS, Columbia Environmental Research Center
4200 New Haven Rd., Columbia MO 65201
phone (###) xxx-xxxx fax: (###) xxx-xxxx
email: Email_address@usgs.gov

Christine Bridges, Ph.D.
USGS, Columbia Environmental Research Center
4200 New Haven Rd., Columbia, MO 65201
phone (###) xxx-xxxx ext. 1744 fax: (###) xxx-xxxx
email: Email_address@usgs.gov

Abstract:
Many amphibians breed and undergo early life-stage development in shallow, temporary pools. These habitats are vulnerable to chemical contamination from aerial transport, direct application, and surface water runoff that can cause immediate mortality or sublethal effects manifested over extended periods of time. Environmental hazards seldom exist in the absence of other harmful conditions. Contaminants may impact amphibians simultaneously with other stressors such as solar ultraviolet radiation (SUV). SUV can be directly harmful to developing amphibians and can also potentiate the injury associated with contaminant hazards.

Background:

Statement of Issue-

Reports of amphibian deformities have increased over recent years. Many causes have been proposed for having caused deformities, including contaminants (Bonin et al., 1999; Ouellet et al., 1997), UV radiation (Ankley et al., 2000), and parasites (Johnson et al., 1999), either singly or in combinations. Short-term toxicity tests with extracts obtained from semipermeable membrane devices (SPMDs) offer a rapid means of evaluating contaminants in amphibian habitats (Huckins et al., 1993). The SPMDs contain lipids that accumulate contaminants in much the same way that organisms do (Petty et al., 2000). The daily accumulation of fat-soluble contaminants by SPMDs approximates the daily accumulation of lipophilic compounds by aquatic organisms. Thus, environmental doses can be estimated from SPMD extracts based on the duration of deployment in the environment. This approach, currently being applied at sites of amphibian deformities in Minnesota, provides a quick answer to the question "Is there anything toxic to developing frogs in this habitat?" We have used SPMD extracts in an experiment to determine the potential contaminants possess to trigger malformities among frogs in northern-Minnesota ponds.

In north-central Minnesota, some ponds have demonstrated high proportions of deformed frogs (nearly 80% in some species). Since the mid-1990s researchers have been attempting to find cause for these deformities in these ponds. Currently we are researching whether contaminants found at the sites are responsible for deformities by exposing native tadpoles to extracts of SPMDs. We used two sets of SPMDs that had been deployed for 30 days, one set in a reference and one in a contaminated site. Because some contaminants are photoenhanced, we also used UV radiation as a factor in our experiments. We found that the SPMD extracts were not acutely toxic to tadpoles, even at concentrations of up to 30x a daily dosage. Extracts from each of our two sites caused an increase in embryonic deformity when exposed to UV light. Hatching success and tadpole weight after 10 days were lower in the contaminated site in the presence of UV light. We are currently attempting to determine whether there are differences in deformity rates among tadpoles reared for 45 days in the SPMD extracts from the two sites, with and without UV light.

Relevant literature-

- Ankley, GT, JE Tietge, GW Holcombe, DL DeFoe, SA Diamond, KM Jensen, and SJ Degitz. 2000. Effects of laboratory ultraviolet radiation and natural sunlight on survival and development of Rana pipiens. Can. J. Zool. 78:1092-1100.

- Blaustein, AR, JM Kiesecker, DP Chivers, RG Anthony. 1997. Ambient UV-B radiation causes deformities in amphibian embryos. Proc. Nat. Acad. Sci. 94: 13735-13737.

- Bonin, J, M Ouellet, J. Rodrigue, J-L DesGranges. 1997. Measuring the health of frogs in agricultural habitats subjected to pesticides. Herpetological Conservation 1:246-257.

- Huckins, JN, GK Manuweera, JD Petty, D Mackay, JA Lebo. 1993. Lipid containing semipermeable membrane devices for monitoring organic contaminants in water. Environ. Sci. Technology. 27:2489-2496.

- Johnson, PJT, KB Lunde, EG Ritchie, AE Launer. 1999. The effect of trematode infection on amphibian limb development and survivorship. Science 284: 802-804.

- Ouellet, M., J. Bonin, J. Rodrique, J. DesGranges, and L. Lair. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. J. Wildl. Dis. 33:95-104.

- Petty, JD, SB Jones, JN Huckins, WL Cranor,. JT Parris, TB McTague, TP Boyle. 2000. An approach for assessment of water quality using semipermeable membrane devices (SPMDs) and bioindicator tests. Chemosphere 41:311-321.

Scope of study-

Given the extent of amphibian deformities and declines, we plan to determine the toxicity of complex environmental contaminant mixtures obtained from amphibian habitats in several Fish and Wildlife Refuges and National Parks, including Sequoia and Kings Canyon National Parks.

Intended use of results-

The addition of UV in the experimental design will allow the elucidation of this potential environmental hazard to amphibians relative to environmental intensities and will also provide an assessment of the potential for the additivity of stressors that may impinge upon amphibians.

Thus, the outcome of this research will provide guidance in management of Parks/Refuges for amphibian conservation relative to contaminants and SUV. Protocols, guidance documents, reports, and peer-reviewed publications will be prepared during the course of our three-year effort. Nothing of commercial use will be derived from this study.

Objectives:

The objective of our research is to determine whether contaminants are present in amphibian habitats in Sequoia, and if so whether they have the potential to cause amphibian mortality or deformity. Furthermore, because ultraviolet (UV) radiation has been known to increase the toxicity of various compounds, we also set out to investigate whether UV interacts with contaminants.

Methods:

Description of study area-

Possible study areas include: the Tablelands, Crescent Meadows, and Sycamore Creek.

Procedures-

SPMDs will be deployed to sequester environmental mixtures of bioconcentratable contaminants that are present in amphibian habitats. Water chemistry including pH, alkalinity, dissolved organic carbon, and temperature will be taken. SPMDs will be deployed for a minimum of 30 days. SPMD extracts will be prepared for standard 96-hour laboratory toxicity tests with larval amphibians. The extracts will include the complex of bioconcentratable chemicals present at each site. In addition SPMD controls, including extracts from appropriate solvent controls, will be tested. Larvae will be exposed to 4 concentrations of extract including a control treatment. The extract exposures will be conducted under three different UV irradiance intensities including a UV control. For each treatment, 10 organisms will be exposed in each of 3 replicate treatments. When significant toxicity is observed, longer-term exposures at environmental concentrations will be conducted to determine site-specific impacts on amphibian development. When appropriate, SPMDs extracts from particular sites could be archived for detailed chemical analysis at a later date.

Collections-

Water samples and the lipophilic compounds sequestered by the SPMDs will be collected at sample sites. Five frog larva per site, where found, of non-threatened or endangered species will be collected from each site for subsequent analysis of contaminant residues and comparison with results from SPMDs.

Analysis-

Standard statistical tests will be implemented to analyze the data using SAS.

Schedule-

SPMDs will be deployed the second week in September, and retrieved the second week in October, 2000. Chemists at the USGS Columbia Environmental Research Center will extract the compounds from the SPMDs during November and December. In February or March 2001, eggs

from locally breeding frogs (e.g., chorus frogs; Pseudacris triseriata) will be collected and exposures will begin.

Budget-

This project is being funded by the USGS Amphibian Recovery and Monitoring Initiative. Either no cataloging costs or negligible costs.

Products:

Publications and reports-

See "intended use of results" section above.

Collections-

All water and frog specimens collected during this project will be consumed during analysis. Any remaining specimens designated as permanent voucher collections will be fixed in 10% buffered formalin and deposited in a repository acceptable to the National Park Service and documented using NPS standards.

Data and other materials-

n/a

Literature cited:

See 'relevant literature' above.

Qualifications:

Both investigators have had experience deploying and retrieving SPMDs as well as carrying out the proposed research with the extracts. See attached curriculum vitae for more information. No previous collections have been made by, or permits issued to either investigator.

Supporting documentation and special concerns:

Safety-

n/a

Access to study sites-

All sites will be accessible from main roads and will involve little hiking.

Use of other equipment-

Three SPMD canisters will be deployed at each site. They will be strung together on a length of airline cable, which will be anchored near the water's edge.

Chemical use-

SPMDs contain a lipid that mimics biological lipids. Because it is contained within the SPMD, it cannot be released into the environment.

Ground disturbance-

n/a

Animal welfare-

Larva collected will be placed into Chloretone solution, a recognized quick and painless method for humane collecting of amphibian larva.

NPS assistance-

During the trip in September, it may be necessary for a park staff member to guide us to the study sites.

VITA

Edward E. Little
Ecological and Contaminants Research Center
U.S. Geological Survey/Biological Resources Division
Columbia, MO 65201
Telephone: ###/xxx-xxxx

PROFESSIONAL EXPERIENCE:

1970 - 1974 Research Assistant, SUNY/Stony Brook

1972 - 1973 Teaching Fellow, SUNY/Stony Brook

1974 - 1978 Research Associate, Florida State University

1978 - 1996 Leader, Behavioral Toxicology Section, National Fisheries Contaminant Research Center, Columbia, MO
1988 - Research Associate, School of Natural Resources, University of Missouri, Columbia,MO

1993 - Adjunct Professor, Institute of Wildlife and Environmental Toxicology. Clemson University. Pendleton, SC.

1991 - Chairman: Subcommittee E47.12: Behavioral Toxicology. American Society of Testing and Materials.

1994 - NBS Representative to Interagency Task Group for National UVB Monitoring Network

1996 - Chief: Ecology Branch, Ecological and Contaminants Research Center

EDUCATION:

1966-1970 Hiram College, Hiram, OH B.A. Psychology and Biology

1970-1974 SUNY/Stony Brook, NY Ph.D. Biology

SCHOLARSHIPS AND HONORS:

1970- 1974 Teaching scholarship SUNY/StonyBrook

1975- 1978 NIH Training Scholarship Florida State University

1989- 1991, 1993 US Fish and Wildlife Service Special Achievement Awards

1995- National Biological Survey Superior Performance Award

1996-American Fisheries Society MOST SIGNIFICANT PAPER Award

PROFESSIONAL SOCIETIES:

American Society of Limnology and Oceanography

American Society for Photobiology

American Society for Testing and Materials

Missouri River Interstate Cooperative Research Association

Society for Environmental Toxicology and Chemistry

UNIVERSITY SERVICE:

Presented Lectures in:

Global Climate Change Seminar - Department of Atmospheric Sciences

Department of Biological Sciences

Department of Civil Engineering

Department of Fisheries and Wildlife

Department of Entomology

Thesis advisor for three Masters Degree candidates, one Ph.D.

Postdoctoral advisor

Served on Graduate Committees for five Masters Degree candidates and two Ph.D. Candidates

Served on the Executive Committee for the UMC Conservation Biology Program

PUBLICATIONS:

Books Edited:

Environmental Toxicology and Risk Assessment. In Press. Volume 7 Edited by Edward E. Little, Aaron J. DeLonay and Bruce M. Greenberg. ASTM STP 1265. American Society for Testing and Materials, Philadelphia.

Environmental Toxicology and Risk Assessment. Volume 4 Thomas W. LaPoint, Fred T. Price, and Edward E. Little ASTM STP 1262. American Society for Testing and Materials, Philadelphia. 280 pp.

Student- authored publications in review

Zaga, A., E.E. Little, C.F. Rabeni, M.R. Ellersieck. In Preparation. Photoenhanced toxicity of a carbamate insecticide to early lifestage amphibians. Environmental Toxicology and Chemistry.

Hurtubise, R.D., J.E. Havel, and E.E. Little. In press. The Effects of ultraviolet B radiation on Freshwater invertebrates: Experiments with a solar simulator. Limonology and Oceanography.

Student -authored publications in press

Hurtubise, R.D., E.E. Little and J.E. Havel. In press. Methods for assessing the impacts of UV-B Radiation on Aquatic Invertebrates. In: Environmental Toxicology and Risk Assessment: Vol 4, ASTM STP 1265. American Society for Testing and Materials, Philadelphia.

Student-authored publications

Kiffney, P.M., E.E. Little, and W.H. Clements. 1997. Influence of ultraviolet-B radiation on the drift response of stream invertebrates. 37:485-492.

DeLonay, A.J., E. Little, W. Brumbaugh, et al. 1993. The response of California golden trout (Salmo aguabonita) to stress from deposition of atmospheric pollutants. Environmental Toxicology and Chemistry 12:1223-1232.

Publications in preparation:

Little, E.E. In preparation. Toxic Response of the Neurobehavioral System. In W.H. Benson and D. Schlenk, editors, Target Organ Toxicity In Marine and Freshwater Teleosts. Taylor and Francis Publishers, Washington DC

Fabacher, D.F. and E.E. Little. In review. Substance that appears to protect fish from ultraviolet-B radiation occurs primarily in outer layers of the skin Environ Science and Pollution Research.

Etc. Etc. Etc.

RESEARCH INTERESTS: Basic and applied laboratory and field research on the influence of sublethal contaminant exposure on the behavioral function of aquatic organisms. Technological standardization for hazard and risk assessment. Assessment of critical habitat and competition of Missouri River fishes. Determination of the impacts of ultraviolet-b radiation on amphibians and aquatic organisms and identification of biotic and environmental factors affecting UV injury. Sensory basis of animal behavior.