Water Resources Research Institute

Annual Technical Report

FY 1998

Introduction

Research Program

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Assessment of environmental estrogens in wastewater: Potential for developmental and reproductive toxicity in fish
Project Number	C-17 1434-HQ-96-GR-02679
Start Date	08/01/1997
End Date	10/31/1999
Research Category	Biological Sciences
Focus Category #1	Toxic Substances
Focus Category #2	Waste Water
Focus Category #3	Water Quality
Lead Institution	Mississippi State University

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Alice C. Layton	Unknown	University of Tennessee	01
William H. Benson	Unknown	University of Mississippi	01
Daniel K. Schlenk	Unknown	University of Mississippi	01
T. Wayne Schultz	Unknown	University of Tennessee	01
Fred Tilton	Unknown	University of Mississippi	01

Problem and Research Objectives

Environmental estrogens refer to substances in the environment that mimic the effects of the endogenous hormone estrogen. A number of environmental contaminants possess estrogenic activity including chlorinated hydrocarbon pesticides and degradation products of high-use industrial surfactants. Included in this list are potent natural and synthetic estrogens which find their way to the aquatic environment through municipal sewage effluent.

The overall goals of this two-year project were to assess wastewater effluents for the presence of environmental estrogens and evaluate the potential for microbial degradation of steroid hormones in activated sludge. In support of the above, during the first year of investigation, wastewater effluent from several locations were biologically screened for estrogenic activity using a caged fish model. In addition, the extent to which microbial degradation of steroid hormones modulates the fate of these compounds was investigated. During the second year of investigation, chemicals of concern, with respect to estrogenic activity, were identified by use of trace enrichment applications and solid-phase extraction technology.

Methodology

The overall approach of this two-year project was as follows:

Phase Ia - Biological Screening of Wastewater. During the first year of investigation, mature, male channel catfish (Ictalurus punctatus) were used in caged experiments. Blood and other biological samples were used to assess the estrogenic activity of wastewater effluents.

Phase Ib - Evaluation of Microbial Degradation of Steroid Hormones. During the first year of investigation, the microbial transformation of 14C-17-ß-estradiol and other 14C- compounds were assessed in two activated sludge samples.

Phase IIa - Toxicity Identification and Evaluation. The estrogenic activity of wastewater was identified by use of trace enrichment applications and solid-phase extraction technology.

Phase IIb - Environmental Fate of Steroid Hormones. The environmental fate of 14C-17-ß- estradiol was determined in at least three different activated sludges. These included activated sludge from a range of plant sizes which had different waste streams such as urban and suburban municipalities, or waste of primarily industrial origin.

Principal Findings and Significance

In the first phase of this two year project, municipal wastewater was biologically screened for estrogenic activity by examining the induction of vitellogenin in male channel catfish. Vitellogenin, a protein precursor of egg yolk, is induced by estrogenically active compounds and is an indicator of estrogenic activity. Seasonal release of estrogenically active wastewater was observed at the two municipal wastewater treatment plants examined. The seasonal trends were correlated to common wastewater parameters utilized to monitor metabolic activity of microbial populations responsible for the aerobic degradation of municipal wastewater. In the second phase of the project, the compound(s) responsible for the estrogenic activity was (were) identified using solid phase chromatography columns and analysis for estradiol using an enzyme-linked immunosorbent assay. Effluent concentrations for estradiol ranged

from no detection to 14 ng/L. Regarding the environmental fate of steroid hormones, results suggest that municipal wastewater treatment plants utilizing activated sludge processes effectively remove steroid hormones. Results of the present investigation demonstrated that degradation and seasonal release of natural and/or synthetic estrogens by municipal sewage treatment facilities are dependent on common wastewater parameters as well as the efficiency of the respective wastewater treatment facility.

Descriptors

Toxic Substances, Reproductive Effects, Wastewater, Water Quality

Articles in Refereed Scientific Journals

Schultz, T.W., G. D. Sinks, and J.R. Seward. 1999. Estrogenecity of benzophenones evaluated with a recombinant yeast assay: Validation of a rules-based system of prediction. Environ. Toxicol. Chem In press.

Tilton, F., D.D. Schlenk, and W.H. Benson. 1998. Biochemical indices of endocrine effect: An in situ approach to assess the estrogenic activity of municipal wastewater. The Toxicologist. 48 (1-S), A1779.

Book Chapters

Dissertations

Water Resources Research Institute Reports

Benson, W.H., D.K. Schlenk, and F. Tilton. 1998. Assessment of environmental estrogen in wastewater: Potential for developmental and reproductive toxicity in fish (Interim report). Mississippi Water Resources Research Institute, Mississippi State, Mississippi. 16 pp.

Conference Proceedings

Tilton, F., Schlenk, D.K., and W. H. Benson. 1999. Seasonal release of estrogenically active municipal wastewater: An assessment utilizing *in situ* biomarkers. In Proceedings of the Mississippi Water Resources Conference, Mississippi Water Resources Institute, Mississippi State, Mississippi. 32-39.

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Chemical mixtures: Consequences for water quality
Project Number	C-18 1434-HQ-96-GR-02679
Start Date	09/01/1997
End Date	10/31/2000
Research Category	Biological Sciences
Focus Category #1	Sediments

Focus Category #1	Sediments
Focus Category #2	Toxic Substances
Focus Category #3	Water Quality
Lead Institution	Mississippi State University

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Jeffrey A. Steevens	Unknown	University of Mississippi	01
William H. Benson	Unknown	University of Mississippi	01
David S. Block	Unknown	University of Mississippi	01

Problem and Research Objectives

Pesticides and metals have the potential to occur as mixtures in surface waters and sediments, as well as bioaccumulate in aquatic organisms resulting in adverse toxicological effects. Presently, assessments of contaminated sediments are based upon single chemical toxicological studies and the assumption of additive toxicity. Therefore, the use of these studies and assumptions for assessments of contaminated sediments may not represent the "real world" toxicological effects of chemical mixtures. This research utilizes a novel approach to evaluate the toxicological effects of bioaccumulative chemical mixtures.

The overall goal of the three-year project is to evaluate the interactions of bioaccumulative chemical mixtures having the potential for toxicological effects not predicted from single chemical toxicity experiments. During the first year of the investigation (1997-1998), toxicological effects of the individual model chemicals were assessed using *H. azteca*. The second year (1998-1999) has been spent conducting exposures to assess chemical-chemical interactions resulting from combinations of the model chemicals. Finally, during the third year of investigation (1999-2000), experiments will be designed to explore the underlying biochemical mechanisms by which the chemical mixtures interact.

Methodology

The overall approach of this three-year project is as follows:

Phase I - Single Chemical Exposures. *H. azteca* were exposed to single chemicals to determine concentration threshold values at which adverse toxicological effects occur. Four-day toxicity experiments using juvenile organisms were conducted as range-finding studies, with survival as the toxicological endpoint. Additional ten-day exposures were conducted to evaluate the effects of individual chemicals on survival as well as growth. Organisms from ten-day exposures were utilized to develop selected biochemical toxicity indices reflecting the mechanisms of action of the individual toxicants.

Phase II - Multiple Chemical Exposures. Mixture toxicity experiments were conducted to evaluate chemical-chemical interactions of chlorpyrifos, dieldrin and methyl mercury using a five by five matrix concentration design. Each experiment included eight single chemical concentrations and a control group. Replication of 15 organisms, using greater than 500 organisms for each experiment, was necessary to adequately meet the requirements of the statistical model. Juvenile *H. azteca* were exposed

to selected concentrations derived from single-chemical exposures. Adult *H. azteca* were exposed at selected points within the five ray design using static-renewal bioassays for ten days, with survival and growth as toxicological endpoints.

Phase III - Assessment of Bioconcentration and Biochemical Toxicity of Chemical Mixtures. During the third year of investigation, experiments using adult *H. azteca* will be water-only, static-renewal exposures at selected concentrations and two-chemical combinations determined from results of the initial comprehensive mixture toxicity exposures. Additional ten-day experiments will be conducted using spiked formulated sediment at environmentally relevant sediment concentrations. Mechanisms of chemical interactions will be investigated through the use of biochemical indices relevant to the known modes of action of the model compounds.

Principal Findings and Significance

Three chemicals that were dissimilar in structure and toxicological mechanisms were evaluated for possible chemical mixture interactions. Current risk assessment models would have suggested that the model compounds would have interacted independently. However, chlorpyrifos and methyl mercury had different interactions for different endpoints that were measured. The two chemicals interacted additively with survival, antagonistically with in vivo enzyme activity, and chlorpyrifos also enhanced the accumulation of methyl mercury. These effects would not have been predicted from the current models. It is these types of chemical interactions which are difficult to predict and require additional investigation.

Chemical mixtures present a complicated challenge to the field of toxicology. The scientific process has, for the most part, successfully addressed the challenge of elucidating the toxicological effects of single chemicals. However, chemicals rarely occur by themselves. Current assessments of chemical mixtures assume a dose additive contribution towards the overall toxicity. This assumption is clearly an oversimplification of a more complex challenge. The characteristics and mechanisms of toxicological interactions of "real world" chemical mixtures must be conducted to improve our current understanding of chemical mixtures and the risks associated with exposure to chemical mixtures.

Descriptors

Ecosystems, Mixtures, Pesticides, Residues, Sediments, Toxic Substances, Water Quality

Articles in Refereed Scientific Journals

Steevens, J.A., and W.H. Benson. 1998. *Hyalella azteca* 10 day sediment toxicity test: Comparison of growth measurement endpoints. Environ. Toxicol. Water Qual. 13 (3):243-248.

Steevens, J.A. and W. H. Benson. 1999. Toxicological interactions of chlorpyrifos and methyl mercury in the amphipod, *Hyalella azteca*. Toxicol. Sci. Accepted.

Book Chapters

Dissertations

Steevens, J. A. 1999. Chemical mixture interactions: Toxicity of Chlorpyrifos, Dieldrin, and Methyl Mercury to the amphipod *Hyalella azteca*, "Ph.D. Dissertation," Department of Pharmacology, School

of Pharmacy, University of Mississippi, University, MS. 146 pp.

Water Resources Research Institute Reports

Benson, W.H., J. A. Steevens, and J. C. Allgood. 1998. Chemical mixtures: Consequences for water quality (Interim report). Mississippi Water Resources Research Institute, Mississippi State, Mississippi. 25 pp.

Conference Proceedings

Steevens, J. A., and W.H. Benson. 1999. Characterizing the chemical mixture interactions of Chlorpyrifos, Dieldrin, and Methyl Mercury. In Proceedings of the Mississippi Water Resources Conference, Water Resources Research Institute, Mississippi State, Mississippi. 156-169.

Other Publications

Information Transfer Program

The publication and distribution of project reports are a major function of the Institute's information dissemination activities. After peer review, final approval, and printing, the reports are distributed to other institutes which request them and to research libraries in and out of state. The quarterly newsletter, *LORE*, announces research in progress, completed research, Institute activities, calls for abstracts and proposals, and news items of interest to our readers. The annual digest of the Institute, In Review, is sent to the entire Mississippi Legislature as well as to scientists and others who have an interest in the Institute. A web site is maintained at http://www.wrri.msstate.edu. Technical reports and other materials that are housed in the Institute library are used by students and faculty.

Annually, the Institute sponsors a water resources conference. In April 1998, it conducted the Mississippi Water Resources Conference in cooperation with the Mississippi District U.S. Geological Survey, the Mississippi Office of Land and Water Resources, and the Mississippi Water Resources Association. The Keynote Address was delivered by Marcelo P. Merino of the North American Coal Company in Dallas, Texas. Dan Ferry with the Tennessee Valley Authority in Knoxville, Tennessee, was the luncheon speaker. Information was provided on Coastal Issues, the Ecology, Surface Water, and Toxins. There were several sessions dedicated to the sharing of information on the National Water Quality Assessment Program. The Department of Health and the Office of Pollution Control approved the conference for continuing education credits for water treatment personnel. A *Proceedings* of the conference has been published.

LIST OF PRINCIPAL INFORMATION TRANSFER PUBLICATIONS

Daniel, B. Jean, Editor. *Proceedings of the 29th Mississippi Water Resources Conference.* Mississippi Water Resources Research Institute, Mississippi State University, Mississippi State, Mississippi. 203 pp.

LORE - quarterly newsletter

1998 in Review - annual program digest

USGS Internship Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	0	0	0	0	0
Masters	1	2	0	0	3
Ph.D.	1	2	1	0	4
Post-Doc.	0	N/A	0	0	0
Total	2	4	1	0	7

Awards & Achievements

Publications from Prior Projects

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications