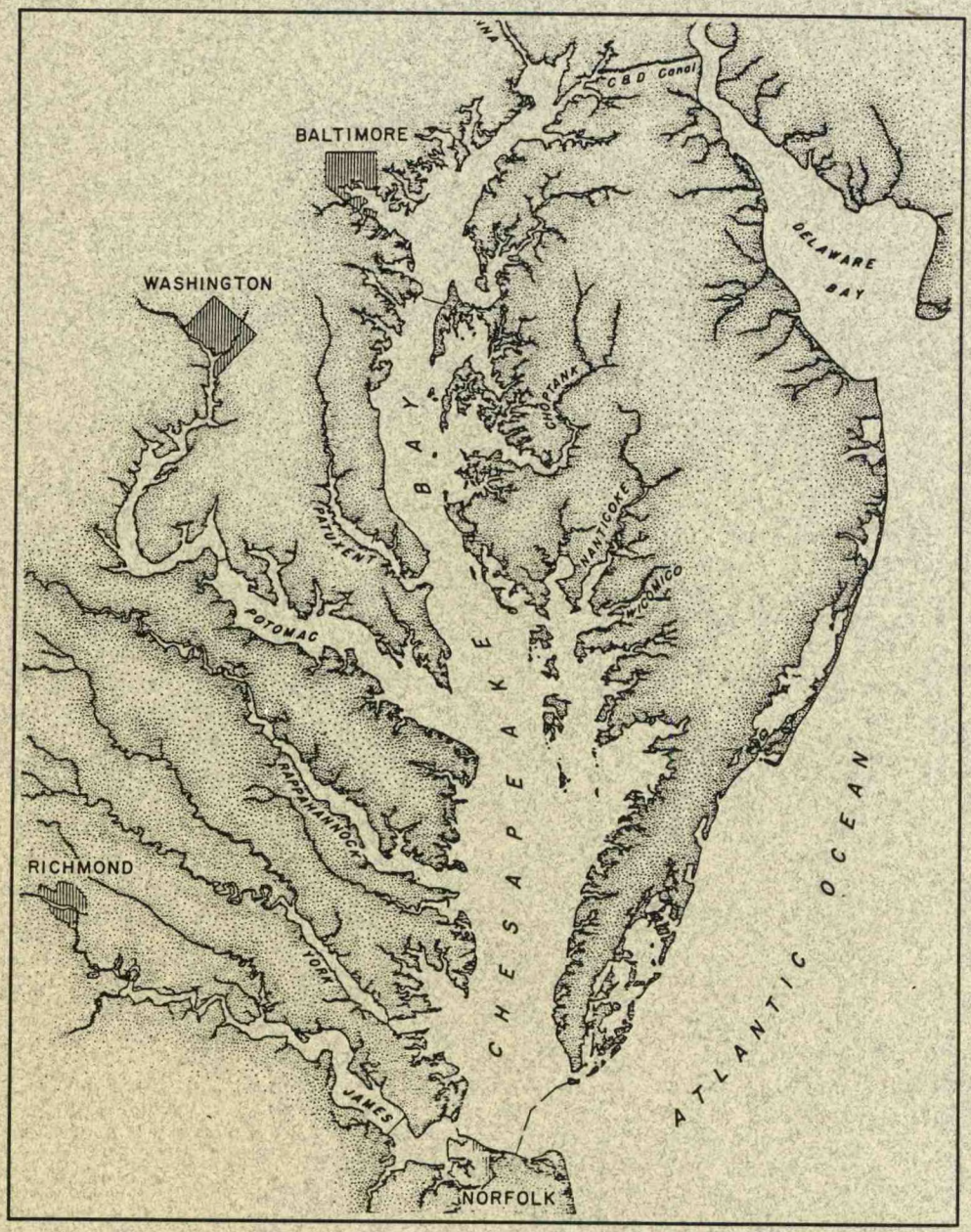
Maryland and Virginia Sea Grant College Programs

Environmental Effects Research on Chesapeake Bay

Toxics Research Program



Maryland Sea Grant

Virginia Sea Grant College Program

Submitted to the
National Sea Grant College Program
National Oceanic and Atmospheric Administration

June 1994

U.S. Department of Commerce
National Oceanic and
Atmospheric Administration

ASSURANCE OF COMPLIANCE WITH THE NONDISCRIMINATION CLAUSE
Applicable to :

SEA GRANT COLLEGE PROGRAM

UNIVERSITY OF MARYLAND
COLLEGE PARK, MARYLAND

(1)hereby warrants, covenants, agrees and assures that it will conduct the program/project described by the above-identified application/proposal, or as it may be revised or modified prior to any grant award or subsequent to any grant award, in compliance with all requirements of the "recipient" imposed by or pursuant to the non-discrimination clause appended hereto, which clause shall also be incorporated into any grant awarded on the basis of such proposal, and

(2)agrees and acknowledges that this assurance of compliance is a prerequisite condition to approval of the proposal or any grant or grant modification or amendment extending and Federal financial assistance which may be extended to it by the U.S. Department of Commerce will be in reliance on the representation made by this assurance and that the United States shall have the right to seek judicial enforcement thereof, and that this assurance of compliance shall be binding upon it, its successors, assignees, and transferees.

6/16/94
Date

Signature of official authorized to sign.
Robert Hando, Vice President
Administration and Financial Affairs

University of Maryland Biotechnology
Institute

July 1, 1994

Proposal to: The National Sea Grant College Program
NOAA, U. S. Department of Commerce

Submitted by: VIRGINIA GRADUATE MARINE SCIENCE CONSORTIUM
Virginia Sea Grant College Program
MADISON HOUSE - 170 Rugby Road
University of Virginia
Charlottesville, VA 22903

Title: Chesapeake Bay Toxics Research Program:
Virginia Portion for 1995

Amount requested from Sea Grant:	$ 467,672
Matching funds proposed:	$ -000-
Duration of proposed activity:	twelve months
Proposed starting date:	1 January 1995

We, the undersigned, certify that, in the event this proposal is accepted, in whole or in part, our signatures on this proposal constitute acceptance of and compliance with statutes and regulations of the U. S. Government and the U. S. Department of Commerce as detailed in Part Three, "The National Sea Grant Program, Background and Suggestions for Proposals", dated March 1, 1972, and that pages 63 to 107 of that publication are incorporated by references as part of this proposal.

Principal Investigator

William L. Rickards
Signature

William L. Rickards, Director
Virginia Sea Grant College Program

221-28-3691

Virginia Graduate Marine Science
Consortium
MADISON HOUSE - 170 Rubgy Road
University of Virginia
Charlottesville, Virginia 22903

Institutional Representative

Signature

D. Wayne Jennings
Director of Sponsored Programs

223-42-1185

Office of Sponsored Programs
P. O. Box 9003
Carruthers Hall
University of Virginia
Charlottesville, Virginia 22903

U. S. Department of Commerce
National Oceanic & Atmospheric Administration

ASSURANCE OF COMPLIANCE WITH THE NONDISCRIMINATION CLAUSE
Applicable to:

Title: Chesapeake Bay Toxics Research Program:
Virginia Portion for 1995

VIRGINIA GRADUATE MARINE SCIENCE CONSORTIUM
Virginia Sea Grant College Program
MADISON HOUSE - 170 Rugby Road
University of Virginia,
Charlottesville, VA 22903

(1) hereby warrants, convents, agrees, and assures that it will conduct the program/project described by the above identified application/proposal, as it may be revised or modified prior to any grant award, in compliance with all requirements of the recipient imposed by or pursuant to the Nondiscrimination clause appended hereto, which clause shall also be incorporated into any grant awarded on the basis of such proposal, and

(2) agrees and acknowledges that this assurance of compliance is a prerequisite condition to approval of the proposed or any grant or grant modification or amendment extending any Federal financial assistance which may be in reliance on the representative made by this assurance and that the United States shall have the right to seek Judicial enforcement thereof, and that this assurance of compliance shall be binding upon it, its successors assignees, and transferees.

6/15/94
Date

Signature

D. Wayne Jennings
Director of Sponsored Programs
Office of Sponsored Programs
P. O. Box 9003
Carruthers Hall
University of Virginia
Charlottesville, Virginia 22903

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Program Information

Project Proposals

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1995 GRANT/PROJ. NO:
University of Maryland, Maryland Sea Grant College
PRINCIPAL INVESTIGATORS: Gail B. Mackiernan, Acting Director

DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	3.40	5.20	34,013	0
b. Associates	2.30	0.00	12,939	0
Sub Total			46,952	0
2. OTHER PERSONNEL				
a. Professionals	0.00	0.00	0	0
b. Research Associates	1.00	6.00	12,642	0
c. Res. Asst. Grad. Std	23.00	0.00	26,700	0
d. Prof. School Student	0.00	0.00	0	0
e. Pre-Bac Students	0.00	0.00	4,000	0
f. Secretarial-Clerical	1.20	0.00	3,200	0
g. Technical-Shop	0.00	0.00	0	0
Total Salaries/ Wages			93,494	0
B. FRINGE BENEFITS			27,421	0
Total Salaries,Wages and Fringe Benefits			120,915	0
C. PERMANENT EQUIPMENT			5,000	0
D. EXPENDABLE SUPPLIES AND EQUIPMENT			22,500	0
E. TRAVEL				
1. Domestic	5,200	0		
2. International	0	0		
Total Travel			5,200	0
F. PUBLICATIONS AND DOCUMENTATION COSTS			2,550	0
G. OTHER COSTS				
1. Computer costs			500	0
2. Copying,Library and Communication			1,692	0
3. Analytical and Shop Services			12,000	0
4. Fuel,Boat Time and Vehicle Usage			10,100	0
5. Word Processing			0	0
6. Institutional Allowance			0	0
7. Tuition			2,500	0
8. Waste Disposal			500	0
9. Service Contracts			4,845	0
Total Other Costs			32,137	0
TOTAL DIRECT COSTS			188,302	0
INDIRECT COSTS				
(On Campus) : % of			74,336	0
(Off Campus) : % of			0	0
Total Indirect Costs			74,336	0
TOTAL COSTS			262,638	0

NOAA FORM 90-4 (ADAPTED) NOAA, U.S. DEPT OF COMMERCE

SEA GRANT BUDGET

GRANTEE:
Virginia Graduate Marine Science Consortium

GRANT/PRO. NO:
CBT-TRP

PRINCIPAL INVESTIGATORS:
W.L. Rickards, VGMSC

DURATION:
1/1-12/31/95

A. Salaries and Wages 1. Senior Personnel	No.	Man-Mo.	Sea Grant Funds	Grantee Funds
a. Prin. Investigator	5	5.70	29826	
b. Associates:			0	0
Sub Total:			29826	0
2. Other Personnel				
a. Professionals	4	20.0	59375	
b. Research Assoc.				
c. RA Grad. Stud.	3		43610	
d. Prof. School Stud.				
e. Pre-Bac. Stud.				
f. Secret./Clerical				
g. Technical/Shop	3		24518	
h. Hourly Labor	3		11792	0
Total Salaries and Wages			169121	0
B. Fringe Benefits			27405	0
Total Sal. Wages & Fringe Benefits			196526	0
C. Permanent Equipment			0	0
D. Expendable Supplies			29250	0
E. Travel				
1. Domestic - US & Possessions		1.	9425	
2. International		2.	0	0
Total Travel			9425	0
F. Pub. and Documentation Costs			4317	0
G. Other Costs				
1. vessel rental			4500	
2. tuition			3300	
3. gas cylinder rental			365	
4. photocopying, phone, postage			900	
5. graphic arts			1251	
6. subcontract to UMDNJ			6400	
7. contractual services			2400	
8. R/CBT-26 and R/CBT-27 renewals			150000	
9.				
10.				
Total Other Costs			169116	0
TOTAL DIRECT COSTS (A through G)			408634	0
Indirect Costs: On Campus:			48868	
Off Campus:			10170	
Total Indirect Costs			59038	0
TOTAL COSTS			467672	0

MARYLAND SEA GRANT COLLEGE

Activity Budget Sheet for 1995

	NOAA FUNDS	MATCHING FUNDS
Marine Resources Development Biological Oceanography	$312,328	$0

VIRGINIA SEA GRANT COLLEGE PROGRAM

CHESAPEAKE BAY ENVIRONMENTAL EFFECTS STUDIES

TOXICS RESEARCH PROGRAM - 1995

ACTIVITY BUDGET

	NOAA FUNDS	MATCHING FUNDS
MARINE RESOURCES DEVELOPMENT		
Pollution - Toxics	$ 285,455	$ 000
PROGRAM MANAGEMENT AND DEVELOP.		
Program Development	$ 182,217	$ 000
TOTAL	$ 467,672	$ 000

INTRODUCTION

CHESAPEAKE BAY ENVIRONMENTAL EFFECTS STUDIES: TOXICS RESEARCH PROGRAM

Joint Maryland and Virginia Sea Grant College Programs

Gail B. Mackiernan, Interim Director
Maryland Sea Grant College Program

William Richards, Director
Virginia Sea Grant College Program

BACKGROUND

In order to restore the productivity and ecological health of the Chesapeake Bay, the federal/state Chesapeake Bay restoration program has set as a goal the reduction of nutrients and of toxic substances into the estuary, Implementation of effective management strategies is basic to improvement of habitat for ecologically and economically important species, and to the many benefits associated with a properly functioning ecosystem. Such actions require a sound scientific basis, however, and many questions need to be addressed as to the impact of anthropogenic materials in the estuary.

To fill this information need, the National Oceanic and Atmospheric Administration (NOAA), through the Maryland and Virginia Sea Grant College Programs, began a major effort to address the issue of environmental effects due to low dissolved oxygen in the Bay. This study, initiated in September 1985, has shed light on both the causes and consequences of hypoxia, and results of this research are now being used by state and federal management and regulatory agencies. Funding levels from NOAA averaged about $400,000 annually, transferred within NOAA's Estuarine Programs Office to be administered by the National Sea Grant Office (NSGO). In 1987, the Chesapeake Bay Environmental Effects Committee (CBEEC) was established by NOAA to oversee this research effort and to provide direction for future programs addressing other critical environmental problems. The committee includes representation from the Maryland, Pennsylvania and Virginia scientific and management communities.

NOAA was reorganized in 1989 and the EPO disbanded, with many of its functions being folded into the new Coastal Oceans Program (COP). Currently, funding from NOAA is transferred directly from National Marine Fisheries Service to NSGO, and thence to the state Sea Grant Programs. In 1990, the Environmental Protection Agency's (EPA) Chesapeake Bay Liaison Office, with approval from the Chesapeake Bay Toxics Subcommittee, joined the Environmental Effects research program by augmenting the fiscal support with the stipulation that the Program be

redirected from hypoxia to studies of toxic contaminants in Chesapeake Bay, an area where considerable information is needed to support planned management actions.

CBEEC recognized the need to address the issue of toxics and accepted the task of developing a cooperative long-range, multidisciplinary, multi-institutional research effort patterned after the Hypoxia Research Program. In so agreeing, the Committee noted that there were several continuing hypoxia projects needing a final year's support. CBEEC further recommended that the initial focus of toxics research be on the ecological effects of contaminants, which complemented previous studies of hypoxia. This focus reflected an awareness that the total funding level in 1990, $783,000, was inadequate to support the type of broad-range toxics research program envisioned by the Bay Program's Research Planning Council (RPC 1989). CBEEC, RPC, and others have recommended that increased resources be directed to the toxics issue and that the research areas be broadened as the funding permits.

The present proposal covers the fourth year in which emphasis is placed upon the ecological effects of toxics in the Bay. Several of the projects (R/CBT-22, 23, 24 and 25) are presented in very brief form since they are renewals of projects previously approved for subsequent funding as long as initial progress was judged acceptable by CBEEC. The balance of the projects (R/CBT- 26, 27, 28 and 29) in this proposal are new to the Program.

HYPOXIA RESEARCH PROGRAM

The hypoxia research program began in 1985, and was funded annually at the level of approximately $400,000. The program, which was conceived as an ecologically oriented study focussing on system-level effects has greatly refined the understanding of the complex relationships between nutrients, production of organic material, carbon cycling and the development of hypoxia. In addition, the impact of low dissolved oxygen on the functioning and productivity of key species and communities was found to be significant.

The results of this research program have become widely recognized in the scientific community, and are receiving attention from managers because of their applicability to similar problems throughout the world. Special sessions devoted to this research were convened at the January and December 1988 American Geophysical Union meetings and at the October 1989 Estuarine Research Federation meeting in Baltimore. In addition, a contributed sessions focussed on the effects of hypoxia was included in the December 1990 Chesapeake Research Consortium conference on Bay research.

A recently published book, *Oxygen Dynamics in Chesapeake Bay,* summarizes the current level of understanding of the interaction of physical, chemical and biological processes that create hypoxic conditions, was edited by the Virginia and

Maryland Sea Grant programs. A workshop convened in late 1991 to achieve agreement among the researchers involved with the hypoxia program resulted in the document Dissolved Oxygen in the Chesapeake Bay: A Scientific Consensus. This report highlights the history of hypoxia in the Bay, the primary causes and what would be the predicted response of the system to nutrient reductions.

TOXICS RESEARCH PROGRAM

As indicated above, the hypoxia program had an ecological focus on system-level environmental effects. In conceiving the toxics research program, CBEEC felt that it was important to keep, as much as possible, the ecological focus of the predecessor program on hypoxia and to begin a smooth transition to the study of toxics. The idea of developing a well-integrated program with continuity to previous research was particularly attractive to CBEEC, given the limited funding available, and given the inherently great expense of conducting research in a subject area as broad as toxics. However, in deciding on the ecological focus of the initial phases of the toxics research program, CBEEC clearly recognized that as the program builds and out-year funding increases, proportionally more resources will have to be invested in projects dealing with organismal, cellular, molecular and physiological processes, and in the promising and burgeoning area of biomarkers.

The following sections review the goals of the early phases of the toxics research program, describe the processes leading up to the selection of the successful proposals, and provide a brief precis of those proposals.

Initial GOALS of the CBEEC Toxics Research Program:

- To understand how Chesapeake Bay ecosystem processes influence the transport, fate and effects of toxicants; and

- To understand the effects that representative toxicants have upon ecological processes, including trophic dynamics, in the Bay.

These goals are to be accomplished through the establishment of a multi-year, interdisciplinary and inter-institutional research program, supported initially by funding from NOAA and EPA, and administered jointly by the Maryland and Virginia Sea Grant College Programs.

Priorities

Long-term objectives for the toxics research program were based on the Chesapeake Bay Program Research Planning Committee's "Toxic Research Prioritizations" document, and focus on increasing the understanding of the source,

transport, fate and effects of toxicants in support of the development of ecological risk assessments for the Chesapeake Bay. The Chesapeake Bay Environmental Effects Committee prepared an RFP based on these needs, emphasizing an ecosystem approach to the issue of toxics in the Chesapeake Bay. Copies of the RFP are available, upon request, from the Virginia Sea Grant College Program.

Principal research needs were identified and noted in the Request for Proposals:

1. Understanding of the factors controlling the distribution of toxics in various parts of the Bay ecosystem, including a quantitative assessment of the dynamics of these processes which can later serve as input to models.

2. Determining the existence of potential biological effects of anthropogenic substances, especially those affecting ecosystem processes such as trophic relationships, reproductive success, and productivity. This information can later be used to evaluate the effects upon populations and biotic communities, a major need for ecological risk assessment.

Proposal Review and Selection Process

The sequence of events for the proposal selection and review follows:

•The Request for Proposals was drafted by CBEEC, reviewed by the Toxics Subcommittee, revised and distributed throughout the Bay Region.

•24 project preproposals plus 4 renewals were received by CBEEC;

•All CBEEC members (and Toxics Subcommittee liaisons) received all preproposals for screening and selection of those to be developed into full proposals (14 of 24);

•Full proposals were peer-reviewed through the independent National Sea Grant review process: for each proposal, in addition, an ad hoc review panel convened by National Sea Grant critiqued each new project proposal; CBEEC representatives observed this panel discussion;

•CBEEC met with NOSG to discuss the reviews for each proposal, the merits of each relative to the objectives of the Toxics Research Program, and to make final funding recommendations based on programmatic criteria and resources.

Final selections were made using two basic criteria: excellence of the science as determined by the review process and relevance to programmatic goals. The committee recognized that resources, as well as the pool of proposals, would not allow complete coverage of all relevant issues, but the final list of projects

constitutes an excellent fourth-year program that builds on earlier efforts. In future years, continued focusing of the RFP and additional funding resources will assist in filling gaps in needed research. From an ecosystem perspective, CBEEC feels that the program will produce information on a number of important processes affecting the behavior of toxicants in the Bay. A conceptual diagram of these processes (Fig. 1) compared to the selected proposals indicates good coverage of major pathways. The projects will also address many of the specific recommendations in the most recent RPW 1990 Research Priorities document (May, 1990).

Projects

Projects to be funded in 1995:

•Anderson, R.S. E.M. Burreson and M. Unger. "The effects of environmental contaminants on the progression of Perkinsus marinus infection in the Eastern oyster." (Continuing). This project will attempt to elucidate the role which toxic substances may play in the cell-mediated changes that occur in oysters which become infected with the disease Perkinsus marinus. Immune functions in oysters exposed to toxic substances appear to be mediated so that the disease organisms more readily infect the host. The results will be of use in oyster management, culture and restoration programs.

•Kramer, J.G. "Metallothionein in marine coccoid cyanobacteria: cloning, transcriptional analysis and application to the assessment of metal stress in natural communities of pico-plankton in Chesapeake Bay." (Continuing). This research will employ cloned genes as probes to determine the relative effects of metal exposure upon the expression of metallothioneins in cyanobacteria from the lower Bay and its tributaries. The results will assist managers in assessing the impact of heavy metals upon the microbial community in the Bay.

•Wright, D.A. and R. Dawson. "A risk assessment for Dimilin use in the northern Chesapeake Bay: a model study for nonpoint-source runoff." (Continuing). This project will characterize the role of Dimilin in the Bay, and it will form the basis of a hazard assessment for this compound's mode of action. Chemical application, run-off and toxicity data will be combined to generate a model risk assessment for non-point source pesticide run-off.

•Dickhut, R.M. and L.C. Schaffner. "Organic contaminant metabolite production, elimination, and bioavailability in benthic macrofauna of lower Chesapeake Bay." (Continuing). This research will further examine the role of benthic organisms in the flux of toxic substances from the sediments and the transformations of such substances which occur as a result of ingestion, digestion and excretion by the organisms. The results will find application in modeling the mass balance of toxic contaminants in the Bay while providing insight into the role

of benthic organisms in the bioavailability of such substances via trophic transfer to demersal consumers.

•Madden, C.J., J.R. Kucklick, and J.E.Baker. "Quantitative evaluation of contaminants in a Chesapeake Bay region of concern: A simulation model of exposure and bioaccumulation in Baltimore Harbor." (New) This project will synthesize existing contaminants data and use simulation modeling techniques to explore the processes that control exposure, transfer and accumulation of contaminants in the water column biota and eventual delivery to the sediments. The eventual goal is understand and quantify the impacts of toxic organic compounds on estuarine systems.

•Mason, R.P. and D.A. Wright. "Factors controlling the food chain accumulation and transfer of inorganic and methylmercury in Chesapeake Bay organisms." (New) This project will investigate the bioconcentration of mercury and methylmercury in both benthic and pelagic food chains, and the factors which control the uptake and assimilation of these contaminants by organisms. The study will seek to determine the transfer of mercury between lower trophic levels to fish, and will provide guidance for managers in developing sediment criteria for mercury.

•Schaffner, L.C. and R.M. Dickhut. "Role of benthic macrofauna in trophic transfer of organic contaminants (PAHs, PCBs) to demersal predators." (New) This research will investigate the distribution of contaminants and their metabolites in prey species and the uptake, accumulation and metabolism of these compounds by demersal predators. Earlier research has documented rapid uptake and bioaccumulation of organic pollutants by a variety of Bay macrobenthos; body burdens varied depending on biological and metabolic characteristics of the different species. This project will seek to make the link between prey and representative predators, fish and crabs, and will facilitate risk assessment for contaminants in estuarine and coastal environments.

•Weis, J.S. and P. Weis. "Impacts of CCA pressure-treated wood structures in Chesapeake Bay." (New) Studies will be conducted to evaluate the impact of chromium, copper and arsenic leaching from pressure-treated bulkheads on the Bay's food web. The effect of new versus older structures will be compared, as well as differences due to the amount of tidal flushing at study sites. The PIs will assess direct impacts on organisms, as well as transfer up the food web, bioavailability and spatial distribution of the contaminants.

Full copies of each proposal are elsewhere in this proposal package.

PROGRAM MANAGEMENT

Funds are requested by discrete projects in order to clarify program activities and to facilitate program review and evaluation. The administration of funds will be through the Sea Grant Program of the individual investigator's institution. A series of meetings devoted specifically to data exchange and analysis will be held at appropriate times during the study. Data management, exchange and analysis will be assisted by the Maryland Sea Grant Program through the Sea Grant Computer Facility. General assessment of these efforts is provided by the Environmental Effects Committee, an advisory group which offers guidance to NOAA Chesapeake Bay research efforts. Grants management and programmatic oversight are provided by the Office of Sea Grant, NOAA.

REFERENCE

Chesapeake Bay Program Research Planning Committee 1989. Toxics Research Prioritizing. EPA Chesapeake Bay Liaison Office, Annapolis, MD.

*** MARYLAND SEA GRANT PROJECT SUMMARY 1995 ***

Title: The effects of environmental contaminants on the progression of *Perkinsus marinus* infection in the eastern oyster

Project Number: R/CBT-22
Grant Number:
Status: continuation
Revision Date: June 15, 1994
Initiation Date: January 1, 1994
Completion Date: December 31, 1996

Principal Investigator: Robert S. Anderson
Affiliation: Chesapeake Biological Laboratory, UMCEES
Months Committed: 2.2

Principal Investigator: Eugene M. Burreson & Michael A. Unger
Affiliation: Virginia Institute of Marine Science
Months Committed: 2.5

Proposed Federal Funds: $97,266
Current Federal Funds: $72,113
Federal Funds to Date: $72,113
Proposed Matching Funds: $
Current Matching Funds: $
Match to Date: $
Related Projects:
Sea Grant Classification#: 45
Keywords: oyster, *Perkinsus marinus*, disease progression, pollutant effects

Objectives: The main objective is to test the hypothesis that pollutant stress may predispose aquatic organisms to infectious diseases or accelerate the progression of the disease. The host-parasite system proposed is *"Crassostrea virginica - Perkinsus marinus;* the environmental toxicants under study will include a lower MW polycyclic aromatic hydrocarbon (naphthalene) and tributyltin. An attempt will be made to associate pollutant-induced changes in disease progression to changes in cell-mediated immune function.

Methodology: Progression of the disease in individual oysters will be followed during chronic exposure to the toxicants. Exposure will be via water and/or food at environmentally relevant concentrations. Disease diagnosis will be carried out on small hemolymph samples repeatedly taken from individual oysters. Immune status will be measured by quantifying hemocyte phagocytic capacity and chemiluminenscence, an indication of cellular antimicrobial activity. Contaminant analysis will be carried out to determine exposure levels and bioaccumulation of the toxicants in question.

Rationale: Environmental chemicals are immunosuppressive and potentiate reduced resistance to infectious diseases in mammals; this relationship needs further evaluation with regard to pollutant impact on economically important aquatic species and the incidence of their diseases. Analysis of oyster cellular immunity, as influenced by the exposure protocols, will provide information on physiological mechanisms related to altered disease susceptibility. Knowledge gained from this project should find practical application in shellfishery management by contributing to understanding the effects of environmental pollutants on the severity and progression of a major disease of oysters.

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1995 GRANT/PROJ. NO: RCBT-22
University of Maryland, Cheasapeake Biological Laboratory
PRINCIPAL INVESTIGATORS: R. S. Anderson

DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	1.00	1.20	8,225	
b. Associates				
Sub Total			8,225	0
2. OTHER PERSONNEL				
a. Professionals				
b. Research Associates	1.00	6.00	12,642	
c. Res. Asst. Grad. Std.				
d. Prof. School Students				
e. Pre-Bac Students				
f. Secretarial-Clerical				
g. Technical-Shop				
Total Salaries/ Wages			20,867	0
B. FRINGE BENEFITS			5,608	
Total Salaries,Wages and Fringe Benefits			26,475	0
C. PERMANENT EQUIPMENT				
D. EXPENDABLE SUPPLIES AND EQUIPMENT			5,500	
E. TRAVEL				
1. Domestic	1,000			
2. International				
Total Travel			1,000	0
F. PUBLICATIONS AND DOCUMENTATION COSTS			500	
G. OTHER COSTS				
1. Computer costs				
2. Copying,Library and Communication			400	
3. Analytical and Shop Services				
4. Fuel,Boat Time and Vehicle Usage				
5. Word Processing				
6. Institutional Allowance				
7. Rapid Response and Program Development				
8. Shipping Charges				
9. Service Contracts			3,102	
Total Other Costs			3,502	0
TOTAL DIRECT COSTS			36,977	0
INDIRECT COSTS				
(On Campus) : 39 % of 36,977			14,421	
(Off Campus) : % of Total Indirect Costs			14,421	0
TOTAL COSTS			51,398	0

**** SEA GRANT BUDGET ****

GRANTEE:
Virginia Graduate Marine Science Consortium

PROJECT NO:
R/CBT-22b

PROJECT TITLE:
Effects of environmental contaminants on the progression of Perkinsus marinus in the eastern oyster

PROJECT STATUS:
2

PRINCIPAL INVESTIGATORS:
Burreson, E.M. & M.A. Unger

DURATION:
1/1/95 - 12/31/95

A. SALARIES AND WAGES	No. of Person.	Months	Sea Grant Funds	Grantee Funds
1. Senior Personnel				
a. Prin. Investigator	2	2.5	11,426	0
b. Associates:	0	0.00	0	0
Sub Total:			11,426	0
2. Other Personnel				
a. Professionals	2	4.0	9,375	0
b. Research Assoc.			0	0
c. RA Grad. Stud.			0	0
d. Prof. School Stud.			0	0
e. Pre-Bac. Stud.			0	0
f. Secret./Clerical			0	0
g. Technical/Shop			0	0
h. Hourly Labor			2,268	0
Total Salaries and Wages			23,069	0
B. FRINGE BENEFITS			5,790	0
Total Sal. Wages & Fringe Benefits (A+B)			28,859	0
C. PERMANENT EQUIPMENT			0	0
D. EXPENDABLE SUPPLIES			3,000	0
E. TRAVEL				
1. Domestic - US & Possessions	1.		2,000	0
2. International	2.		0	0
Total Travel			2,000	0
F. PUBLICATION AND DOCUMENTATION COSTS			0	0
G. OTHER COSTS				
1.			0	0
2.			0	0
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
Total Other Costs			0	0
TOTAL DIRECT COSTS (A through G)			33,859	0
INDIRECT COSTS: On Campus: 35.47% of MTDC			12,009	0
Off Campus:			0	
TOTAL INDIRECT COSTS			12,009	0
TOTAL COSTS			45,868	0

EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON THE PROGRESSION OF *PERKINSUS MARINUS* INFECTION IN THE EASTERN OYSTER

Robert S. Anderson
Professor
Chesapeake Biological Laboratory
University of Maryland System (CEES)
Solomons, MD 20688

Eugene M. Burreson
Professor
Virginia Institute of Marine Science
The College of William and Mary
Gloucester Point, VA 23062

Michael A. Unger
Assistant Professor
Virginia Institute of Marine Science
The College of William and Mary
Gloucester Point, VA 23062

INTRODUCTION

The possible relationships between contaminant exposure, immunosuppression, and altered susceptibility to infectious disease need to be better defined, especially in the case of aquatic invertebrates. In this proposal, we request funds for the second year of this grant to study the effects of xenobiotic exposure on the immunological status and the progression of *Perkinsus marinus* infection in the oyster, *Crassostrea virginica*. *Perkinsus marinus* infections pass through several stages of increasing intensity until the hemolymph contains thousands of parasites per ml. These organisms exist both free in the serum and within the hemocytes, eventually exhausting any cellular or humoral defense mechanisms available to the oyster. One component of this study will quantify processes central to the cells' ability to kill microbes and establish resistance to infectious disease: phagocytic capacity and the production of cytotoxic reactive oxygen intermediates (ROIs). Luminol-augmented chemiluminescence (CL) will be used to quantify ROI production by hemocytes; this method has been successfully used to measure killing capacity of leukocytes (Horan et al, 1982) and to screen for immunomodulatory chemicals (Tam and Hinsdill, 1990) in macrophages. Phagocytic activity of the hemocytes will be quantified by measuring the uptake of fluorescently-labeled particles (Hed, 1986). These observations on cellular

OBJECTIVES

Overall Objectives

The major objectives are to determine (1) the dynamics of TBT bioaccumulation in oysters as a function of route of administration, (2) if chronic exposure of *Crassostrea virginica* to model aquatic pollutants will increase susceptibility to (and/or alter the progression of) *Perkinsus marinus* infection and, (3) if these physiological changes can be correlated to changes in hemocyte immune function.

1995 Objectives

(1) To conclude TBT studies started in 1994.
(2) To evaluate the effects of naphthalene on *P. marinus* progression and oyster immune parameters.
(3) To attempt to establish the dose-dependency of the TBT and PAH responses outlined above.

PROJECT CHANGES

The most substantial change is the addition of Dr. Unger to the project. Although the review of the initial proposal was favorable, it was suggested that the study would be significantly strengthened by the addition of an analytical chemist. Therefore, this add-on initiative was developed in response to the proposal review comments, and it was subsequently approved by CBEEC. As a result we will not only have precise knowledge of xenobiotic levels in the water and oyster tissues, but also will be able to predetermine the optimal dosing procedures required, particularly with regard to the merits of feeding contaminated food vs. exposure via the water.

Studies of immunotoxicological effects of TBT and naphthalene on oyster hemocytes following in vitro exposure will be added by Dr. Anderson. The same immune parameters to be measured in hemocytes withdrawn from in vivo exposed oysters will be quantified in cells exposed in vitro. These assays will supplement and complement the in vivo findings; their perceived merits are briefly described below, under "anticipated results."

Analytical Methods (TBT exposures). All water samples will be analyzed for TBT and its degradation products, dibutyltin (DBT) and monobutyltin (MBT), by the gas chromatographic technique developed by Unger et al (1986) for the analysis of TBT in environmental water samples. This technique required extraction of the butyltins from the sample matrix and derivatization with hexylmagnesium bromide to form stable tetraalkyltin compounds. After fractionation by open column chromatography, the derivatized butyltins are easily separated and quantified by a capillary gas

immune parameters will be carried out to complement and provide mechanistic insight regarding the studies of disease progression. Disease progression itself will be followed by quantifying the numbers of *P. marinus* in the hemolymph of experimentally-infected oysters held in the presence or absence of model environmental pollutants. The concentration of the pollutants will be measured to provide quantitative data on toxicant exposure and bioaccumulation throughout the course of the experiment.

The CL response of *C. virginica* hemocytes was first shown to be affected by various environmental toxicants including metals, pesticides, and other organics by Larson et al (1989) and Fisher et al (1990). Chemically-induced inhibition of luminol-augmented CL responses of oyster hemocytes has also been produced in this laboratory by exposure to particulate brass, cadmium, copper, and pentachlorophenol (Anderson et al, 1992a & b; Roszell and Anderson, 1992; Anderson et al, 1993). Such evidence implicates these xenobiotics as potential immunotoxicants, by extension of the criteria already developed for mammals. It should be noted that the organometallic compound (TBT) selected for this current proposed study is orders of magnitude more immunotoxic (at least to mammals) than any of the metals studied previously. Studies by Chu and Hale (1992) indicate exposure of oysters to aqueous extracts of PAH-rich Elizabeth River sediments increased their levels of *Perkinsus marinus* infection. Their immunological findings included enhanced hemocyte CL and chemotaxis following in vitro exposure of the cells to the extracts, but CL was either reduced (2-3 wks) or unchanged (5 wks) in cells withdrawn from exposed oysters. Therefore, the available preliminary results suggest that modulation of hemocyte immune functions can be produced by exposure to xenobiotics, and that this may be expressed as reduced resistance to disease. However, more direct evidence is required to demonstrate the phenomenon convincingly and to show that it has significance with regard to intensity of infection.

The effects of important Chesapeake Bay toxicants on the progression of a major parasitic disease of oysters will be quantified. The proposed study is an initial step in determining these effects on oyster populations in the field; it concentrates on laboratory-maintained populations exposed to individual priority pollutants. The contaminants in question are representative of pollutants found in the Bay. Specifically, they are tributyltin and a low molecular weight aromatic (naphthalene) commonly found in aqueous extracts of PAH-contaminated sediments. Naphthalene and tributyltin (TBT) are recognized as toxicants of immediate or potential threat to the Chesapeake Bay system (EPA, 1991a; 1991b); these compounds have also been implicated as immunotoxicants of oysters (Larson et al, 1989; Fisher et al, 1990).

Our approach is to examine the effects that these pollutants might have on immune competency and the progression of a parasitic disease in individual oysters. Effective diagnosis and staging of *Perkinsus* infections will be carried out using small hemolymph samples periodically withdrawn from the adductor muscle of individual *C. virginica*. This kinetic approach will provide a sensitive technique to measure in vivo immunosuppression by toxicants, and the immunoassays will provide in vitro correlates.

chromatograph equipped with a modified flame photometric detector. This method has been adapted for the analysis of tissue samples (Rice et al., 1987) and will be used to analyze TBT concentrations in oysters and algae used in these experiments. Designated tissue samples will also be analyzed by selected ion monitoring mass spectrometry (Greaves and Unger, 1988) to confirm the identity of tributyltin in the samples.

Analytical Methods-Naphthalene exposures. All water and tissue samples will be analyzed for naphthalene by gas chromatography/mass spectrometry. Samples will be spiked with deuterated-naphthalene as an internal surrogate standard prior to extraction with methylene chloride. This will compensate for any volatility losses of the analyte during sample preparation. An additional internal standard (p-terphenyl) will be spiked into extracts just prior to mass spectrometric analysis to quantify total recovery of the surrogate. Tissue samples will be desiccated with sodium sulfate and precipitated silica prior to soxhlet extraction to minimize volatility losses.

Anticipated Results. This proposed work will examine the relative importance of contaminated diet (algae) and water on the rate of TBT uptake by the eastern oyster. Previous studies (Salazar, et al., 1987) have shown that long exposure periods are necessary before shellfish tissue concentrations reach steady state with ambient water concentrations of TBT. This study will identify the kinetics of TBT accumulation by the eastern oyster and will determine how contaminated food (algae) influences the uptake kinetics. Results from this work will identify the relative contributions from these routes of uptake and give insight into the best mechanism for exposing oysters and monitoring concentration levels during laboratory experiments examining immunosuppression by TBT. This will allow a comparison between ambient TBT water concentrations, TBT concentrations in phytoplankton and oyster tissues, and the progression of disease.

The results from the proposed in vitro studies will be useful from a comparative point of view in the assessment of the utility of in vitro models of immunotoxicological effects. The development of such models is of increasing importance in the search for alternatives to whole animal toxicity tests. These tests will serve as screening assays to identify potential immunotoxicants and provide strong rationale for more extensive tests, such as those proposed here. The resultant data will have value in their own right. There is only one paper available on TBT effects on bivalve hemocytic responses (Fisher et al., 1990); our findings will extend these observations by quantifying the TBT actual levels (vs. the nominal concentrations), quantifying the hemocyte lethality associated with TBT dose (LD_{50}), define a dose-response curve for CL activity as effected by TBT and determine the EC_{50}, and determine the effects of TBT on the kinetics of the CL response (as well as on the peak CL response).

Methods for staging *P. marinus* infections and quantifying hemocyte immune parameters. These methods remain as described in the original proposal. Hemolymph diagnosis is by a modification of the method described by Gauthier and Fisher (1990). Phagocytosis of fluorescent test particles (zymosan) by hemocytes will be measured via a Pandex® fluorescence concentration analyzer (Hed, 1986). Hemocyte-mediated chemiluminescence in the presence of luminol will be measured before and

after phagocytic stimulation (Anderson et al, 1992c).

Experimental design remains the same as described in the original proposal, except regarding possible changes in chemical dosing route and schedule based on the preliminary studies of Drs. Unger and Burreson. It is also likely that the majority of the actual exposure studies will be carried out at VIMS rather than at CBL, as originally proposed. This is advisable because of technical details regarding routes of administration, holding facilities, and other logistic considerations. A small increment in Dr. Burreson's budget is requested to cover these expenses, please see the budget justification for the details.

PROGRESS

Progress to date. The first year's objectives include establishing optimal *P. marinus* dosing procedures for the experimental infections, establishing optimal TBT dosing procedures, and carrying out TBT exposure studies to study its effects on immunocyte functions and disease progression. As of this report (~3 months into year one), some progress has been made in each of these areas:

Experimental infection. Cultured *P. marinus* will be used so that the experiments can be conducted without regard to seasonal fluctuations in the natural abundance of the parasite. Evidence gathered during the last year by VIMS scientists and others suggests that *P. marinus* cells taken directly from culture and introduced as a single dose into aquaria do not reliably produce infections. Evidence also suggests that if the culture cells are held in estuarine water for as little as 24 h. infectivity increases, but a single dose is still unreliable. Dr. Burreson has started a series of preliminary experiments to determine the most appropriate dosing method, amount and schedule of *P. marinus* to be used in the subsequent studies of xenobiotic effects on disease progression. Two dosing techniques are under investigation, mantle cavity injection and repeated feeding. For mantle cavity injection, 3 aquaria were established each with 10 uninfected oysters. Each oyster in a tank received an injection of either 10^5, 10^6 or 10^7 *P. marinus* cells. Cells were injected with needle and syringe after a very small notch was cut in the shell. Mantle cavity fluid was drained and *P. marinus* cells in estuarine water were injected into the mantle cavity. Cells were not injected directly into oyster tissue. Hemolymph will be withdrawn for *P. marinus* diagnosis every 3 weeks for 9 weeks. This technique has produced reliable infections in previous studies. For the repeated feeding experiments six aquaria were established each containing 10 uninfected oysters. Two replicate tanks received 10^4 *P. marinus* cells/oyster, 10^5 cells/oyster or 10^6 cells/oyster every day for 10 consecutive days. These exposures yield a total inoculum per oyster equal to that of the injection studies above. *Perkinsus marinus* cells were transferred from culture medium to 20 ppt. sterile estuarine water for 48 h. before dosing. One control tank was established with 10 uninfected oysters; this tank received no *P. marinus* cells. Hemolymph will be withdrawn for *P. marinus* diagnosis every 2 weeks for 10 weeks from one the replicate tanks series, and every 3 weeks for 9 weeks from the other replicate series. The dosing level selected will be the lowest that produces infections in all of the oysters; the bleeding schedule will be

the one that yields the lowest mortality.

TBT dosing. The kinetics of TBT uptake by oysters after in vivo exposure to several doses in the water column or after uptake of TBT-treated algae are being studied by Dr. Unger. It is important to identify the best method available to achieve the dosing/sampling regime for subsequent experiments examining the effect of TBT on oyster immune responses and disease progression. Aquaria are being maintained by static renewal and TBT concentrations in the water, algae (food), and oyster tissues are being monitored by gas chromatography. The experiment is being conducted using four treatments: 1) uncontaminated water and food, 2) uncontaminated water, TBT-dosed algae, 3) TBT-dosed water, uncontaminated algae, and 4) TBT-dosed water and food. Water concentrations are held at high environmental TBT concentrations (pptr), and algae are equilibrated with aliquots of the same water prior to feeding.

Effects of TBT on hemocyte immune parameters. During the initial period of this grant, while dosing techniques and sampling schedules are being optimized, Dr. Anderson has been studying the immunotoxicity of TBT on oyster hemocytes after short-term in vitro exposure. He has also been sending media samples to Dr. Unger for TBT analysis in order to be able to more accurately quantify exposure levels, knowledge which is now limited to nominal concentrations. TBT exposure for 1 hr at a wide range of concentrations (0.5ppb -1ppm, nominal) produced no lethal effects on the hemocytes, on the basis of trypan blue exclusion assays. Analysis of TBT concentrations in the exposure medium indicated: control = ND = $<$0.003ppb; 0.5ppb = ND; 5.0ppb = 0.5ppb; 50 ppb = 10ppb; 500ppb = 110ppb; and 1 ppm = 240ppb (nominal = analyzed value). The necessity of knowing the actual vs. the nominal TBT exposure concentrations is apparent. Although not cytolethal at these levels after 1 hr exposure, the higher TBT concentrations produced significant reduction of luminol-dependent chemiluminescence (Fig. 1). Figure 2 shows that longer exposure (20hr) produced a more marked suppression of chemiluminescence, even though TBT-induced hemocytic lethality was still minimal ($<$85%). Hemocytic production of chemiluminescence (CL) in response to phagocytic stimulation (by the addition of zymosan) is a quantitative indication of the immune status of the cells. The hemocytes' main means of killing ingested pathogenic microbes is via the actions of certain lysosomal hydrolases and reactive oxygen intermediates (ROIs); CL, as measured with the probe luminol, quantifies activity of a major hemocyte defense mechanism: the myeloperoxidase/hydrogen peroxide pathway. In Fig. 1 a dose-dependent CL inhibition is seen for the higher TBT concentrations. This provides a strong rationale for our planned in vivo experiments by directly demonstrating immunomodulation by TBT at sublethal concentrations. The implications of immunosuppression regarding altered resistance to parasitic disease will be explored further during the course of this study.

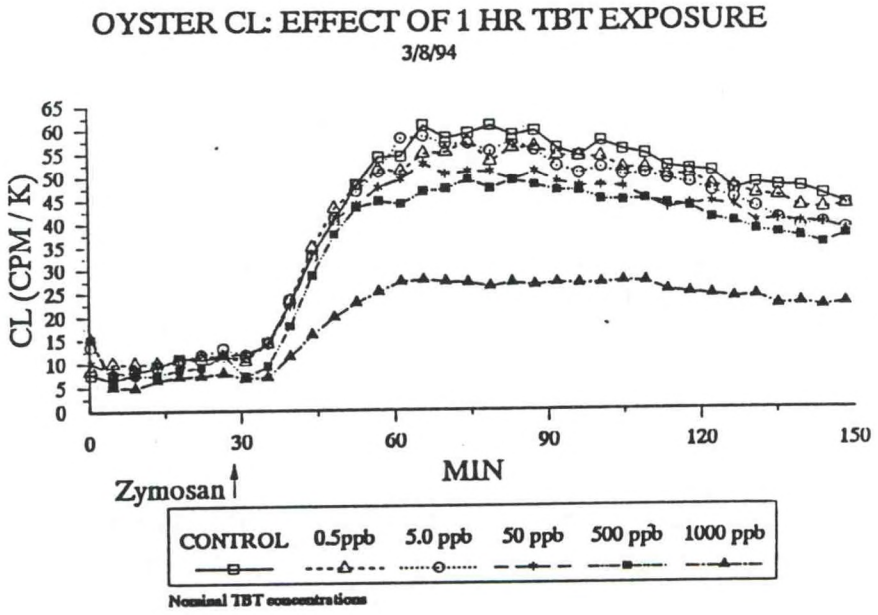

Figure 1. Effects of 1 hr. TBT exposure on hemocytic chemiluminescence

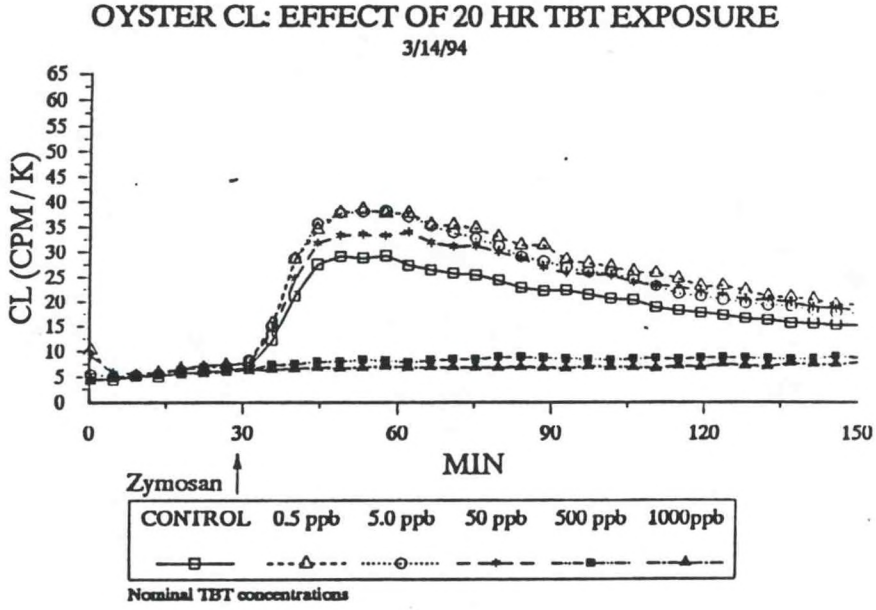

Figure 2. Effects of 20 hr. TBT exposure on hemocytic chemiluminescence

REMAINING PROJECT ACTIVITY

Since this project has been under way for only a few months almost the entire scope of work (as originally outlined) remains to be completed. Chemical exposure dose and route (in food vs. in water), and *P. marinus* infection protocol and hemolymph sampling times, will be determined by the preliminary studies already started. Remaining activity includes: analysis of contaminant bioaccumulation data, and carrying out periodic sampling of oysters during progression of *P. marinus* infections to stage disease, quantify hemocyte immune parameters, and to determine contaminant levels in the oyster tissues. Please see attached Project Schedule page.

DISSEMINATION OF RESULTS

In addition to publication of the results in peer-reviewed literature, findings will be presented at scientific meetings via contributed and invited papers. The prospects for dissemination at national and international symposia are very good; the PIs have ongoing records of being invited to participate in and/or organize symposia at meetings such as National Shellfisheries Association, Society for Invertebrate Pathology, Pollutant Responses in Marine Animals, Society of Environmental Toxicology and Chemistry, Modulators of Fish Immune Responses, World Congress on Cell and Tissue Culture, etc. We also are periodically asked to present our data to various state and federal agencies.

Budget Justification (CBEES Toxics Research Program)

A. Salaries and Wages: The P.I. (R.S. Anderson, Ph.D.) requests funding for 10% (1.2 man-months) effort on this project; his Senior Research Assistant (Lisa Brubacher, M.S.) will devote 50% (6 months) of her effort to the project.

C. Permanent equipment: none requested

D. Expendable Supplies and Equipment:

Animal expenses (cost of acquisition, housing, maintenance)	$ 500.00
Sterile plasticware and glassware	2,500.00
Reagents, fluorescent probes, other laboratory chemicals	2,500.00
	$ 5,000.00

E. Travel: $1,000 is requested for domestic travel to permit P.I. to present data from this project at the Workshop on Modulators of Immune Responses to be held July 8-15, 1995 at Breckenridge, Colorado. This biennial meeting attracts some of the best known comparative immunologists and immunotoxicologists and is an excellent venue for discussing this work.

F. Publication costs: $500 is requested to cover preparation of graphic materials and slides for manuscripts and meeting presentations.

G. Other costs: The chemiluminescence assay for reactive oxygen species is a key component of the study. It requires a dedicated liquid scintillation counter modified for single photon counting; this unit carries a service contract at $2,200 per annum. The Chesapeake Biological Laboratory maintains service contracts on balances, microscopes, centrifuges, etc. for which it imposes 1.5% TDC surcharge on all grants and contracts ($902 in this case). $50 is requested for communication (phone, FAX, etc.) expenses. Water and tissue samples from this project will need to be sent to Dr. Unger for chemical analysis and tissue samples to Dr. Burreson for disease diagnosis; $350 is requested to cover this expense.

BUDGET NOTES

for R/CBT-22B (E. Burreson and M. Unger, VIMS)

Supplies

Funds for the following supplies are requested:

Solvents and chemicals for TBT analysis	$1,000
Glassware	150
GC column, gases and miscellaneous supplies for gas chromatography	600
Mass spectrometry supplies	250
Glass aquaria, 10 gal.	150
Cartridge water filters	225
Instant ocean salt	100
Thioglycollate medium	25
Penicillin/streptomycin	50
Culture flasks	200
Disposal tubes for thioglycollate analysis	150
Scalpel blades and miscellaneous supplies	100
Total	$3,000

Travel

Travel funds are requested for the following:

Trips (x2) to CBL from VIMS, 320 mi. @ $0.24/mi.	$154
Lodging ($60/night)	120
Meals ($41.00/day x two days x two people)	164
Trip to annual progress meeting (x2 people)	250
Trip to annual national scientific meeting (x2), partial support	1,312
Total	$2,000

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[UMCEES]CBL 93-038a

PROJECT NUMBER: _____

PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	YEARS 1994	1995		
1. Preliminary studies to establish optimal *P. marinus* dosing and sample times	xxx			
2. Chemical uptake studies, food as an effective dosing technique	xxx			
3. In vitro immunotoxicological assays to complement in vivo assays	xxxx	xxxx		
4. Disease progression, TBT effects	xxxxxxxx	xxx		
5. Immunoassays, TBT effects	xxxxxxxx	xxx		
6. Disease progression, naphthalene effects		xxxxxxxx		
7. Immunoassays, naphthalene effects		xxxxxxxx		
8. Establish dose-dependency relationship for each xenobiotic	xxx	xxx		
*PROJECTED BUDGET: FEDERAL	$ 48,896	$ 51,398		
MATCH				
*RSA's portion only				

NOAA FORM 90-2 (ADAPTED) U.S. DEPARTMENT OF COMMERCE

*** MARYLAND SEA GRANT PROJECT SUMMARY 1995***

Title: Metallothionein in Marine Coccoid Cyanobacteria: Cloning, Transcriptional Analyses and Application to the Assessment of Metal Stress in Natural Communities of Picoplankton in Chesapeake Bay.

Project Number: R/CBT-23
Grant Number:
Status: continuation
Revision Date: June 15/94
Initiation Date: January 1, 1994
Completion Date: December 31/96

Principal Investigator: Jonathan G. Kramer
Affiliation: Center of Marine Biotechnology, UMBI
Months Committed: 4 months

Principal Investigator:
Affiliation:
Months Committed:

Proposed Federal Funds: $51,962
Current Federal Funds: $37,616
Federal Funds to Date: $37,616
Proposed Matching Funds: $
Current Matching Funds: $
Match to Date: $
Related Projects:
Sea Grant Classification#: 45
Keywords: Toxic metals, metallothionein, marine *Synechcoccus spp.* molecualr biology

Objectives: The objective of this program is to assess the extent and degree that metals impact the picoplankton in Chesapeake Bay and selected tributaries. This objective includes isolation and cloning of the gene encoding metallothionein (MY) from representative marine cyanobacteria (*Synechococcus spp.*). The cloned genes will be used as probes to examine variations in transcription rates in natural populations isolated from the lower Bay, Elizabeth River and James River. The goal of this program is to apply the newest molecular biological techniques to the problem of determining how metal stress impacts the Chesapeake Bay ecosystem.

Methodology: The structural gene encoding the cyanobacterial MT cloned during the first year of this program will be used in quantitative Northern hybridizations to assess variations in transcription patterns of the gene. RNA will be purified from natural populations of *Synechococcus spp.* collected by bulk, differential filtration techniques from selected sites in the Lower Bay, and two tributaries. The results of these field studies will be related to results of experiments conducted with laboratory grown cells exposed to a suite of toxic metals.

Rationale: Understanding the impact of toxic metals upon the estuarine biota is complicated by the fact that exposure is often chronic and at sublethal levels. Development of high resolution techniques to examine specific organisms represents a viable means to assess how environmentally relevant concentrations of toxic materials impact a community. The molecular biological methods utilized here represent a novel approach to this problem and exploit specific, metal-responsive systems in an important group of phototrophs found in Chesapeake Bay. This program will merge field studies with detailed laboratory development of molecular biological tools. This will lead to a fundamental understanding of the physiology of metal responses in the target species and their ramifications in the natural environment. Therefore, this study will establish a new basis for understanding how metals affect the planktonic community of Chesapeake Bay.

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1995 GRANT/PROJ. NO: RCBT-23
University of Maryland, Center of Marine Biotechnology
PRINCIPAL INVESTIGATORS: Jonathan G. Kramer

DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.		4.00	14,333	
b. Associates				
Sub Total			14,333	0
2. OTHER PERSONNEL				
a. Professionals				
b. Research Associates				
c. Res. Asst. Grad. Std.				
d. Prof. School Students				
e. Pre-Bac Students				
f. Secretarial-Clerical				
g. Technical-Shop				
Total Salaries/ Wages			14,333	0
B. FRINGE BENEFITS			3,727	
Total Salaries,Wages and Fringe Benefits			18,060	0
C. PERMANENT EQUIPMENT				
D. EXPENDABLE SUPPLIES AND EQUIPMENT			6,000	
E. TRAVEL				
1. Domestic	1,000			
2. International				
Total Travel			1,000	
F. PUBLICATIONS AND DOCUMENTATION COSTS			750	
G. OTHER COSTS				
1. Computer costs			500	
2. Copying,Library and Communication				
3. Analytical and Shop Services				
4. Fuel,Boat Time and Vehicle Usage			7,800	
5. Word Processing				
6. Institutional Allowance				
7. Tuition			500	
8. Waste Disposal			500	
9. Service Contracts				
Total Other Costs			9,300	0
TOTAL DIRECT COSTS			35,110	0
INDIRECT COSTS				
(On Campus) : 48 % of 35,110			16,853	
(Off Campus) : % of				
Total Indirect Costs			16,853	0
TOTAL COSTS			51,963	0

METALLOTHIONEIN IN MARINE COCCOID CYANOBACTERIA: CLONING, TRANSCRIPTIONAL ANALYSES AND APPLICATION TO THE ASSESSMENT OF METAL STRESS IN NATURAL COMMUNITIES OF PICOPLANKTON IN CHESAPEAKE BAY

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INTRODUCTION

Sensitive measures are required to develop a better understanding of how chronic exposure to toxic compounds impacts the ecosystem of Chesapeake Bay. Ultimately any understanding of how stress is propogated through the various components of this ecosystem will depend upon discerning first how individual organisms respond. Priority has been given to understanding the stress caused by several toxic metals found on the Chesapeake Bay Program's Toxics of Concern List. The goal of this research program is to understand how one component of the planktonic ecosystem: the autotrophic picoplanktonic cyanobacteria (*Synechococcus* spp.) are effected by metal stress. In specific terms, the intent is to examine the molecular biological basis of this response and to utilize these subcellular characteristics as a means to assess the impact of metals upon natural populations in impacted and non-impacted areas of Chesapeake Bay. The molecular genetic signature of metal exposure in these organisms can provide a sensitive biomarker useful for tracing how a specific toxic stress is distributed into the estuary via these impacted populations.

Metallothioneins are a class of low molecular weight proteins known to be induced by exposure to a variety of metals. The importance of these proteins has been well characterized in eukaryotic organisms (Hamer 1986) Recently, a novel metallothionein was isolated and characterized in the freshwater cyanobacteriun *Synechococcus* spp. PCC 6301 (Olafson et al. 1988). The gene encoding this protein called *smtA*, as well as a separate regulatory element (*smtB*) were cloned and sequenced Robinson et al. 1990, Gupta et al. 1993). Characterization of these clones revealed that while there was only slight homology with other known

metallothionein genes, there was a rapid increase in transcription when the cyanobacterium was exposed to sublethal quantities of toxic metals (Gupta et al. 1993). It was also noted that chronic exposure caused *Synechococcus* sp. PCC 6301 to develop an enhanced tolerance (Gupta et al. 1992, 1993). This adaptation could be traced to an amplification in the number of *smtA* genes in the organism's chromosome. In addition, cells exposed to cadmium were found to have an altered *smt*-operon. In this case, a region upstream from the *smtA* structural gene was deleted. This region encodes a repressor (*smtB*). Once deleted, the organism maintained a higher constitutive level of transcription of the metal responsive element (Gupta et al. 1993).

Together, these factors suggest that cyanobacteria exposed to metals have a unique molecular signature. Application of appropriate molecular biological techniques designed to recognize this signature will provide a sensitive measure of metal exposure and stress. The typical coccoid cyanobacteria found in Chesapeake Bay are closely related to the *Synechococcus* spp. used in the initial characterization of the *smt*-operon and its regulation. Isolation of the gene encoding metallothionein in these marine *Synechococcus* spp. will enable us to apply these techniques to examine metal stress in natural populations within Chesapeake Bay. *Synechococcus* spp. are important components of the planktonic ecosystem. They are also nearly ideal candidates for studies such as this. They can be grown in laboratory culture as well as easily identified and sampled in the field (Kramer and Singleton 1993). By applying a molecular biological approach to an ecologically important organism, we have the opportunity to develop the means to assay how a critical component of the microbial community in the estuary is impacted and responds to toxic metals.

OBJECTIVES

Overall Objectives: The overall objectives of this program are to isolate and clone *smtA* from two marine *Synechococcus* spp. found in Chesapeake Bay. These cloned genes will be characterized and then used as probes to examine variations in the transcription of the metallothionein gene after exposure to selected metals in laboratory studies. The same probes will then be utilized to examine variations in transcription patterns of natural populations of *Synechococcus* spp. in the lower Chesapeake Bay as well as the Elizabeth and James Rivers.

1994 Objectives During this current, first year of the program the objectives are:

1. To isolate and clone *smtA* from *Synechococcus* spp. WH 5701 and WH 7803,

2. To examine how *smtA* transcription varies after exposure to Cd, Cr, Cu, Hg, Pb, and Zn and,

3. To determine the minimum concentration of each metal that induces *smtA* transcription

1995 Objectives The specific objectives for the second year of the program are:

1. To conduct two surveys to collect Synechococcus spp. from the lower Chesapeake Bay, the Elizabeth River and James River. RNA purified from these cells will be probed for *smtA* transcript levels. Sampling will be conducted once in the spring and once in late summer.

2. To determine through laboratory experiments if marine *Synechococcus* spp. develop tolerance to metals through molecular genetic pathways similar to those discerned in the freshwater models.

PROJECT CHANGES

There have been no changes in the objectives or methodologies proposed in the original submission of this proposal.

PROGRESS

This program was initiated in January 1994. Since that time significant progress has been made. Primary efforts have been directed to the isolation and cloning of the *smtA* gene from *Synechococcus* sp. WH 5701. This is a phycocyanin dominant cyanobacterium found in Chesapeake Bay. It is very closely related to the freshwater species used in the initial cloning of the gene. The strategy employed thus far has taken two tracks. First, synthetic oligonucleotides (32-mers) complementing sequences flanking a 200 base pair (bp) region of *smtA* were synthesized at C.O.M.B. They were then utilized in conjunction with PCR techniques to amplify the putative fragment from template DNA prepared from the marine strain. Analysis of the products of that amplification revealed the presence of a fragment at approximately 200 bp. The conditions of the amplification are presently being optimized to enhance the production of this fragment which will then be cloned and sequenced. A second route presently being pursued involves direct probing for the *smtA* gene. Initial efforts have utilized *smtA*-specific probes generated by end-labelling the synthetic oligonucleotide primers used in the PCR amplifications. These probes have been hybridized to restriction digests of genomic DNA purified from both *Synechococcus* sp. WH 5701 as well as the phycoerythrin-dominant strain WH 7803. Initial studies using hybridization conditions of low stringency revealed that the probes were in fact sufficiently homologous to hybridize to the chromosomal digests. The degree of hybridization was greater to DNA from *Synechococcus* sp. WH 5701 although bands were found for

Synechococcus sp. WH 7803 as well. At present, the a second set of probes are being designed and will be synthesized. These longer oligonucleotides as well as the cloned PCR-products will then be used in additional hybridization studies. These results, strongly suggest that the marine/estuarine strains of *Synechococcus* have the *smtA* gene. Characterization of the gene by sequence analysis and utilization of probes generated from the cloned fragments to analyze transcription patterns will begin soon. Given the progress to date, the program is on schedule based upon projections made in the original submission of this proposal.

REMAINING PROJECT ACTIVITY

During the renewal period, activities will be directed chiefly to the collection and analysis of field samples from the Chesapeake Bay and the Elizabeth and James Rivers. Samples of the picoplankton fraction will be collected at all three sites at two times during 1995. A spring and late summer sampling will be conducted. Filters containing the target fraction will be processed for RNA purifications. The abundance of *smtA* transcripts in these samples will be assessed and compared in relation to the perceived toxic metal load at the respective sites. In addition to the field studies, laboratory cultures will be utilized to examine if tolerance to selected metals is developed in the marine *Synechococcus* spp. after chronic exposure. Stepwise exposure to increasing dosages will be coupled with fine scale analysis of *smtA* dosage and structure by Southern hybridization techniques during the course of these studies. Results will be compared to the patterns noted for the more well described freshwater cyanobacteria.

DISSEMINATION OF RESULTS

The results of these studies will be disseminated by a variety of means. Publication of findings in peer reviewed journals will comprise the primary route. In addition, it is anticipated that findings will be presented at national level conferences. Importantly, data will be synthesized and presented upon request to all agencies designated by the CBEEC administration. Efforts will be made to present the molecular methodologies in a manner that is easily utilized by a variety of audiences. All clones isolated from this program will be made available as will the sequences of the relevant genes via the GENBANK database.

BUDGET JUSTIFICATION

Funds requested for the second year of this program refect the costs required for successful completion of the proposed research. There have been two changes in the budget relative to what was anticipated for this time period in the original submission of the proposal. The first, is a change in the indirect cost rate. The University of Maryland Biotechnology Institute has increased its overhead rate by 2 % (46% to 48%). This increase is reflected in the current budget. In addition, there has been a small increase in salary support over what was originally projected for the second year of the program. All other items remain unchanged.

The budget for this program includes a limited amount of salary (4 mos.) for the principal investigator. Additional funding is requested for expendable supplies. This request is justified on the basis of costs incurred during the course of molecular biological studies. An itemized accounting of these costs includes:

Radioisotopes (for probe labelling)	$750
Restriction and modification enzymes, sequencing kits, labelling kits etc.	$1,250
Electrophoresis and blotting supplies	$500
Oligonucleotide synthesis	$500
Chemicals	$750
Glassware and culture supplies	$500
RNAse-free plasticware and misc. plastic supplies	$750
Filtration equipment and supplies (field supplies)	$1,000

Funds are requested to travel to one scientific conference. In addition some travel funds will be used to defray the costs incurred in travel to meet the ship used in the field program. A request is made for 5 days of shiptime. The amount requested reflects the projected costs based upon the current hourly rates for the *RV AQUARIUS* (Univ. MD. System). Additional limited funding is requested for computer use fees. Access to network databases is essential to the sequence analyses in this program. In addition, a limited request is made for equipment maintenance as well as the removal of the toxic chemical and radioactive wastes generated during the course of the program

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PROJECT NUMBER: _____

PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	YEARS 1994	1995
1. smtA isolation and cloning	XXXXX	
2. Sequence analyses	XXX	
3. Studies of transcription patterns in metal exposed laboratory cultures	XXXXX	XXXX
4. Spring collection of cyanobacteria from Chesapeake Bay and tributaries		X
5. Analysis of spring samples		XX
6. Summer/Autumn Collections		X
7. Analysis of summer/autumn samples		XX
8. Tolerance studies in laboratory		XXX
PROJECTED BUDGET: FEDERAL	$37,616	$51,962
MATCH		

*** MARYLAND SEA GRANT PROJECT SUMMARY 1995 ***

Title: Risk Assessment for Dimilin use in northern Chesapeake Bay. A model study for non point-source run off.

Project Number: R/CBT-24
Grant Number:
Status: continuing
Revision Date: June 15, 1994
Initiation Date: January 1, 1994
Completion Date: December 31, 1996

Principal Investigator: David A. Wright
Affiliation: Chesapeake Biological Laboratory
Months Committed: 1.2

Principal Investigator: Rodger Dawson
Affiliation: Chesapeake Biological Laboratory
Months Committed: 1.2

Proposed Federal Funds: $94,216
Current Federal Funds: $90,217
Federal Funds to Date: $90,217
Proposed Matching Funds: $
Current Matching Funds: $
Match to Date: $
Related Projects:
Sea Grant Classification#: 45
Keywords: Dimilin, Chesapeake Bay, non point-source, toxicity

Objectives: Specific objectives will be to map the usage patterns of Dimilin in the northeast Chesapeake Bay catchment area, and monitor Dimilin run-off at two field sites. Bioassay results from two crustacean species at these field sites will be compared with bioassay data from a (Dimilin-free) reference site. End points from these and laboratory bioassays will characterize the role of food in Dimilin bioavailability and will form the basis of hazard assessment for this compound's specific mode of action. Chemical application, run-off and toxicity data will be combined as a model risk assessment for non-point source pesticide run-off.

Methodology: Chemical analysis for Dimilin residues in water and sediments using a combination of HPLC and LC-HS techniques will be coupled with sensitive laboratory and field bioassay procedures employing as end points mortality, fecundity and cuticular abnormalities of two crustaceans. Field bioassays will be coordinated with Dimilin applications adjacent to watersheds of rivers in the Northern Chesapeake Bay., Data from the field application of Dimilin, run-off levels, environmentl half-life, ecology of test animals and bioassayus will be used to construct a risk assessment for this pesticide in these locations. Field studies will be supported by mesocosm studies during the 1995 scope of work.

Rationale: In order to construct a risk assessment, two components are necessary: exposure assessment and hazard assessment. In constructing a model for non-point-source risk assessment it is important to choose a compound and test organism(s) for which there is a high probability of achieving food quantiative estimates of both of these parameters. There are several reasons why Dimilin and the test psecies proposed here form an excellent basis for such a model.

Exposure Assessment relies for success on accurate data for both environmental chemical loading and distribution of test organisms. In the case of Dimilin, usage in Maryland is carefully monitored and controlled by the Agency responsible for its application (MD Department of Agriculture). Detailed spatial and temporal records are kept and recorded centrally. With respect to species distribution, unusually detailed records are available from several sources for both *E. affinis* and *L. plumulosus*.

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1995 GRANT/PROJ. NO: RCBT-24
University of Maryland, Cheasapeake Biological Laboratory
PRINCIPAL INVESTIGATORS: David A. Wright
Rodger Dawson
DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	1.20		6,655	
b. Associates	1.20		7,139	
Sub Total			13,794	0
2. OTHER PERSONNEL				
a. Professionals				
b. Research Associates				
c. Res.Asst.Grad.Std.	12.00		13,200	
d. Prof. School Students				
e. Pre-Bac Students				
f. Secretarial-Clerical	1.20		2,200	
g. Technical-Shop				
Total Salaries/ Wages			29,194	0
B. FRINGE BENEFITS			9,860	
Total Salaries,Wages and Fringe Benefits			39,054	0
C. PERMANENT EQUIPMENT			5,000	
D. EXPENDABLE SUPPLIES AND EQUIPMENT			6,000	
E. TRAVEL				
1. Domestic	1,200			
2. International				
Total Travel			1,200	
F. PUBLICATIONS AND DOCUMENTATION COSTS			600	
G. OTHER COSTS				
1. Computer costs				
2. Copying,Library and Communication			792	
3. Analytical and Shop Services			12,000	
4. Fuel,Boat Time and Vehicle Usage			1,500	
5. Word Processing				
6. Institutional Allowance				
7. Tuition			2,000	
8. Waste Disposal				
9. Service Contracts			1,038	
Total Other Costs			17,330	0
TOTAL DIRECT COSTS			69,184	0
INDIRECT COSTS				
(On Campus) : 39 % of 64,184			25,032	
(Off Campus) : % of				
Total Indirect Costs			25,032	0
TOTAL COSTS			94,216	0

A RISK ASSESSMENT FOR Dimilin® USE IN THE NORTHERN CHESAPEAKE BAY. A MODEL STUDY FOR NON POINT-SOURCE RUNOFF.

David A. Wright, Associate Professor, Chesapeake Biological Laboratory
Rodger Dawson, Associate Professor, Chesapeake Biological Laboratory

ABSTRACT

This is the second year of a two year study designed to characterize the effects of non-point source pesticide runoff on fresh and brackish water crustaceans in the northern Chesapeake Bay area. We have chosen the benzoylurea pesticide diflubenzuron (Dimilin®) and its effects on non-target crustaceans as a model for this risk assessment because of the potential for acquiring accurate information on its usage and dispersal, and because of the fact that preliminary data from our laboratory indicates a potential for significant effects of this compound at environmentally realistic ambient concentrations. Primary objectives of the study will be to (1) conduct a survey of Dimilin® usage in the northern Chesapeake Bay catchment area, (2) conduct extensive field studies at two sites to determine (a) runoff, (b) environmental half-life and (c) lethal and sublethal effects on two abundant crustacean species found in the receiving water.

These studies will involve a combination of in situ field bioassays, laboratory bioassays, a mesocosm study and chemical monitoring to evaluate selected parameters which will indicate acute or chronic stress.

In Year One, in situ toxicity tests will be conducted at an impacted site and a reference site. Site selection will be based on information obtained from spraying activities. Tests will be coordinated with spraying regimes and location and timing will be carefully selected to avoid extraneous effects from other chemicals. Rainfall, Dimilin®, and other chemical concentrations and water quality conditions will be monitored at both test and reference sites using automated sampling devices.

In Year Two, further laboratory toxicity tests will be conducted with the amphipods Leptocheirus plumulosus, Hyalella azteca and the copepod Eurytemora affinis exposed to Dimilin® levels measured in Year One and under similar water quality conditions. Additional field experiments using the most sensitive indicators will be conducted in order to identify the spacial extent of Dimilin® effects. A mesocosm study will be designed to simulate field conditions. It will specifically address leaf washoff resulting from rain events, soil percolation and ensuing exposure of animals to Dimilin®-contaminated water and sediments.

Anticipated results of this study would fulfill the Toxics Research Program stated need to better understand the responses of Chesapeake Bay organisms to exposures of toxic substances. Dimilin® source, transport, fate, exposure and effects would be incorporated into a Risk Assessment which would serve as a model for other non point-source chemicals in the Chesapeake Bay ecosystem.

INTRODUCTION

This proposal addresses the second year (Jan 1-Dec31, 1995) scope of work for the currently funded project 'A risk assessment for Dimilin® use in the northern Chesapeake Bay. A model for non-point source runoff'.

The project addresses the need for information on the fate and effects of toxic chemical runoff in the Chesapeake Bay catchment area at environmentally realistic levels, and the effect which trophic transfer is likely to have on bioavailability (and toxicity). The problem essentially has the elements of a risk assessment in that chemical application is related to exposure concentration which is, in turn, compared with toxicity data. Pesticides are excellent candidates for risk assessment particularly where exposure and toxicity data overlap.

Rationale

In order to construct a risk assessment, two components are necessary: exposure assessment and hazard assessment. In constructing a model for a non-point-source risk assessment it is important to choose a compound and test organism(s) for which there is a high probability of achieving good quantitative estimates of both of these parameters. There are several reasons why Dimilin® and the test species proposed here form an excellent basis for such a model.

Exposure Assessment relies for success on accurate data for both environmental chemical loading and distribution of test organisms. In the case of Dimilin®, usage in Maryland is carefully monitored and controlled by the Agency responsible for its application (Md. Department of Agriculture). Detailed spatial and temporal records are kept and recorded centrally.

With respect to species distribution, unusually detailed records are available from several sources for both E. affinis and L. plumulosus (see page 4).

Hazard Assessment: Data which have been gathered in this laboratory are virtually unique in the Chesapeake system in demonstrating toxicity of Dimilin® to a widespread indigenous species (E. affinis) at very low levels which have been documented in the ambient

aquatic environment (Smucker 1988; Wright et al 1992; Savitz et al. 1994; Table 1). Toxicity data were reported from 10‰ (Savitz et al 1994). Although we have yet to investigate Dimilin® toxicity in the lower salinities/freshwater found at the study sites proposed here, we anticipate the toxicity would be even greater. The characteristic cuticular abnormality seen in Dimilin®-exposed E. affinis (Savitz et al 1994) is likely to prove particularly useful in identifying specific Dimilin® toxicity and provide a means of differentiating it from other potentially toxic agents in the water. Spatial and temporal considerations of test-site selection should also minimize complicating effects of other toxic agents. Information from laboratory experiments will further refine the hazard assessment.

We therefore anticipate a high probability of obtaining precise data for both components of the Risk Assessment. The high, and characteristic, toxicity of Dimilin® in this regard, give the study a good probability of achieving characteristics of a "worst case" model and one which may aid in similarly modelling other non-point source toxics.

Dimilin® Usage and Toxicity

Diflubenzuron (DFB), marketed under the trade name Dimilin® has been approved by the U.S. Environmental Protection Agency for control of several insect pests including the gypsy moth (1976) and foliar feeders on soybeans (1982). It is presently in wide use in Maryland for gypsy moth (Lymantria dispar (Smucker and Speith, 1987). In aquatic habitats DFB is present on particulates, in sediments, and in the water itself as a result of mobilization from forests that have been aerially sprayed (Smucker and Speith 1987, Smucker 1988). In 1988 Maryland Department of Agriculture listed 28,000 lbs. (active ingredient) Dimilin® usage. More current figures are currently in preparation but are not yet available.

DFB (TH-6040, or 1-(4-chlorophenyl)-3-(2,6-difluorobenzoylurea) is an insect growth inhibitor, specifically targeting the molting process. Following the use of DFB as a pest control measure, residues may enter the aquatic environment in runoff, or by direct over aquatic systems. Because of its specific mode of action, there is little concern over direct effects on non-target organisms, such as birds and mammals, which do not have chitin-containing exoskeletons (Mauchamp and Perrineau 1987, Granett 1987). Similarly, effects on fish occur only at extremely high concentrations (Julin and Sanders 1978, Lee and Scott 1989). Chitin production in diatoms and fungi surprisingly is not affected by DFB treatment (Booth et al 1987; Mauchamp and Perrineau 1987). In crustaceans, however, the mode of action of DFB is similar to that in insects (Christiansen et al 1984; Antia et al 1985). In the past it was thought that DFB directly inhibited the enzyme chitin synthetase which is required for arthropod molting (Gijswijt et al 1979). However, recent studies have demonstrated that its action is less direct. For example, it may act as a serine protease, inhibiting the activation of the chitin synthetase (Clarke and Jewess 1990; Lee et al 1990), or it may affect hormonal processes in the molting cycle such as B-ecdysone metabolism (Gulka et al. 1980; Lee et al. 1990). Alternatively, it may affect substrate or cofactor availability (Marks and Ward 1987). Finally, a post-synthetic process such

as chitin polymerization, deposition or integration may be the target (Antia et al. 1985; Cohen 1987; Grosscurt and Jongsma 1987). Impairment of chitin incorporation may, in turn, be the result of effects on transport or synthesis of chitin precursors or cuticular proteins (Lee et al 1990). Histological studies have shown that deposition of the endocuticle is disturbed by DFB in both crustaceans as well as insects (Mulder and Gijswijt 1973; Christiansen and Costlow 1982; Gulka et al 1982; Retnakaran and Wright 1987).

Because of its mode of action Dimilin® is likely to elicit some highly characteristic symptoms in exposed test organisms. This facilitates a highly specific hazard assessment, even in the presence of other toxic chemicals. Savitz et al (1994) and Savitz and Wright (unpublished) have demonstrated that Dimilin® is toxic to the copepod Eurytemora affinis at very low concentrations. Survival and reproductive performance were both affected at Dimilin® concentrations $< 1 \mu g l^{-1}$ and some of the data have been summarized in Table 1. Additionally, cuticular separation in E. affinis was shown to be characteristically associated with Dimilin® exposure (Savitz et al., 1994). It is also interesting that delayed toxic effects have been observed following short-term (96h) exposure to water concentrations as low as 0.6 ppb (Harrahy et al., 1994). Field concentrations of Dimilin®, following spraying, have been shown to be within the range of toxic concentrations reported in Table 1 (Smucker, 1988).

Test Organisms

Brownlee and Jacobs (1987) found that E. affinis made up 20% of the mesozooplankton in the Chesapeake Bay when abundance was averaged over a full year, ranking second in number to the calanoid copepod Acartia tonsa. Together, these two species comprised 65% of the total mesozooplankton collected. While E. affinis dominates in the oligohaline, mesohaline and polyhaline regions mainly in the spring months, it is the dominant copepod in freshwater portions of the Bay throughout the year (Brownlee and Jacobs 1987). Information on distribution and abundance of the species in the Chesapeake Bay is available from Allan et al 1976; Setzler-Hamilton et al. 1981b; Storms 1981; Brownlee and Jacobs 1987; Olsen 1987. In addition to this large body of information, there are several other reasons why E. affinis is an appropriate organism for this study. This species has proven highly sensitive to Dimilin® in controlled assays involving mortality, fecundity and cuticular abnormalities (Savitz et al 1994; Savitz and Wright, unpublished). Additionally, Hartwell et al (1993) have demonstrated the utility of a E. affinis fecundity assay as a means of assessing toxic stress in the ambient environment. E. affinis has a short generation time of approximately two weeks at 20°c (Heinle and Flemer 1975) and it has been successfully cultured in the laboratory. Moreover, its preference for salinities < 15 ppt (Jeffries 1962, from Katona 1970), along with its presence in early spring (Heinle 1972; Bradley 1977; Brownlee and Jacobs 1987; Olsen 1987) make it vulnerable to pest control practices during spring months in the relatively freshwater regions of the Bay and its tributaries.

Leptocheirus plumulosus Shoemaker is an ecologically important infaunal inhabitant of both oligohaline and mesohaline portions of the Chesapeake Bay where nearly 20 years of (almost monthly) population data are now available (Holland et al 1988; Kerhin et al 1988). Its use as a bioassay organism for sediment toxicity testing in the Chesapeake Bay was recently recommended by Schlekat et al. (1992) who demonstrated its wide salinity tolerance.

OBJECTIVES AND HYPOTHESIS

Hypothesis

We hypothesize that receiving waters adjacent to areas subjected to Dimilin® spraying will have concentrations of the pesticide higher than water at a reference site of similar characteristics but distant from Dimilin® application.

We hypothesize that test crustaceans entrained in ambient water containing close to 1μg L^{-1} will show significant toxic effects (mortality, reproductive impairment, cuticular abnormalities) relative to animals from reference sites.

Laboratory bioassays simulating field conditions will be used to refine toxic end-points and investigate the part played by particulate adsorption in Dimilin® bioavailability. We anticipate that both environmental half-life and bioavailability of Dimilin® will be significantly affected by particulate adsorption. The effect of Dimilin® adsorption to particulates on its bioavailability (and toxicity) will be investigated in the laboratory.

We anticipate that a combination of Dimilin® runoff data, test species ecology and results from in situ and laboratory bioassays will enable risk assessment for this pesticide which will act as a model for other non point-source compounds.

Overall Objectives

Specific objectives of this study will be to:

1) Map the usage patterns of Dimilin® in the northern Chesapeake Bay catchment area, document the loading and runoff patterns in the northwest segment of the Bay between the Elk and the Choptank rivers and determine which areas represent the most likely recipients of non-point source runoff from this activity.

2) Evaluate the effects of Dimilin® runoff on Eurytemora affinis, and Leptocheirus plumulosus as determined by mortality, reproductive performance and molting, using a combination of in situ and laboratory bioassays. (Hyalella azteca may be used as an additional bioassay organism in freshwater environments).

3) Examine the role which particulates (food) play in the uptake of Dimilin® by planktonic and benthic crustaceans.

4) Evaluate the most sensitive crustacean screening assay among those tested and use those indicators to characterize the extent of Dimilin® effects down a gradient.

These Objectives remain the same as originally proposed except that we have added a mesocosm study to the 1995 scope of work. We have also incorporated the improvements of our analytical procedures into both the 1994 and 1995 scope of work. These changes are discussed in later sections.

1994 Objectives

The primary focus of the study during the first year is to document Dimilin® application in the catchment area of the northeast Chesapeake Bay region in a quadrant between the Elk and the Choptank Rivers and to assess the proportion of the applied compound which reaches the receiving waters. Effects of Dimilin® will be assessed using in situ bioassays.

Work will begin on laboratory bioassays using amphipods (Leptocheirus plumulosus and Hyalella azteca) designed to test effects of sediment characteristics (particulate profile, TOC, etc.) on Dimilin® bioavailability.

1995 Objectives

Copepod bioassays will test the effect of particulate adsorption on Dimilin® toxicity. Both inorganic (Kaolin) and organic (algae) particulates will be used, effectively characterizing the role of food in Dimilin® bioavailability.

Laboratory bioassays will be conducted on copepods and amphipods using Dimilin® concentrations, water quality and sediment characteristics corresponding to field data gathered in Year One. Mesocosm studies will be performed which will specifically address exposure route (i.e. leaf washoff, soil percolation, etc.).

METHODOLOGY

Documentation of Pesticide Application and Loading

Dimilin® usage in Maryland is documented by the Maryland Department of Agriculture. This agency keeps an inventory of the amount of Dimilin® used and coordinates flight paths and patterns. A detailed application record is, therefore, available which can be coordinated with runoff to the Elk, Bohemia, Sassafras, Chester, Wye and Choptank Rivers. Flow data available from the U.S. Geological Survey will be used in conjunction with field chemical analyses of Dimilin® in constructing a budget of Dimilin® runoff.

Field Analyses of Dimilin®

Analyses of water and sediment will be made at two sites in the study area, selected on the basis of their proximity to spraying activity. One site will be in the Elk river and the other selected from the Wye, the Chester or the Choptank Rivers. One hour integrated water samples will be collected daily over a two week period following a spraying event. Daily sediment samples will be collected from the same site over this 14 day period and weekly thereafter over the next 56 days. Aquatic Dimilin® concentrations will be used to estimate runoff. Daily analysis of a static water sample from the field will be used (together with sediment data) to estimate the environmental half life of the compound. (Breakdown products and derivatives will also be monitored).

Dimilin® is concentrated from natural waters using c-18 solid phase extraction (SPE) after adjustment of pH and the addition of acetonitrile to the samples. After clean-up of interfering compounds using selective elution, Dimilin® is detected by Reversed Phase HPLC with detection by UV spectrophotometry and Photo Diode Array. Detection limits are below the ppb level when using stable, sensitive detectors. Sediments are extracted by exhaustive sonication with acetonitrile-water mixtures, subjected to a multi-stage SPE clean-up and further analyzed by HPLC as above. Recoveries from sediments are quite matrix dependent and have been shown to range from 60-90% (Smucker, 1991) which requires that spiked samples should be analyzed at each sediment sampling site to constrain the range of expected Dimilin® recovery.

With the acquisition of an LC-MS system (scheduled for mid 1994) Dimilin® will also be confirmed by a mass spectrometry. A new technique is currently being developed in collaboration with the Naval Research Laboratory which employs an electrospray interfaced to the mass spectrometer. The initial measurements of the Na+ adduct of Dimilin® (Drs. Mona Shahgoli and John Callahan, NRL pers. comm.March, 1994) indicates that this will be a more sensitive and unambiguous detection system, less prone to interferences with other compounds in environmental matrices. This technique will also enhance our ability to follow the production of breakdown products of Dimilin®, specifically the formation of parachloraniline which has

been detected by HPLC in environmental samples (Smucker,1988). We are proposing to incorporate this new technology with the assistance of the Naval Research Laboratory into our scope of work for 1995 at relatively minor cost for components to complete the interface (see Budget Justification).

1994 Progress to Date

Work to date in the first year of the study has proceeded along three lines: Amphipod toxicity testing in the laboratory; copepod toxicity testing; refinement of analytical techniques.

Toxicity of Dimilin® to Hyalella

Acute toxicity bioassays have now been run for two different size classes of Hyalella azteca; 1.2mm mean length (ra 0.9-1.9) and 2.3mm mean length (ra 1.7-2.8). Data indicate LC50s of 2 and 5 μg L-1 respectively for these two size classes. (Fig 1).

Currently in progress is an assay designed to determine the effect of diflubenzuron on delayed mortality. Hyalella are exposed to a range of diflubenzuron concentrations similar to those used in the above tests, but for only 24-96h. They are then reared in diflubenzuron-free water to determine survivorship over an extended time. The experiment is designed to simulate the pulsed application of Dimilin® resulting from a spraying event and was initiated in the light of the recent paper by Harrahy et al. (1994) who showed delayed effects of Dimilin® on larval aquatic insects at levels as low as 0.6ppb in freshwater.

These aquatic bioassays will be followed by sediment toxicity bioassays to be conducted in the laboratory under controlled conditions. These will encompass a range of Dimilin® concentrations found under field conditions. The physico-chemical characteristics of sediments will be matched to field conditions.

Methodology will essentially follow that used be Schlekat et al.(1992), although experiments will last two weeks and will involve both Leptocheirus and Hyalella. A range of five Dimilin® concentrations plus controls will be used, and assays will be conducted in triplicate. Dimilin® will be analyzed in sediment, interstitial waters and biota using HPLC with photodiode array detection with periodic mass spectral confirmations. As with field assays, survivorship and fecundity will be measured, and particular attention given to molting problems and cuticular abnormalities. A dose response will be developed for all these parameters.

Copepod Bioassays

Laboratory assays have proceeded this year with particular emphasis on the effect of salinity on Dimilin® toxicity to Eurytemora affinis (Figs. 2 and 3). While there appears to be no salinity effect on Dimilin® toxicity per se there is a strong indication that fecundity and survival are affected at low salinities. These data have been prepared as a manuscript (Wright et al.,1994) which has been submitted for publication in Ecotoxicology. A copy of this manuscript is appended to this proposal.

Direct Low-level Detection of Dimilin®

During the early part of 1994 we have been obliged to address the issue of improving the methods in order to decrease detection limits for the analysis of diflubenzuron in environmental samples.

Diflubenzuron (Dimilin®) can be determined in environmental samples by reversed phase HPLC with UV detection (e.g. Sundaram et al. 1989). Unfortunately DFB is a weak UV absorber and the lower limit of detection is at best 0.5ng on column which translates to an environmental concentration in water of 5ppb for a 100ul injection. Using solid phase extraction (e.g. Savitz et al.1994) water samples were concentrated from 250ml to effectively 4ml prior to injection of 40ul. The limit of detection was thus 25 times lower at 0.2ppb. Dimilin® has been shown to be toxic to Eurytemia at or approaching the 0.5 ppb level and the above methods assume at least 250ml of seawater is processed. For studies of sediment interstitial waters only some 10- 20ml may be available. The initial analyses using our HP1050 HPLC system with photo diode array detection confirmed these existing limits of detection, provided additional spectral peak purity and library information, validated the recoveries of Dimilin® from SPE cartridges at better than 95% and verified the percentage of active compound in the kaolin formulation as close to 25% as stated by the supplier.

Since no overall further improvement in detection limits could be achieved from the detector itself, we have developed a method involving trace enrichment by focussing a large (10ml) injection volume onto a C-18 enrichment column which is time programmed to backflush onto the analytical column after loading with low solvent strength eluant. Effectively the on-column detection limit is the same at around 0.5ng but we have a 100 fold increase in sample applied. Direct injection of seawater is hence possible if there is 0.25 ppb of Dimilin® in the sample. If we process some 50ml of seawater or interstial water using SPE this is reduced by a factor of 5 to 50ppt.

We are now confident that we can detect Dimilin® in environmental samples well below acute toxicity levels and we are preparing a manuscript describing these analytical procedures. Sediment samples have been obtained and we are proceeding to describe the sorption and desorption behavior of the insecticide through controlled laboratory studies. Electrospray mass-

spectrometry of Dimilin® standards have been performed in conjunction with the Naval Research Laboratory. Further tests on spiked environmental samples are currently being planned. It is envisaged that this technique will achieve detection limits approaching the photodiode array.

Field Studies

Preparations are well underway for the first field program which is scheduled to begin during the first week of May 1994. The field bioassay and sampling effort is being coordinated with Robert Tichenor of Maryland Department of Agriculture who oversees the pesticide spraying program.

Three platforms have been built for deployment of copepod containers in the field. Each platform carries beneath it four x 1.5L polypropylene chambers. Each chamber has four 62 μm Nitex windows which allow water circulation while retaining animals as small as stage 1 nauplii. Benthic (amphipod) chambers are rectangular polypropylene boxes with Nitex windows on each side above and below the sediment-water interface. Each container contains 2" of sediment. Field assays run between 10 days (for copepods) to 3 weeks (for amphipods) with some adjustments for ambient temperature. Toxic endpoints will be mortality, growth, fecundity and cuticular abnormalities. Water and sediments at all bioassay sites will be monitored for a broad range of trace metals and organic chemicals using AAS and GC-MS. Organic carbon content and grain size characteristics of sediments will also be analyzed.

1995 Laboratory Assays

Laboratory assays will be conducted using Eurytemora affinis to determine the effect of Dimilin® adsorption to particulates on its bioavailability/toxicity. Both inorganic (kaolin) and organic (algae) particles will be studied. Savitz et al. (1994) used a 1:1 mixture of Thallasiosira fluviatilis and Isochrysis galbana for E. affinis culture. Kaolin will be used as the inorganic particulate in view of the fact that Dimilin® is normally applied as a kaolin-adsorbed powder or suspension. Two assays will be performed:

1. The toxicity of 1μg L^{-1} Dimilin® will be tested as (a) pure compound, (b) pure compound plus kaolin suspension (same density ad field application), (c) pure compound plus algal density of 10^5 cells/ml, (d) pure compound plus algal density of 10^4 cells/ml, (e) kaolin only control, (f) algal control (10^5 cells/ml). Mortality of nauplii will be followed in (6) replicated containers over a 6-day period (Savitz et al 1994).

2. Toxicity of 1μg L^{-1} Dimilin® will be tested as (a) pure compound, (b) pure compound plus kaolin suspension, (c) pure compound plus kaolin suspension plus algae (10^5 cells/ml), (d) kaolin only control.

1995 Mesocosm Study

In addition to laboratory and field assays, a mesocosm study will be conducted at the Chesapeake Biological Laboratory using Dimilin®-contaminated leaves collected from the field. We consider it important to have good quantitative information on the degree and time course of wash-off from foliage. A representative sub-sample of leaves will be taken from the field collected sample and analyzed for Dimilin® content. Rainwater will be directed onto Dimilin®-contaminated foliage via a sprinkler system designed to deliver a measured volume of water per unit time. Rate of wash-off will be determined by analyzing foliage at three different time intervals following the initiation of the simulated rain event. Water from the leaves will be allowed to percolate through soil (collected from an uncontaminated field site) and directed into a through flow mesocosm.

The mesocosm itself consists of a 7' fiberglass channel containing 6" of sediment. Water flowing through the channel consists of well water adjusted to approximately 1‰ salinity with Patuxent River water. Entrained in the water column will be quadruplicate copepod chambers of identical design to those deployed in the field. Four benthic chambers containing amphipods will be placed in the sediments to a depth of two inches. Each chamber will initially contain 150 copepods (E.affinis) nauplii and 15 juvenile amphipods (L.plumulosus) respectively.

Assays will be run for periods of 10d (copepods) to 21d (amphipods) with possible minor adjustments made according to ambient water temperature. Toxic end points for copepod and amphipod bioassays will be the same as field assays, i.e. mortality, growth, fecundity and cuticular abnormalities.

Assays will be conducted following a single simulated rain event and (in separate experiments) multiple rain events. Follow-up experiments will encompass multiple Dimilin® applications and will incorporate into the design the concept of delayed toxicity (see Harrahy et al., 1994, and Progress to date).

1995 Field Assay (Transect)

Initial chemical analyses will be performed in the field in order to characterize a concentration gradient for Dimilin®, following a spraying event. A 3-station transect will be established down this gradient and in situ bioassays will be conducted at these sites using end-points characterized

in Year One and refined in the light of laboratory experiments on both test species. Mortality, growth, fecundity and cuticular abnormalities will all be considered as end points.

Statistical Analyses

Results obtained from all field and laboratory bioassays will be analyzed using appropriate parametric or non-parametric statistics, dependent on the requirements of each data set. Whenever feasible, analysis of variance will be used to compare sites or between treatments to detect significant differences ($p < 0.05$). The 96h LC_{50} and EC_{50} values and 95% confidence limits will be determined for toxicity tests using parametric (probit) and non-parametric (Trimmed Spearman Karber) procedures.

TABLE 1

Effects of Diflubenzuron on Maturating and Reproductive Success of *Eurytemora affinis* (from Savitz and Wright 1992)

	Continuous Exposure				Early Exposure				Late Exposure			
	Control	0.5 µg/L	1.0 µg/L	1.25 µg/L	Control	0.5 µg/L	1.0 µg/L	1.25 µg/L	Control	0.5 µg/L	1.0µg/L	1.25 µg/L
Percent Survival±S.D	96.25±4.79	98.33±2.89	67.5^±8.66	22.5^±21.02	90.00±7.07	87.50±6.46	41.25^±7.50	8.75^±4.79	86.25±8.54	92.50±11.90	83.75±11.09	82.50±11.90
Percent Adults ± S.D.	93.40±3.00	98.33±2.89	71.78^±11.00	6.35^±7.45	95.59±5.63	97.21±3.22	86.11±3.98	62.50±7.87	100±	96.25±3.80	80.80±7.90	81.90±6.90
Percent Females w/Broods ±SD	70.80±10.69	52.30±20.18	0.	0.	81.55±7.86	58.33±21.51	45.83±1.67	37.50±7.87	50.60±1.48	81.61±2.76	0^	6.25^±0.13
Naupli/Female					9.5±4.16	13.00±9.48	2.56±5.13	0^	10.27±3.85	7.30±5.94	0	0
Percent Exuviae attached	0.00	0.00	13.00	8.25	0.00	0.00	0.00	0.00	0.00	0.00	17.00	30.80
Total / Survivors n=80	77	59(n=60)	60	18	74	70	33	7	69	74	67	66

^Significantly different from control at $p<0.05$

96 Hour Toxicity of DIMILIN to Hyalella azteca

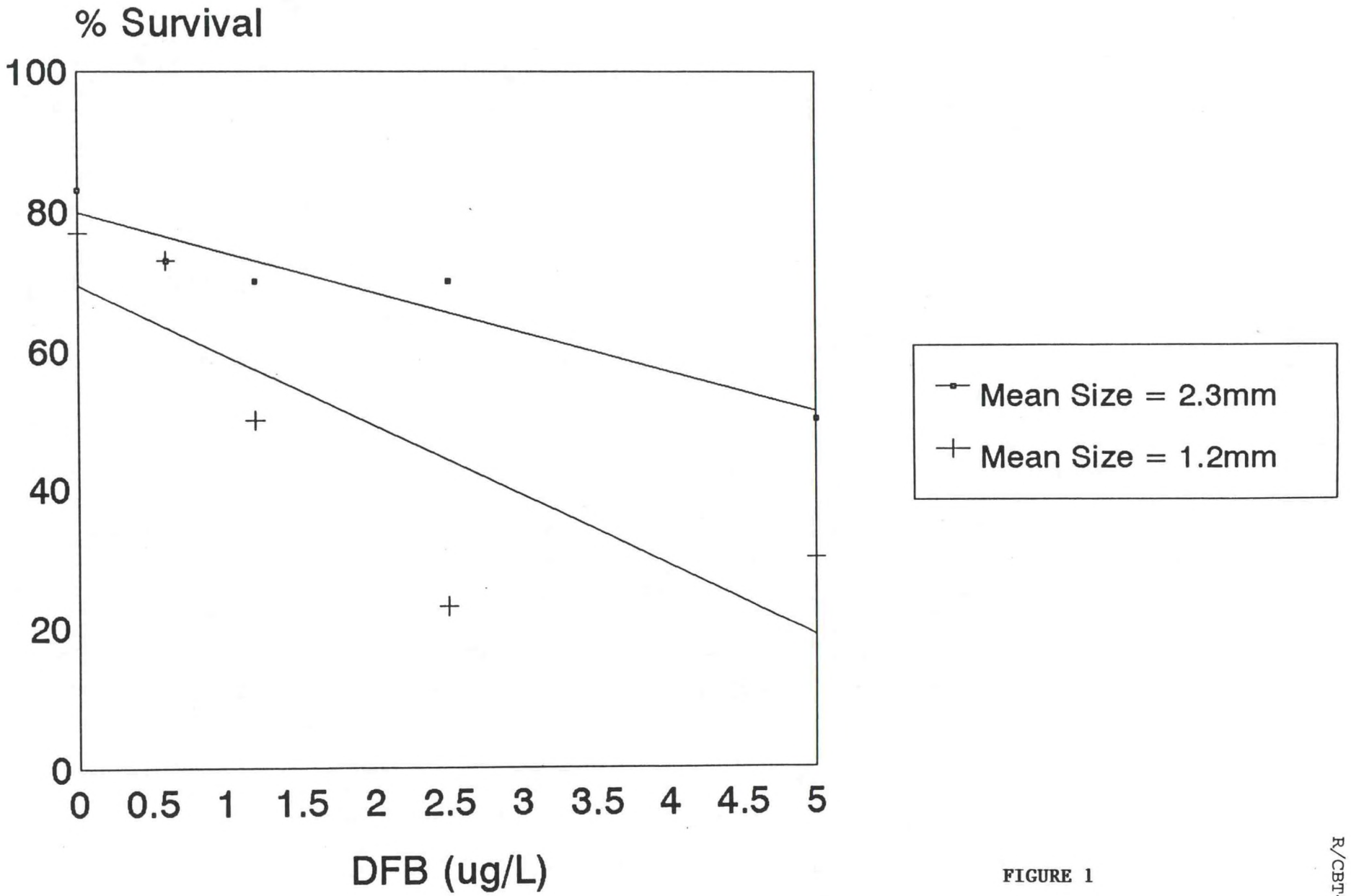

FIGURE 1

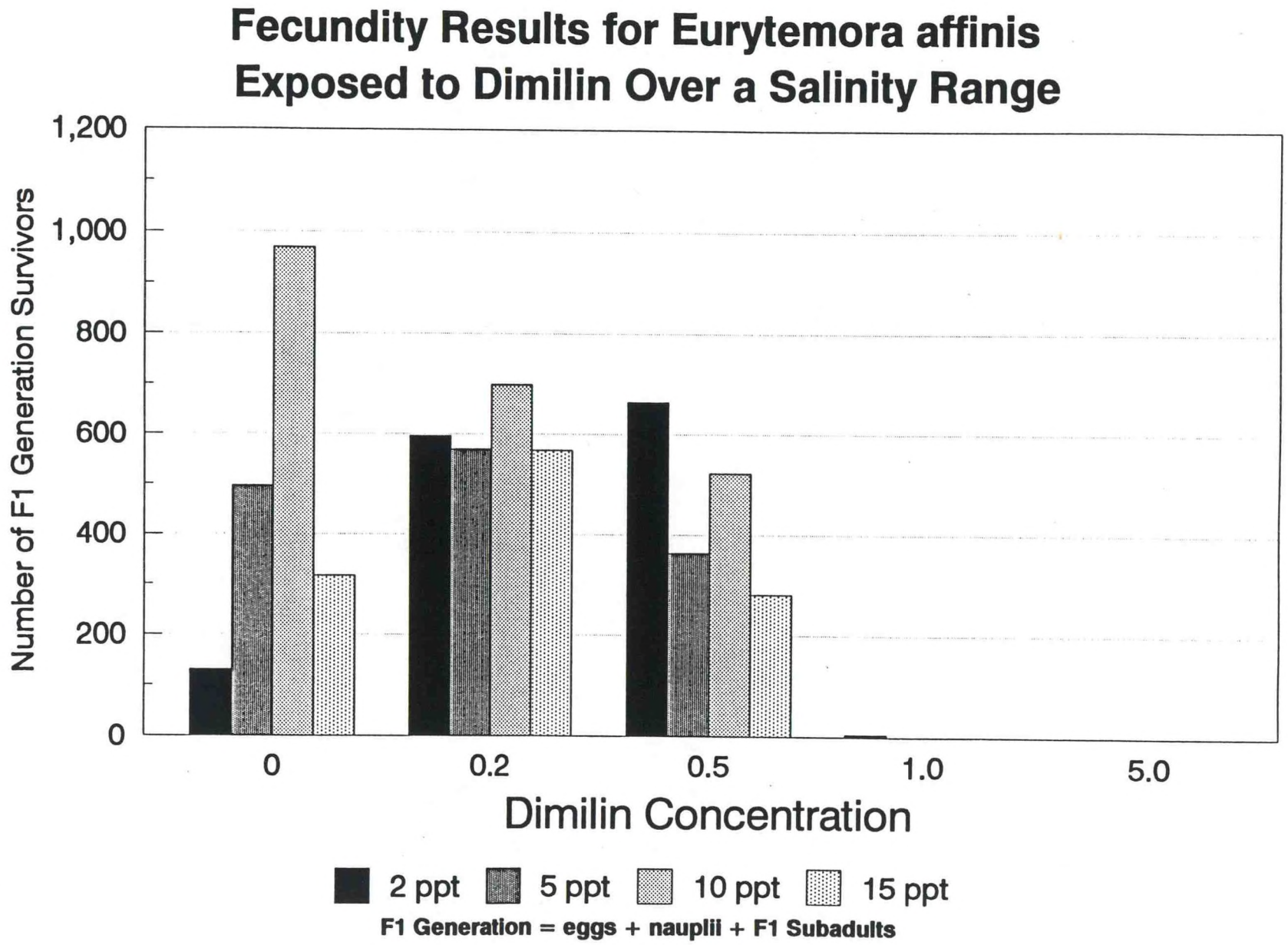
Fecundity Results for Eurytemora affinis
Exposed to Dimilin Over a Salinity Range
Number of F1 Generation Survivors
1,200
1,000
800
600
400
200
0
0
0.2
0.5
1.0
5.0
Dimilin Concentration
2 ppt
5 ppt
10 ppt
15 ppt
F1 Generation = eggs + nauplii + F1 Subadults

FIGURE 2

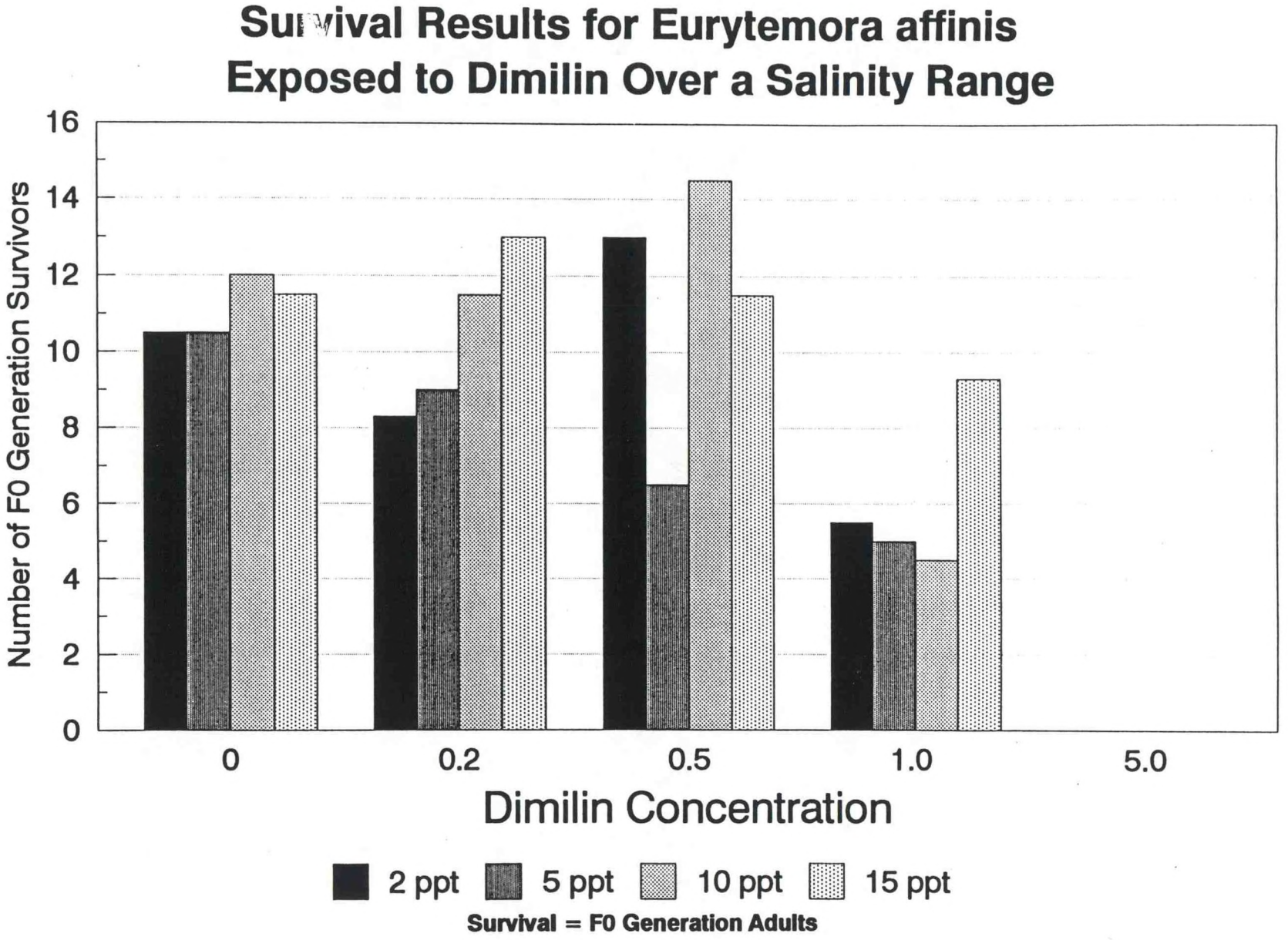
Survival Results for Eurytemora affinis
Exposed to Dimilin Over a Salinity Range
Number of F0 Generation Survivors
16
14
12
10
8
6
4
2
0
0
0.2
0.5
1.0
5.0
Dimilin Concentration
2 ppt
5 ppt
10 ppt
15 ppt
Survival = F0 Generation Adults

FIGURE 3

BUDGET JUSTIFICATION

Funds are requested for partial (10%) salary support for the P.I. to conduct this study. Specifically Dr Wright will be responsible for the design and conduct of the laboratory and field toxicological experiments, statistical design and reporting of bioassay results. Dr Dawson will be responsible for chemical measurements of field and laboratory samples and the design and execution of field sampling for sediment cores and integrated water samples.

A graduate research assistant will be expected to assist in the field program as well as in the maintenance of cultures and sample preparation for chemical and biological analysis. It is expected that the GRA would develop aspects of the research into an independent thesis research leading to a Masters degree.

Secretarial assistance is requested to cover 10% of salary support.

Travel funds have been calculated to cover costs of transport and accommodations for field work at remote sites and for attending local conferences and planning sessions with Agencies involved in the Program.

Expendables and supplies have been budgeted according to past experience in operating organic chemistry and toxicology laboratories and include: compressed gases, HPLC solvents, HPLC and GC supplies and standards, columns, sampler vials, filters, replaceable parts, septa, piston seals, glassware and shop services.

Analytical service costs are projected at $12,000 each year and are based on the current costs for TOC, DOC, and CHN analyses for ancillary parameter characterization, as well as for determinations of trace metals by AAS and organic analyses by GC-MS including mass spectrometer usage for electrospray LC-MS.

A daily charge is made for the use of small boats (25ft) belonging to the CBL fleet. This has been estimated at $1,500 annually.

Investigators are required to contribute to general service contracts on common use equipment at the laboratory and for access to Decstation computing facilities and electronic mail (1.5% of TDC).

PERMANENT EQUIPMENT

Funds are requested ($5000) for the purchase of components to upgrade the mass spectrometer (HP5989 MS engine) with an electrospray inlet for LC-MS. These include a low vacuum diffusion pump, a regulated high voltage supply and specialized fittings and capillaries. We shall draw upon the expertise of the Naval Research Laboratory in the construction of the inlet.

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[UMCEES]CBL 94-

PROJECT NUMBER: _____

PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	YEARS 1995			
Chemical analyses of field samples from 1994.	January			
Copopod bioassays. Effect of particles on bioavailability.	Jan - April			
Mesocosm study. Effect of single rain event/single Dimilin application.	March - April			
Mesocosm study. Effect of multiple rain events/single Dimilin application.	May - June			
Laboratory study of Dimilin partitioning in sediments.	May - June			
Field studies. Bioassays along Dimilin concentration gradient and at reference site.	May - June			
Mesocosm study. Effect of multiple Dimilin applications/multiple rain events.	June - July			
Mesocosm study. Effect of different soil and sediment types.	August - September			
Completion of chemical analyses from field and laboratory studies.	September - October			
Collation of field and laboratory data. Preparation of final report.	November -December			
PROJECTED BUDGET: FEDERAL	$ 94,216			
MATCH				

SEA GRANT PROJECT RECORD FORM

SG-SID-No.:_____
Specialist:_____
SG Class: _____

I. PROJECT SUMMARY INFORMATION

INSTITUTION: Virginia Graduate Marine Science Consortium
ICODE: 5100
TITLE: Organic contaminant metabolite production, elimination,and bioavailability in benthic macrofauna of lower Chesapeake Bay

PROJECT NUMBER: R/CBT-25
PROJECT STATUS: 2
SUB PROGRAM: Toxics/CBEEC

REVISION DATE: 06/15/94
INITIATION DATE: 01/01/94
COMPLETION DATE: 12/31/95

PRINCIPAL INVESTIGATOR: Rebecca M. Dickhut
AFFILIATION: Virginia Institute of Marine Science
CO-PRINCIPAL INVESTIGATOR: Linda Schaffner
AFFILIATION: Virginia Institute of Marine Science

EFFORT: 0.6
AFFILIATION CODE: 5101
EFFORT: 0.6
AFFILIATION CODE: 5101

S.G. FUNDS: 0
LAST YEAR'S SG FUNDS: 0
PASS-THROUGH FUNDS: $ 89,414

STATE MATCHING FUNDS: 0
LAST YEAR'S MATCH FUNDS: 0
LAST YEAR'S PASS-THROUGH: $104,029

RELATED PROJECTS: All R/CBT projects
PARENT PROJECTS: R/CBT-15
SEA GRANT CLASSIFICATION: Pollution-other-toxics (45)
KEYWORDS: organic contaminants, PAH, PCB, macrofauna, metabolism

OBJECTIVES: Using Chesapeake Bay macrofauna as representative estuarine species we will (1) quantitatively evaluate the rates of uptake, transformation, metabolite binding and elimination of a series of representative organic contaminants; (2) examine the rates of uptake and metabolism of representative organic contaminants as a function of food source material; and (3) determine the fate of organic contaminant metabolites produced for a series of representative organic contaminant parent compounds.

METHODOLOGY: A microcosm approach will be used to evaluate uptake, transformation, binding and elimination processes in selected estuarine benthic macrofauna as a function of food source and contaminant properties. Benthic macrofauna will be selected for study based on ecological importance, abundance, and demonstrated ability to metabolize organic contaminants. Polycyclic aromatic hydrocarbons and polychlorinated biphenyls will be selected for study based on their hydrophobicity, anticipated difference in uptake, transformation, and elimination rates. Microcosms prepared with various combinations of sediment or sterile, solvent extracted fine sand substrate, presence of absence of benthic macrofauna and contaminated food source material will be used to resolve microbial versus macrofauna release of metabolites from the sediment into the water column. Elimination rate experiments will be used to assess rates of contaminant elimination from body tissues. Radiolabeled organic chemical analyses will be used to identify: 1) parent compound, 2) primary metabolites which are more polar than the parent compound but still extractable with organic solvents, 3) secondary, conjugated metabolites which are water soluable, and 4) bound metabolites which are resistant to extraction.

RATIONALE: The proposed research emphasizes quantification of the rates of organic contaminant uptake, and subsequent metabolite production, binding and elimination by representative benthic organisms. Our ongoing research (R/CBT-15) indicates that metabolite production and subsequent cycling is a potentially important process governing contaminant transfer and fate at the sediment-water interface. The biogeochemical cycling of metabolites is virtually unknown but has major implications for a variety of ecosystem processes including trophic transfer. This effort will allow determination of the disposition of organic contaminant parent compound and metabolites within the benthic region and delineation of associated risks after initial exposure of the benthos to organic pollutants. The proposed research is highly relevant to understanding the bioavailability of organic contaminants from the benthos to demersal predators.

**** SEA GRANT BUDGET ****

GRANTEE:
Virginia Graduate Marine Science Consortium

PROJECT NO:
R/CBT-25

PROJECT TITLE:
Organic contaminant metabolite production, elimination and bioavailability in benthic macrofauna of lower Chesapeake Bay

PROJECT STATUS:
2

PRINCIPAL INVESTIGATORS:
Dickhut, R.M. & L.C. Schaffner

DURATION:
1/1/95 - 12/31/95

A. SALARIES AND WAGES	No. of Person.	Months	Sea Grant Funds	Grantee Funds
1. Senior Personnel				
a. Prin. Investigator	2	1.2	10,400	0
b. Associates:	0	0.00	0	0
Sub Total:			10,400	0
2. Other Personnel				
a. Professionals			0	0
b. Research Assoc.			0	0
c. RA Grad. Stud.	2		29,400	0
d. Prof. School Stud.			0	0
e. Pre-Bac. Stud.			0	0
f. Secret./Clerical			0	0
g. Technical/Shop	1		8,049	0
h. Hourly Labor			3,024	0
Total Salaries and Wages			50,873	0
B. FRINGE BENEFITS			5,213	0
Total Sal. Wages & Fringe Benefits (A+B)			56,086	0
C. PERMANENT EQUIPMENT			0	0
D. EXPENDABLE SUPPLIES			8,500	0
E. TRAVEL				
1. Domestic - US & Possessions	1.		3,100	0
2. International	2.		0	0
Total Travel			3,100	0
F. PUBLICATION AND DOCUMENTATION COSTS			1,000	0
G. OTHER COSTS				
1. ship/vessel rental			1,500	0
2. tuition			1,800	0
3. gas cyliner rental			165	0
4. photcopying			300	0
5. graphic arts			1,001	0
6.				
7.				
8.				
9.				
Total Other Costs			4,766	0
TOTAL DIRECT COSTS (A through G)			73,452	0
INDIRECT COSTS: On Campus: 35.47% of MTDC*			14,455	0
Off Campus: 5% of A2c			1,507	0
TOTAL INDIRECT COSTS			15,962	0
TOTAL COSTS			89,414	0

* less A2c, G1, G2, G5

ORGANIC CONTAMINANT METABOLITE PRODUCTION, ELIMINATION, AND BIOAVAILABILITY IN BENTHIC MACROFAUNA OF LOWER CHESAPEAKE BAY

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INTRODUCTION

Our work (Schaffner and Dickhut, 1991) and that of others (*e.g.* McElroy 1985 and 1990; Weston, 1990) illustrates the strong capability of a variety of benthic macrofauna to accumulate and transform organic contaminants, in particular, polycyclic aromatic hydrocarbons (PAHs) and low molecular weight polychlorinated biphenyls (PCBs). Bioaccumulation and biotransformation vary both within and among major taxa, and with the organic contaminant (OC) physical-chemical properties (*e.g.* desorption rate, diffusivity, octanol/water partition coefficient - K_{ow}) (Dickhut *et al.*, 1994). Moreover, large fractions of various organic contaminants in certain benthic macrofauna exist as metabolic products (Dickhut *et al.*, 1994; McElroy 1985 and 1990) and little is known about the bioavailability and effects of these substances. Metabolites resulting from benthic macrofauna biotransformation of OCs in sediments can potentially adversely effect the organisms directly or be transferred through trophic interactions with subsequent effects on consumers. Thus, to fully understand the effects of organic contaminants on benthic organisms and the influence of benthic biota in the transfer of toxic substances to other aquatic species, it is necessary to quantitatively evaluate the production, binding and elimination of OC metabolites in benthic macrofauna.

This research emphasizes quantification of the rates of organic contaminant (OC) uptake, and subsequent metabolite production, binding and elimination by selected benthic macrofauna from lower Chesapeake Bay. This research effort will allow determination of the disposition of organic contaminant parent compound and metabolites within the benthic region and delineation of associated risks after initial exposure of the benthos to organic pollutants.

OBJECTIVES

Overall Objectives

Using Chesapeake Bay macrofauna as representative estuarine species we will:

[1] quantitatively evaluate the rates of uptake, transformation, binding and elimination of a series of representative organic contaminants;

[2] examine the rates of uptake and metabolism of representative organic contaminants as a function of food source material;

[3] determine the fate of organic contaminant metabolites produced for a series of representative organic contaminant parent compounds.

1995 Objectives

[1] Complete microcosm and elimination rate studies.

[2] Conclude data analysis and prepare manuscripts for publication.

PROGRESS

We have developed an analytical protocol to quantify organic contaminant parent compounds, polar and conjugated metabolite fractions, and nonextractable, cellular bound organic contaminant fractions in benthic organisms. Briefly, organic contaminants and degradation products are extracted from the organism using a combination of methanol, dichloromethane, and water. After extracting the tissue twice, the sample residue is removed via centrifugation and reextracted with dichloromethane. Subsequently, the residual tissue is then dried and combusted at 1000°C with evolution of cellular bound radioactive chemical as $^{14}CO_2$ or tritium gas which is trapped in phenethylamine and quantified using liquid scintillation counting (LSC). The solvent extracts are fractionated into organic and aqueous soluble components and subsequent radioactivities determined via a combination of high performance liquid

chromatography (HPLC) and LSC. Parent compounds and polar metabolites associated with the organic fraction are resolved using HPLC; polar metabolites elute prior to parent compounds on a reversed phase column which allows for separation and LSC analysis. Evaluation of radioactivity in the aqueous fraction results in quantification of secondary, conjugated metabolites. We have implemented this extraction procedure in our lab for our winter, benthic community microcosm experiment (Schaffner and Dickhut, 1991).

REMAINING PROJECT ACTIVITY

During 1994 and 1995, we will be conducting microcosm experiments with selected benthic macrofauna and contaminated food source material (*i.e.* surface sediment or plankton) to evaluate organic contaminant uptake, metabolite production, binding and egestion/elimination in benthic macrofauna from lower Chesapeake Bay. We will be conducting experiments on two benthic macrofauna species (*Leptcheirus plumulosus*, *Paraprionospio pinnata* or *Loimia medusa*) during 1994; at least one of these sets of experiments will be conducted for a Masters' thesis project. The remaining experiments will be conducted during 1995. Our first experiment is scheduled for late spring and the second for late summer, 1994. Data from these experiments will be used to model organic contaminant uptake, metabolite formation, elimination and binding rates for a series of organic contaminants ranging in hydrophobicity (*e.g.* log K_{ow}'s between 4 and 7) in selected benthic organisms from Chesapeake Bay. Each process will be examined for relationships to species and chemical contaminant characteristics.

DISSEMINATION OF RESULTS

The results of this work will be of interest to those involved in making management decisions for the Chesapeake Bay and to the scientific community in general. Results will be distributed to appropriate agencies and individuals as advised by the local Sea Grant office. Our results will also be presented at national scientific meetings (*e.g.* Society of Environmental Toxicology and Chemistry, Estuarine Research Federation), and manuscripts prepared for publication in appropriate peer-reviewed scientific journals.

BUDGET JUSTIFICATION

Supplies for this project total $8,500 in year two which includes: $2000 for radioactive organic chemicals and $5000 for scintillation cocktail and vials, organic solvents, HPLC columns and supplies, metaboite standards, materials for enzyme assays, biological oxidizer scintillation cocktail, cataylst, and combustion tubes, glassware and general lab supplies.

Travel costs requested are $3100 for student and principal investigator travel to a national meeting (*e.g.* SETAC, ACS, ERF/IAGLR) to present results from the research during year two of the project.

LITERATURE CITED

Dickhut, R.M., L.C. Schaffner, P.W. Lay and S. Mitra. 1994. *Bioaccumulation and Biotransformation of Selected Organic Contaminants by Benthic Macrofauna from Lower Chesapeake Bay*. In preparation.

McElroy, A.E. 1990. *Polycyclic aromatic hydrocarbon metabolism in the polychaete Nereis virens*. Aquatic Toxicology, 18:35-50.

McElroy, A.E. 1985. *In vivo metabolism of benz[a]anthracene by the polychaete Nereis virens*. Marine Environ. Res., 17:133-136.

McElroy, A.E., J.W. Farrington and J.M. Teal. 1990. *Influence of mode of exposure and the presence of tubiculous polychaete on the fate of benz[a]anthracene in the benthos*. Environ. Sci. Technol. 24:1648-1655.

Schaffner L.C. and R.M. Dickhut. 1991. *Role of benthic communities in sediment-associated toxic organic chemical fate and transport in lower Chesapeake Bay*. Proposal to the Chesapeake Bay Environmental Effects Studies (CBEES) Toxics Research Program. Project No. R/CBT-15.

Weston, D.P. 1990. *Hydrocarbon bioaccumulation from contaminated sediment by the deposit-feeding polychaete Abarenicola pacifica*. Mar. Biol. 107:159-169.

PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	Y E A R			
	1994	1995	1996	1997
Develop analytical protocol for analysis of radiolabeled organic parent compound and metabolites	———			
Perform microcosm studies examining organic contaminant uptake and metabolite formation, binding and elimination in benthic macrofauna	———	———		
Evaluate data and prepare manuscripts for publication		———		
PROJECTED FEDERAL BUDGET	$104,029	$ 89,414	$	$
PROJECTED MATCH BUDGET	$ 0	$ 0	$	$

*** MARYLAND SEA GRANT PROJECT SUMMARY 1994 ***

Title: Quantitative Evaluation of Contaminants in a Chesapeake Bay Region of Concern: A Simulation Model of Exposure and Bioaccumulation in Baltimore Harbor

Project Number: R/CBT-26
Grant Number:
Status: New
Revision Date:
Initiation Date: September 1, 1994
Completion Date: December 31, 1996

Principal Investigator: Christopher J. Madden
Affiliation: Horn Point Environmental Laboratory
Months Committed: 4.0

Principal Investigator: John R. Kucklick & Joel E. Baker
Affiliation: Chesapeake Biological Laboratory
Months Committed: 4.0 & 0.6

Proposed Federal Funds: $49,690
Current Federal Funds: $
Federal Funds to Date: $
Proposed Matching Funds: $
Current Matching Funds: $
Match to Date: $
Related Projects:
Sea Grant Classification#: 45
Keywords: toxic contaminants, HOC, ecosystem modeling, Baltimore Harbor

Objectives: To synthesize available contaminants data and use simulation modeling techniques to integrate existing information and explore processes that control exposure, transfer, and accumulation of contaminants in biota in Baltimore Harbor. Also, to explore how the dynamics of the carbon flow in Baltimore Harbor affects the cycling of toxic organic contaminants in the water column biota and their eventual delivery to the sediments.

Methodology: We will interact with state and national agencies to obtain all available information on organic contaminants in Baltimore Harbor sediments, water and biota, which will be screened for completeness and quality, before incorporation into a calibration database. Concurrent with the database formalization, a simulation model will be developed specific to Baltimore Harbor encompassing both the carbon and contaminant flows in the water column and sediment biota. The database information will then be used to calibrate the model and determine if the model is indeed predicting the observed contaminant concentrations.

Rationale: The eventual fate of toxic organic contaminants in aquatic systems appears to be closely linked to the trophic carbon flow and affected by such parameters as phytoplankton growth, zooplankton grazing rates and benthic production. In addition , the impact of sublethal toxic effects on these processes may mediate the cycling of organic contaminants as they are manifested in ecosystems effects. The project attempts to rationalize a variety contaminant information on a specific Region of Concern in Chesapeake Bay via a contaminant-ecosystem model with the goal of understanding and predicting the impacts of toxic organic contaminants on estuarine systems.

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1995 GRANT/PROJ. NO: RCBT-26

University of Maryland, Chesapeake Biological Laboratory

PRINCIPAL INVESTIGATORS: Chris J. Madden, John R. Kucklick and Joel E. Baker

DURATION (MOS.): 16 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	8.00		22,247	
b. Associates				
Sub Total			22,247	0
2. OTHER PERSONNEL				
a. Professionals				
b. Research Associates				
c. Res. Asst. Grad. Std.				
d. Prof. School Students				
e. Pre-Bac Students				
f. Secretarial-Clerical				
g. Technical-Shop				
Total Salaries/ Wages			22,247	0
B. FRINGE BENEFITS			6,897	
Total Salaries,Wages and Fringe Benefits			29,144	0
C. PERMANENT EQUIPMENT			5,000	
D. EXPENDABLE SUPPLIES AND EQUIPMENT			750	
E. TRAVEL				
1. Domestic	1,300			
2. International				
Total Travel			1,300	0
F. PUBLICATIONS AND DOCUMENTATION COSTS				
G. OTHER COSTS				
1. Computer costs				
2. Copying,Library and Communication			400	
3. Analytical and Shop Services				
4. Fuel,Boat Time and Vehicle Usage				
5. Word Processing				
6. Institutional Allowance				
7. Rapid Response and Program Development				
8. Shipping Charges				
9. Service Contracts			557	
Total Other Costs			957	0
TOTAL DIRECT COSTS			37,151	0
INDIRECT COSTS				
(On Campus) : 39 % of 32,151			12,539	
(Off Campus) : % of				
Total Indirect Costs			12,539	0
TOTAL COSTS			49,690	0

QUANTITATIVE EVALUATION OF CONTAMINANTS IN A CHESAPEAKE BAY REGION OF CONCERN: A SIMULATION MODEL OF EXPOSURE AND BIOACCUMULATION IN BALTIMORE HARBOR

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Horn Point Environmental Laboratory

John R. Kucklick, Assistant Research Scientist
Joel E. Baker, Assistant Professor
Chesapeake Biological Laboratory

INTRODUCTION

In recent years, the watershed of Chesapeake Bay has been undergoing intense urban, agricultural, and industrial development, resulting in a serious degradation in the environmental quality of the estuary. Among the most serious impacts are the chronic loadings of heavy metals, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and other contaminants to receiving waters which affects biota either through direct toxicity (Hall, 1988), or by bioaccumulation of hydrophobic organic contaminants (HOCs) (Schimmel *et al.*, 1979). The deleterious effects of contaminants on estuarine organisms and human health has led to increased impetus at state and national levels to delineate areas in the Chesapeake Bay where toxics problems are likely to occur and determine what impact these problems might have on the ecosystem. As a direct consequence, three sites in Chesapeake Bay which merit particular attention, the Anacostia and Elizabeth Rivers and the Baltimore Harbor, have been tentatively designated by the Chesapeake Executive Council as Regions of Concern (USEPA 1993). In our proposed study, we plan to focus on one of these Regions of Concern, Baltimore Harbor, investigating the effects of HOC contamination on the estuarine food web and the fate of these contaminants in the environment using mathematical modeling techniques.

Sediments in Baltimore Harbor contain high concentrations of many organic contaminants including PCBs, PAHs, chlordanes, and DDT (Morgan and Sommer, 1979; NOAA, 1988; O'Connor and Huggett, 1988; Foster and Wright, 1988). The effects of these toxicants on native and transient organisms and on the general health of the ecosystem have yet to be completely elucidated. From this project will emerge the specific benefit of a better, quantitative understanding of effects of pollution problems in Baltimore Harbor, and in a larger context, provide a protocol for evaluating other Regions of Concern throughout Chesapeake Bay. The proposed study will incorporate three phases:

1. An analysis and synthesis of existing information on toxic HOCs in sediment, biota and water in Baltimore Harbor.

2. The development of an ecosystem simulation model including the principal trophic groups in Baltimore Harbor focusing on biogeochemical processes, HOC kinetics, autochthonous processes, allochthonous inputs, water column biotic components, benthic-pelagic coupling, and toxicant transfer to fish.

3. A model experimentation phase including a detailed examination of intra-system characteristics, by adjusting the model to conduct sensitivity analysis, and an

evaluation of specific scenarios to determine the relationship of contaminant input rates, input levels, pulse frequencies, and congener characteristics to exposure levels, bioaccumulation factors and transfer rates in the environment.

Previous and ongoing field studies in the Baltimore Harbor include long-term monitoring of toxics and biological resources by a number of organizations such as VERSAR, Maryland Department of the Environment (MDE) and the National Oceanographic and Atmospheric Administration (NOAA) Mussel Watch program. These investigations have resulted in the development of several databases which are currently available in various formats, diverse stages of analysis, and stored in different locations. In addition, MDE is now initiating a new study of nutrient loadings to Baltimore Harbor which will add further to the base of available data. Preliminary to the modeling process, we will collate and integrate this information into a coherent database specifically designed as a calibration dataset which we will make available to the public. We will evaluate the HOC data for completeness, analytical integrity, consistency, and accuracy preparatory to it's incorporation into the model.

We have experience with the development and use of models as tools for evaluating and synthesizing information on HOCs in Chesapeake Bay. In a current CBEEC study, we have developed two models which address different aspects of contaminant problems. The first, a 5-stage trophic transfer model, is used to study in a general way the kinetics and transfer mechanisms of HOCs typical of the mesohaline Chesapeake Bay through a food web. The second tool is the product of an integration of the 5-stage model into a more complex carbon flow simulation specific for the mesohaline Chesapeake Bay. This tool has enabled us to answer larger ecosystem-level questions about the mesohaline bay and make predictions about the system with a high degree of confidence. We propose to develop similar models for Baltimore Harbor which will enable us to identify key questions and sharpen the focus and direction of toxics research. We hope that this results in progress toward an understanding of how contaminants cycle in the environment and how to best remediate toxic effects.

Modeling contaminants in aquatic ecosystems

Consumers in the aquatic environment acquire HOCs by direct diffusive partitioning through water and by ingesting contaminated food. Phytoplankton receive contaminants only by diffusive uptake, since there is no feeding route. The equation used to model diffusive uptake by organisms takes the form:

$$dC/dt = -\alpha A_s/V\ (C_t - K_{ow} \times C_w) \qquad (1)$$

where dC/dt is the contaminant flux from the water to the organism occupying a given volume of water (mass HOC/volume water), α is the mass transfer coefficient (length/time), A_S is the organismal surface area (length^2), V is the water volume (length^3), C_t is the HOC concentration at time t (mass/water volume), K_{ow} is the octanol/water partition coefficient and C_w is the ambient water HOC concentration (mass/volume). Adequate description of the diffusive flux requires a knowledge of both the dissolved (unassociated) HOC concentration (C_t), the exchange surface area, and the equilibrium state. The term $-\alpha A_S/V$, which is highly organism-specific, is often consolidated into a single term in most models and is therefore highly vulnerable to error, since the surface

area of small organisms such as phytoplankton, is often large relative to volume (Brown *et al.*, 1982). The mass transfer coefficient is affected by the contaminant's hydrophobicity, steric properties and the nature of the exchange membrane. The diffusive equation for fish is often refined to include variable ventilation rates as a function of temperature (Norstrom *et al.*, 1976, Thomann *et al,* 1992). Since α for a contaminant is not usually known as a function of temperature, it is related to the mass transfer coefficient for oxygen, which is known well as a function of temperature (Thomann *et al.*, 1992).

Contaminants can also accumulate in organisms by feeding routes, generally modeled in the following fashion:

$$dC_{pred}/dt = (A \times C_{prey} \times I) - E \times C_{pred} \quad (2)$$

where dC_{pred}/dt (mg HOC/mg lipid/time) is the time-dependent concentration in the predator, C_{prey} is the prey concentration (mg HOC/mg lipid), C_{pred} (mg HOC/mg lipid) is the predator contaminant concentration, I (mg prey lipid/mg pred lipid) is the predator's ingestion rate of prey, A (mg HOC assimilated/mg ingested) is the HOC assimilation efficiency of the predator and E (mg HOC/day) is the predator's excretion rate (Norstrom *et al.*, 1976; Connolly and Tonelli, 1985; Thomann, 1989; Thomann *et al.*, 1992a,b). Assuming the compound is metabolized little, the amount of contaminant exposure from food increases with the number of trophic links.

The full model contaminant trophic transfer model combines equations (1) and (2) so that there is contaminant uptake and depuration by both feeding and diffusion from the water. Further modifications of this model also include the effects of growth and reproduction, which are dilution terms. Several studies have shown that A for organic contaminants is not constant and varies with the ingestion rate (Harding *et al.*, 1982) and contaminant hydrophobicity, expressed as K_{ow} (Thomann, 1989; Thomann *et al.*, 1991). In addition, E appears to be closely linked with contaminant hydrophobicity, increasing with increasing K_{ow} because of poor gut adsorption and a greater affinity for feces (Gobas *et al.*, 1988, 1989, 1993).

Several investigators have proposed trophic transfer models that attempt to explain contaminant levels in different aquatic food webs (Norstrom *et al.*, 1976; Thomann 1981, 1989; Connolly and Tonelli, 1985; Connolly and Pendersen, 1988). Quantitative descriptions of HOC accumulation in estuarine food webs include those of polychlorinated biphenyls in the Hudson River (Thomann *et al.* 1991) and Kepone in the James River of the lower Chesapeake Bay (Connolly and Tonelli 1985). These models have successfully described the annual averages and long-term changes in HOC inventories in the upper trophic levels. However, these investigators developed a model that was essentially driven by the highest trophic levels, using empirical measurements of contaminants in upper trophic levels and then estimating the transfers through phytoplankton and zooplankton needed to support those levels. Organic contaminant levels in phytoplankton and zooplankton are used as fitting parameters in these models. Indeed, the model results of Thomann (1989) and field results of Oliver and Niimi (1988) and Kucklick *et al.* (1994) specifically implicate lower trophic levels, especially phytoplankton, as crucial mediators of the eventual fate of contaminants is aquatic systems.

Benthic interactions can be added to trophic transfer models to provide a more complete picture of HOC cycling in aquatic systems (Bierman, 1990; DiToro *et al.*, 1991; Thomann *et al.*, 1992a,b). The dominant modeling approach is to use the equilibrium partitioning

theory (EPT) where it is assumed that sediment, water and biotic compartments are at equilibrium and can then be described by a series of partition coefficients, *i.e.* the fugacities in sediment organic carbon, water and the organism's lipid are equal. Exposure of benthic organisms to HOCs in sediments occurs through partitioning from the bioavailable fraction in pore water and the overlying water and ingestion of sediment particles (Thomann *et al.*, 1992a). Contaminant concentrations in sediments pore water and adsorbed to sediments have been successfully modeled by DiToro *et al.* (1992) and Karickhoff (1984) and Mackay and Powers (1987). Obviously, the ultimate exposure of a benthic organism will depend strongly on the way it uses the sediment. For instance, burrowing organisms, such as polychaetes, are likely more exposed to porewater HOCs than epibenthic organisms such as oysters. It is also likely that the fraction of time the organism spends associated with contaminated sediments is important to its measured body burden. In addition, several consumers (*eg.* spot and white perch) eat both benthic and pelagic food items. Therefore, their contaminant burdens are influenced by the relative preference for benthic or pelagic food sources and the associated contaminant concentrations. Our model will resolve ecological issues at this level of detail for the Baltimore Harbor ecosystem.

HOC modeling of Chesapeake Bay

Results of the current CBEEC modeling effort have proven extremely useful in addressing questions about the trophic transfer and biomagnification of HOCs in the mesohaline region of Chesapeake Bay. Our model appears robust and well calibrated to carbon flows, and the simulation system has served as a valuable tool for analysis and interpretation of field data such as PCB concentrations in size fractioned particulates collected by J. Baker and colleagues for mesohaline Bay. It appears that processes in the ecosystem are relatively resilient to acute toxic stresses; however, key populations of interest may by vastly more sensitive, and some unknown mechanisms not included in the models could increase the sensitivity to certain processes.

Model simulations of HOC behavior show consistency with field data based on assumptions about size relations for functional groups (ciliates, phytoplankton and zooplankton). Model studies revealed that HOC concentrations increase along trophic links as expected and that differences among congeners could be predicted by octanol/water partition coefficients (K_{ow}). HOC concentrations associated with lower trophic levels, especially phytoplankton are clearly dynamic and apparently kinetically, not thermodynamically mediated. With the aid of model simulations, however, concentrations measured in limited field surveys could be related to mean and maximum expected values over an annual cycle.

The addition of contaminant variables within each of the organic carbon pools (biomass plus detritus) of our plankton community submodel allow us to consider how HOC congeners accumulate at lower ends of plankton food chains. Each congener behaves differently in terms of its bioaccumulation. Overall, food-chain concentrations are generally higher in the model for compounds with lower K_{ow} values (Fig. 1); an exception was the PCB homologue group octachlorobiphenyl (average log K_{ow} = 7.99). A general pattern of increasing HOC concentrations along the food-chain (phytoplankton <bacteria <protozoa <detrital POC <zooplankton) was observed. We compared model output to field data from the mesohaline region of the Bay, assuming various particle size-classes corresponded to different functional groups: phytoplankton= 64-202μ; bacteria <10μ; protozoa = 10-64μ; zooplankton >202μ; POC = mean. There was generally good agreement between model and data with these assumptions, except that HOC pools in

model zooplankton were often only 10% of those measured in the field, suggesting the importance of lipid-rich materials (e.g., eggs) associated with copepods. Under conditions of constant external inputs, organism-bound HOC pools reached pseudo-equilibrium conditions within 6-10 months. When input conditions were varied at seasonal or greater frequencies, however, plankton biomasses varied accordingly and HOC pools never reached equilibrium within one year simulations.

Model experiments conducted to simulate acute toxicity effects at the level of the integrated planktonic-benthic ecosystem illustrated that phytotoxic effects on phytoplankton production are most pronounced on diatoms and are attenuated along trophic pathways, so that 25% reductions in photosynthesis were barely detectable for copepod abundance (Fig. 2). Seasonally concentrated herbicide additions had the greatest impact on the plankton community when focused in the early spring; summer additions had much smaller effect on copepod abundance.

Direct toxic effects on consumer populations were also examined in several ways. Inhibition of copepod ingestion had substantially greater impact on both zooplankton and phytoplankton abundances than did proportionally similar increases in copepod mortality. In all cases, effects were most pronounced on spring copepods (Fig. 1). We also investigated the top-down cascade of ecosystem effects resulting from toxin-induced reductions in planktivorous fish abundance. In general, top-down effects were confined to the summer months, because planktivore abundance is negligible in winter and spring. As expected, reductions in fish predation resulted in substantial increases in copepod abundance, which, in turn, caused smaller decreases in phytoplankton biomass.

The model has also been instrumental in identifying gaps in our data and directing additional research directions. For example, we found that data are needed for HOC sorption kinetics. Improved information is needed for field concentrations of HOCs and other contaminants associated with major taxonomic or functional groups of organisms rather than size-classes only. Further information is also needed on the rates of metabolism for HOCs and other contaminants after incorporation into/onto organism tissues. Finally, based on model-generated HOC mass balances, it appears that colloidal DOC may be an important pool, which needs further description.

Relevance to the problem

Development of an ecosystem-oriented toxic contaminants model for Baltimore Harbor will provide a means to synthesize existing and incoming information about HOCs in this Region of Concern into a coherent understanding; it will provide a tool which will assist in making management decisions about toxics remediation and in developing criteria for evaluating the status of the Bay's subsystems; it will aid in identifying important areas for future toxics research; and it will provide a protocol for assessing other Regions of Concern. The working simulation model will give us a means of answering specific questions about toxics in the environment, for example the effect that nutrient mitigation, stormwater runoff and dredging will have on contaminant levels in biota, sediments and water. It will the help us to understand if the HOC concentrations that are currently observed in fish are most likely from new inputs (eg. stormwater runoff) or simply recycled from the sediments, and consequently, will provide guidance in management decisions as how to best handle the problem. Modeling will provide insights into the interactions between HOC cycling and the biotic and abiotic components of the bay.

OBJECTIVES

Overall objectives

With this project, our general objective is to synthesize available contaminant data using simulation modeling techniques to explore processes that control exposure, transfer, and accumulation of HOCs in biota in Baltimore Harbor. The resulting model will be used to explore the potential results of various proposed management scenarios for nutrient mitigation, dredging or other activities on the cycling of toxic organic contaminants in Baltimore Harbor. We will evaluate the effects of contaminants on organisms within various ecological functional groups, such as transient versus resident species, pelagic versus demersal dwellers, benthic in- versus epi-fauna. Our other general objective is to address the following specific hypotheses through model experimentation:

a. The benthic community, including filter and deposit feeders serve to facilitate removal of HOCs from the system by sequestering contaminants from the dissolved and particulate phases and passing them to buried organic material pool as feces and detritus.

b. The degree of accumulation of HOCs in fish species which utilize Baltimore Harbor is dependent on habitat utilization, the proportion of time spent in contaminated versus non contaminated areas, and prey selection (eg benthic vs pelagic and the food item's trophic position).

c. The sub-lethal effects of toxicants on the biota through species shifts, reductions in productivity, and interference with the life cycle, influence both carbon and contaminant flows in the ecosystem.

1995 objectives:

a. To compile existing contaminant data for sediments, water, and biota for Baltimore Harbor and the Patapsco River and evaluate this data for completeness, quality and applicability for our modeling efforts.

b. Compile a similar database from existing sources on plankton, fish and benthic species composition and biomass for Baltimore Harbor to incorporate into the model's carbon-flow portion.

c. Begin development of a specific ecosystem model for Baltimore Harbor.

d. Expand the model by including benthic-pelagic coupling and three fish components

e. Calibrate the model to the Baltimore Harbor ecosystem carbon dynamics (from nutrient and biological data).

f. Calibrate the model to the Baltimore Harbor ecosystem toxicant behavior (from HOC data).

1996 objectives:

a. Make structural additions to the carbon-HOC model by including a fish bioenergetics module and associated contaminant relationships

b. Validate the model using nutrient data from the 1994 Baltimore Harbor nutrient loading study.

c. Perform model experiments and sensitivity analysis on the carbon trophic flows, the importance of the benthic community to contaminant behavior, the effects of fish bioenergetics on contaminant and carbon flows, and contaminant types.

d. Evaluate the impact of proposed management strategies (eg. nutrient control and stormwater mitigation, dredging) on contaminant bioaccumulation and long-term residence times.

METHODOLOGY

Data synthesis

During Year 1 we will begin compiling information required to develop and calibrate the carbon and contaminant flow model for Baltimore Harbor. We will obtain data from state and federal agencies including Maryland Department of the Environment, Maryland Department of Natural Resources, Maryland Department of Health and Mental Hygiene and the National Oceanic and Atmospheric Administration (Table 1). Some of the information is contained in the EPA Bay Program database, which we recently queried using Baltimore Harbor's location and organic contaminants as search parameters. We received seven data records which are designated in Table 1 by the "*." The records contain information on organic contaminants in water, sediments, benthic organisms and fishes, the most frequently measured of which was DDT plus metabolites (DDE, DDD) and chlordanes. For the purposes of the study we will request the data from the agency and search for additional information on organic contaminants, water chemistry, species abundance and composition. The results from the data pool will be entered into a database format on a desktop computer using speadsheet or database program, such as Oracle.

The specific criteria used to evaluate the data will include the HOC type, its limit of detection, the number of data records for different sample matrixes and the analytical or quantification method used (eg. packed column versus capillary gas chromatography). For instance, several studies have quantified PCBs by the Aroclor method and not by specific congeners. Since the physico-chemical properties of individual PCBs often span several orders of magnitude, we will only use PCBs quantified using specific congeners. We will limit the database to include only persistent, bioaccumulatable organic contaminants included in the EPA Toxics of Concern list, such as chlordanes, DDTs, PCBs and possibly PAHs.

Part of the data synthesis will be done by interfacing with projects scheduled to begin either late 1994 or early 1995. Currently, MDE is initiating a large study of point- and non-point nutrient sources in Baltimore Harbor to construct a nutrient budget. We have already contacted MDE regarding this and they are eager to provide us with nutrient data that we will use to calibrate our model (see attached letter). In 1995, there will be a large multi-

investigator project, including one of us (JEB) which will focus on the influence of the city of Baltimore on the surrounding Chesapeake Bay including Baltimore Harbor. The study will make measurements of PCBs and PAHs in water. These data will be used in our model to set the initial dissolved phase water concentration.

Model development

We will employ standard simulation modeling techniques using STELLA computer software to develop a carbon flow model for Baltimore Harbor. Initially, the state variables will include: phytoplankton (diatoms, summer flagellated species), zooplankton (copepods, eurytemora), protozoa, POC, DOC, nutrient pools (N, P), benthic organisms (suspension feeders, deposit feeders), phytoplanktivorous fish, zooplanktivorous fish, piscivorous fish, and the HOC tissue concentrations for each of the preceding groups. Each of 7-10 representative congeners we will choose for modeling will be simulated separately to prevent the model becoming to unwieldy, thereby preserving performance. The behavior of HOC congeners will first be modeled using the simple 5-stage model strategy we used successfully in the current CBEEC funding. This will enable us to calibrate the model using static conditions. Congener characteristics (K_{OW}, equilibrium dissolved concentrations) will be then inserted into the larger ecosystem model to observe behavior under dynamic conditions with realistic seasonal fluctuations of light, temperature and nutrient conditions, as well as the movements of transient fish populations and seasonal population cycles of other functional groups.

Differential equations will describe the flows of carbon and other nutrients between state variables during each time step. HOC tissue concentrations will flow in direct proportion to the carbon flow between each trophic group, but assimilation of the toxicant will be modified by carbon and HOC assimilation efficiencies. Additionally, diffusive fluxes will be calculated by equation (1). Numerical solutions for the equations will be resolved by iterative calculation of difference equations. The integration scheme employed will be a Runge-Kutta2 algorithm to reduce errors by numerical approximation of the analytical solution without sacrificing calculation speed. The model time unit will be in d, and the integration time-step will be 0.25 time units. Phytoplankton growth equations will follow first-order Michaelis-Menton kinetics for light, averaged over the mixed depth, and for nutrient levels. Standard values for nutrient half-saturation coefficients (K_S) will be used from the literature. Grazing by first-order consumers (zooplankton and protozoa) will follow standard Lotka-Volterra mechanics modified by a temperature coefficient. Our working model for the mesohaline Chesapeake Bay successfully incorporates the above concepts producing a large simulation system operable on a Quadra desktop computer which can produce two-year simulations in 2-3 min. The HOC flows in the model will be calibrated using congener-specific PCB concentrations in water and best-estimate size fractionated particles determined under a previous CBEEC funded project using the following classifications: dissolved; <10μ: bacteria; 10-64μ: phytoplankton; 64-202μ: microzooplankton and protozoa; >202μ: zooplankton.

Using a small 5-compartment model as a developmental tool, we will determine the relative timescales and magnitudes of PCB incorporation into the system foodweb by diffusional uptake and feeding routes. Current simulations indicate that diffusional uptake, especially by phytoplankton, is apparently kinetically limited and very sensitive to the diffusional mass transfer coefficient and phytoplankton growth. In attempting to calibrate the model for HOCs our efforts are limited by a scarcity of information on such parameters as mass transfer coefficients, assimilation efficiencies and the levels in the lower trophic levels, such as bacteria, phytoplankton and zooplankton. Use of the 5-compartment model as a development tool will assist us in arriving at ecologically relevant numbers for these terms.

Our current CBEEC model has enabled us to calculate the relationship between biomass turnover time within trophic levels and HOC equilibrium kinetics which govern the ability of the the system to come to equilibrium. We will use the model for Baltimore Harbor to generate similar quantitative data on system function and toxic cycling rates. Understanding the relationship between the two processes of carbon flow and HOC kinetics will enable us to predict the tendency of specific trophic levels to reach equilibrium and the frequency and level of HOC contamination required to drive the process of biomagnification. With the information on contaminant concentration and body burden that is available for additional trophic groups (oysters, fish) in Baltimore Harbor, we will enhance the model's calibration of higher order trophic relationships.

In the second year, we will link the core HOC model with other models on two fronts: first, our existing CBEEC model will be integrated with the Baltimore Harbor model, enabling the simulation of the movement of organisms between the mesohaline Bay and Baltimore Harbor sub-systems, providing a spatial component to our analysis of trophic and HOC transfer; secondly, a fish bioenergetics model developed by Steve Brandt and Jiangang Luo (CBL), who are collaborating with us on another Chesapeake Bay modeling project, will be docked with our HOC models to provide detailed simulation of HOC kinetics at the top consumer level in the bay. It is our intention to develop a simulation model which can realistically represent the potential body burdens of nekton species under a range of current and projected contamination scenarios.

EXPECTED RESULTS

The deliverables from this project will be: a compiled database on HOCs, nutrients, and living resources in Baltimore Harbor suitable and available for modeling studies; a working HOC-carbon flow model which we can be used to address questions related to contaminant cycling in Baltimore Harbor; a series of model experiments which will evaluate topics of importance to the issues of toxic contamination in Baltimore Harbor; specific conclusions and recommendations based on these simulations. The simulation experiments will include:

- the effect of organisms feeding in contaminated versus non-contaminated areas
- how feeding preference for organisms that feed in benthic and pelagic zones influences their contaminant concentrations
- the effects that different nutrient scenarios have on the transfer of HOCs to the sediments and in the foodweb
- an examination of contaminant pulse rates, levels, and characteristics, and how these parameters influence contaminant propagation through the foodweb
- an examination of contaminant pulse rates, levels, and characteristics, and how these parameters influence contaminant delivery to the sediments
- how dredging may affect HOC flows, i.e. through increased suspended sediment loads, a reduction in benthic invertebrates or changes in bedded sediment concentrations (up or down)

- the interaction of toxic effects and nutrient enrichment (eutrophication) effects on functional group productivity
- the importance of trophic versus diffusive transfer in determining bioaccumulation rates at various trophic levels
- the impact of different levels of direct toxicity to biological ecosystem processes
- the importance of toxic effects on species composition and system health

An important result of the report produced by this study will be the recommendations section. A series of recommendations will emerge from examination of the data base and its evaluation from a classical ecotoxicology perspective (LDL, direct toxicity effects) and from a second avenue generated by the model analysis, which will address both theoretical and management issues. The model will also enable us to provide assessments as to which additional biological data, contaminant concentration data, and process information will be needed for an improved predictive model.

DISSEMINATION OF RESULTS

We will present the results to MDE (Dr. Elizabeth Casman) and the Bay Program Toxics Subcommittee as well as our suggestions for further studies and any modifications in their data quality objectives regarding contaminants in sediments and biota. We will present our observations at scientific meetings and prepare the final results for publication in quality peer-review journals such as *Ecological Modelling*, *Environmental Toxicology and Chemistry*, and *The Journal of Environmental Quality*.

RELATIONSHIP TO OTHER WORK

One of the most important aspects of the proposed research is the web of interdisciplinary connections to other work that it will foster, and indeed, require. The connections will be two-way, with our results made freely available to the several agencies and investigations named in Table 1, as we hope to draw heavily on past and ongoing studies for calibration, validation and elaboration of the model structure. The proposed simulation modeling exercise builds directly on our current toxics cycling model, developed using CBEEC funding. This new funding request will enable us to make a model specific to Baltimore Harbor, incorporate detailed additional ecosystem components (benthic and fish) and begin to develop a spatial component to the simulation by linking the mesohaline bay model. In addition, our previous mesohaline Chesapeake Bay model used PCB and PAH concentration data in water, sized fractionated particles and sediments as a calibration data set. During the model building process, we will interact with groups developing a model of the Patuxent River ecosystem. One of us (CJM) is the principal party involved in development of this model. Other PIs on this project have recently compiled data and developed a fish bioenergetics model which we will draw on for the inclusion of a detailed fish submodel to our Baltimore Harbor model. Two of us (JRK and JEB) are also completing laboratory experiments on determining mass transfer coefficients for estuarine phytoplankton, which will be directly incorporated into our model. The proposed simulation study also relates to a recently funded (NOAA) project examining trophic transfer of contaminants in Lake Superior.

By nature, the modeling effort will incorporate data from a variety of sources, much of which is contained in the Bay Program toxics data base such as results from the Bay Program ambient toxicity survey, the MDE fish surveillance program and the NOAA Mussel Watch. Two other projects that are set to begin in 1994, the Baltimore Urban Plume Study and MDE's nutrient loading inventories for Baltimore Harbor, will substantially aid in the calibration of the final model. For instance, Baltimore Urban Plume Study will include simultaneous measurements of air and water concentrations of both PCBs and PAHs in Baltimore Harbor and adjacent Chesapeake Bay. The resulting data on dissolved-phase contaminant data will greatly help to calibrate the diffusional portion of the model.

PERMANENT EQUIPMENT

We are requesting $3000 for the first year of the project to purchase and a desk-top computer to run the STELLA-based model. The computer will be located at HPEL. We also need to upgrade our current desk-top computer at CBL and are therefore requesting $2000 for the purchase of an accelerator card and memory upgrades.

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CBEEC Toxics Research Program

Title: Quantitative evaluation of contaminants in a Chesapeake Bay Region of Concern: A simulation model of exposure and bioaccumulation in Baltimore Harbor

Investigators: Christopher J. Madden, John R. Kucklick, and Joel E. Baker

Horn Point Environmental Laboratory
Chesapeake Biological Laboratory
University of Maryland
Center for Environmental and Estuarine Studies

Budget Justification-Year One

Salaries

Chris Madden and John Kucklick will be the principal researchers working in this project. Their time will be spent on travel to various sites where data are stored, collection and analysis of data, development of a database and development of simulation models as outlined in this proposal @ 33% of their time each. Joel Baker will provide 5% of his time for assistance in development of the models.

Permanent Equipment

As this is a computer-intensive endeavor, an accelerator will be needed for an existing computer at CBL ($2,000), and a new computer with expanded RAM, ethernet, and accelerator capabilities will be required for model development at HPEL ($3,000).

Expendables

Computer software, networking and modem supplies will be needed for both sites to ensure communication and data transfer ($750 total).

Travel

We require $1,300 in our Travel budget, for Year 1 for the following:

Three one-day trips from CBL to HPEL for collaboration, data exchange, model building and report writing at 322 mi round trip, @0.22/mi 213.00

Per diem for above trips 15.00

Three one-day trips from HPEL to CBL for collaboration, data exchange, model building and report writing at 322 mi round trip, @0.22/mi 213.00

Per diem for above trips 15.00

Three one-day trips from HPEL to Annapolis (EPA, MDE) for data collection, discussion and research at 90 mi round trip, @0.22/mi 60.00

Per diem for above trips 20.00

Four one-day trips from CBL to Annapolis (EPA, MDE) for data collection, discussion and research at 210 mi round trip, @0.22/mi 140.00

Per diem for above trips 20.00

Travel for two people to one scientific meeting to present results of this work at end of Year 1 ..... 604.00

TOTAL 1300.00

Table 1. A partial list of biological, nutrient, and contaminant data available for Baltimore Harbor.

Study	Organization	Year	Sample Type	Parameters
Maryland Chesapeake Bay Water Quality Program: Phytoplankton Component	[1]BERL/[2]MDE	1984 to present.	Phytoplankton	Species Composition Chlorophyll a Primary Production
Maryland Chesapeake Bay Water Quality Program: Zooplankton Component	BERL/[3]CES/ MDE	1984 to present	Zooplankton	Species Composition Wet weight and Dry Weight Biomass
Maryland Chesapeake Bay Water Quality Program: Benthic Organism Component	[4]DNR/MDE/ VERSAR	1984 to present	Benthic Organisms	Species Composition Species Distribution Biomass (ash free dry wt.) Sediment Characteristics
Maryland Juvenile River Herring Survey	DNR	1983 to present	Herring and Shad	Length, Number, Catch per unit effort
Maryland Chesapeake Bay Water Quality Monitoring Program: Tributary Chemical/Physical component	MDE/[5]DHMH	1986 to present	Water Chemistry	Diss. Oxygen, nutrients, salinity, pH
*Maryland Chesapeake Bay Sediment Toxicant Monitoring Program	MDE/DHMH / CBL	1985 to present	Sediment Contaminants	PAHs, PCBs, Organochlorines, Metals
*NOAA Status and Trends Program- Mussel Watch Program	[6]NOAA/ Batelle/ [7]TAMU/[8]NIST	1986 to present	Mussel and Sediment Contaminants	PAHs, PCBs, Organochlorines, Metals, Sediment Characteristics
*Maryland Finfish Tissue Monitoring Program	MDE/DHMH	1977 to present	Contaminants in Finfish	Organochlorines, metals, PCBs (Aroclors)
*NOAA Status and Trends Program- Benthic Surveillance Project	NOAA/NIST	1984 to present	Contaminants in Sediments and benthic fishes	PCBs, organochlorines, PAHs, metals, species composition
*A Pilot Study for Ambient Toxicity Testing in Chesapeake Bay (years one and two included separately in BPO data base)	UofMD/DNR/MDE/USEPA	1990-91	Ambient Water Toxicity and Contaminants in Water and Sediments	PCBs (Aroclors), PAHs, organochlorines metals
*1983 Crab Survey	MDE	1983	Blue Crab Tissue Contaminants	Organochlorines, metals

[1]Benedict Estuarine Research Laboratory

[2]Maryland Department of the Environment

[3]Coastal Environmental Services, Inc.

[4]Maryland Department of Natural Resources

[5]Maryland Department of Health and Mental Hygiene

[6]National Oceanic and Atmospheric Administration

[7]Texas A&M University

[8]National Institute of Stds. Technology

*From EPA Bay Program database

TROPHIC ACCUMULATION OF PCB CONGENERS

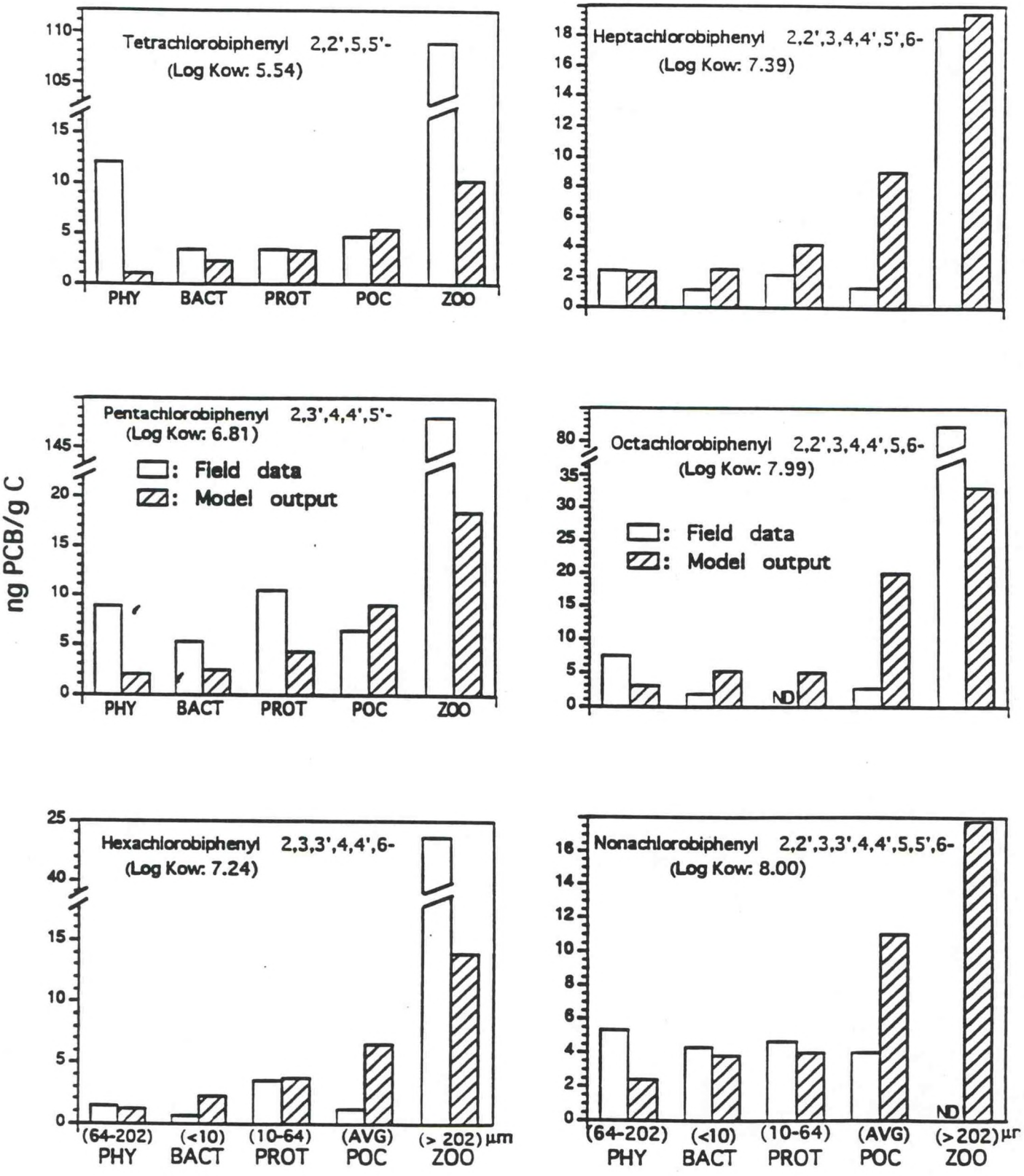


Figure 1.

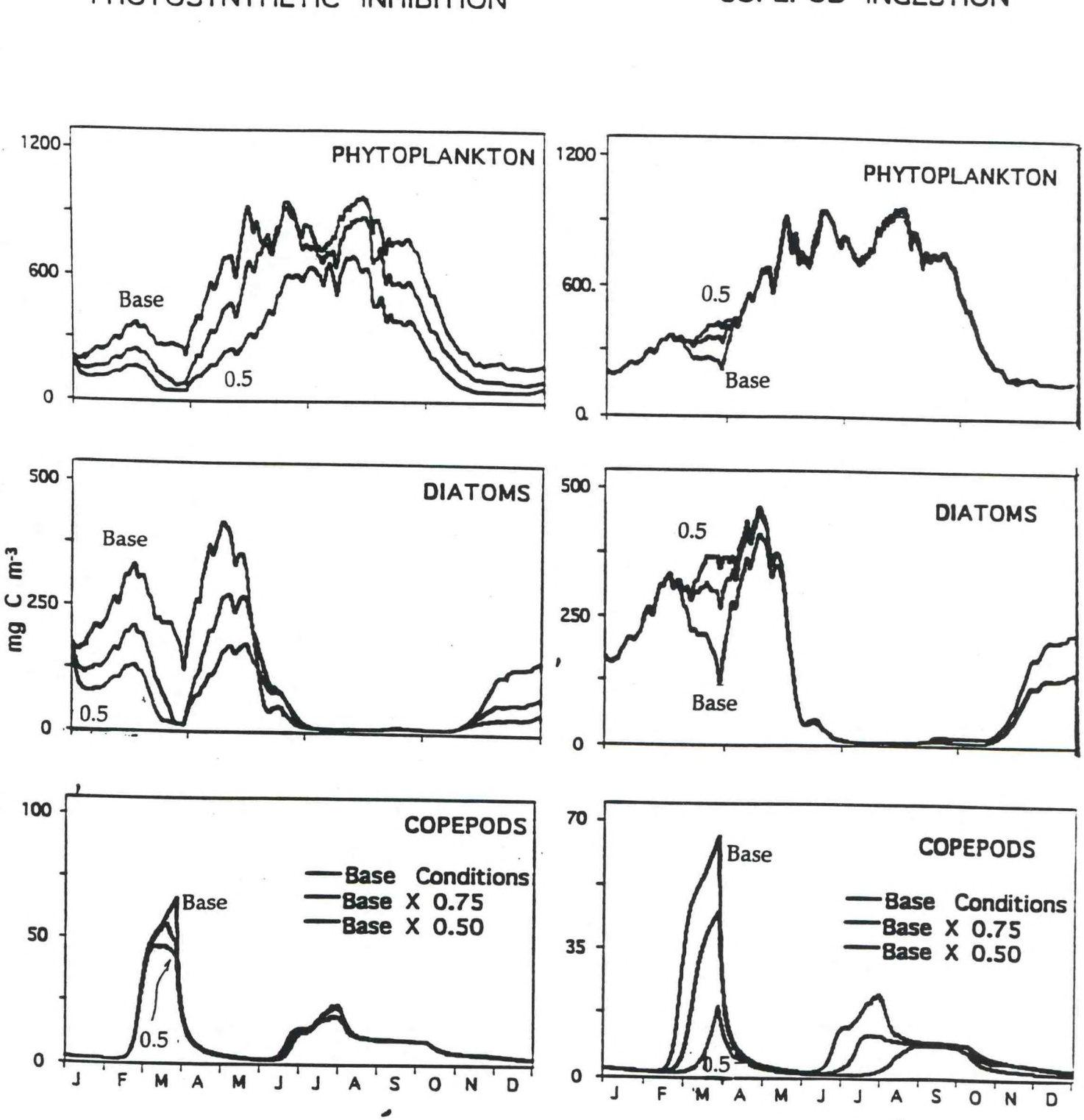

PHOTOSYNTHETIC INHIBITION
COPEPOD INGESTION
PHYTOPLANKTON
Base
0.5
DIATOMS
COPEPODS
Base Conditions
Base X 0.75
Base X 0.50
mg C m-3
J F M A M J J A S O N D

Figure 2.

TITLE: Quantitative Evaluation of Contaminants in a Chesapeake Bay Region of Concern: A Simulation Model of Exposure and Bioaccumulation in Baltimore Harbor

PROJECTED TIME SCHEDULE

PROJECT ACTIVITIES	Year 1 1st	2nd	3rd	4th	Year 2 1st	2nd	3rd	4th
Collect avail. data on toxics	---------->							
Obtain results from urban plume and nutrient studies					---------->			
Database Building		---------->						
Further develop current carbon-flow model and adapt to Balt. Harb.		---------->						
Add benthic-pelagic coupling and fish state variables				---------->				
Calibrate the model with the database information					---------->			
Sensitivity analysis of contaminant-flow parameters					---------->			
Model runs using realistic scenarios						---------->		
Write final report							---------->	

*** MARYLAND SEA GRANT PROJECT SUMMARY 1995 ***

Title: Factors controlling the food chain accumulation and transfer of inorganic and methylmercury in Chesapeake Bay organisms

Project Number: R/CBT-27
Revision Date:
Grant Number:
Initiation Date: January 1, 1995
Status: New
Completion Date: December 31, 1996

Principal Investigator: Robert P. Mason
Affiliation: CBL, UMCEES
Months Committed: 1.2

Principal Investigator: David A. Wright
Affiliation: CBL, UMCEES
Months Committed: 1.1

Proposed Federal Funds: $65,061
Current Federal Funds: $
Federal Funds to Date: $
Proposed Matching Funds: $
Current Matching Funds: $
Match to Date: $
Related Projects:
Sea Grant Classification#: 45
Keywords: methylmercury, bioconcentration, food chain, Chesapeake Bay

Objectives: (1) To investigate the bioconcentration of Hg and methyl Hg in organisms representatives of the Chesapeake Bay from both pelagic and benthic food chains. (2) To ascertain relative assimilation efficiencies of inorganic and methyl Hg by algae (pelagic/benthic diatoms or dinoflagellates) and by zooplankton and zoobenthos. (3) To elucidate water column variables and sediment chemistry controlling uptake by microorganisms and factors controlling assimilation by zooplankton/zoobenthos. (4) To assess transfer of Hg and methyl Hg between primary consumers and fish.

Methodology: Food organisms (algae) will be dosed with inorganic Hg and methyl Hg (measured using atomic fluorescence and/or ^{203} Hg) and feed in controlled amounts to zooplankton. Zoobenthos will be fed labeled detritus. Assimilation efficiencies will be recorded for several species.

Rationale: Few details exist on Hg bioaccumulation at the base of the food chain, particularly in the estuarine environment. Results from this project will provide valuable input data for food chain models as well as highlighting likely differences between Hg cycling in benthic and pelagic food chains. Sediment quality criteria for Hg may require adjustment in light of this information.

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1995 GRANT/PROJ. NO: RCBT-27
University of Maryland, Cheasapeake Biological Laboratory
PRINCIPAL INVESTIGATORS: R.P. Mason
D.A. Wright
DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	1.20		4,800	
b. Associates	1.10		5,800	
Sub Total			10,600	0
2. OTHER PERSONNEL				
a. Professionals				
b. Research Associates				
c. Res.Asst.Grad.Std.	11.00		13,500	
d. Prof. School Students				
e. Pre-Bac Students			4,000	
f. Secretarial-Clerical			1,000	
g. Technical-Shop				
Total Salaries/ Wages			29,100	0
B. FRINGE BENEFITS			8,226	
Total Salaries,Wages and Fringe Benefits			37,326	0
C. PERMANENT EQUIPMENT				
D. EXPENDABLE SUPPLIES AND EQUIPMENT			5,000	
E. TRAVEL				
1. Domestic	2,000			
2. International				
Total Travel			2,000	
F. PUBLICATIONS AND DOCUMENTATION COSTS			700	
G. OTHER COSTS				
1. Computer costs				
2. Copying,Library and Communication			500	
3. Analytical and Shop Services				
4. Fuel,Boat Time and Vehicle Usage			800	
5. Word Processing				
6. Institutional Allowance				
7. Tuition				
8. Waste Disposal				
9. Service Contracts			705	
Total Other Costs			2,005	0
TOTAL DIRECT COSTS			47,031	0
INDIRECT COSTS				
(On Campus) : 39 % of 46,231			18,030	
(Off Campus) : % of				
Total Indirect Costs			18,030	0
TOTAL COSTS			65,061	0

FACTORS CONTROLLING THE FOOD CHAIN ACCUMULATION AND TRANSFER OF INORGANIC AND METHYLMERCURY IN CHESAPEAKE BAY ORGANISMS

R.P. Mason and D. A. Wright
Chesapeake Biological Laboratory, University of Maryland System

INTRODUCTION

Methylmercury accumulation in fish is an increasingly important health concern, with advisories concerning fish consumption becoming more frequent recently (Fitzgerald and Clarkson, 1991). This heightened concern has led to a re-evaluation of the factors controlling the accumulation and transfer of Hg and methylHg in aquatic good chains as it is now generally accepted that the food chain is the primary route of mercury accumulation in fish (Lindqvist et al., 1991.). However, while there have been a large number of studies initiated to look at the factors controlling Hg accumulation in freshwater fish in the USA, Canada and Europe, the ocean has received scant attention. This is surprising considering that estuaries and coastal regions, which are directly impacted by man's activities, are an important source area for both commercial and recreational fish and seafood. The sharp differences in salinity, organic content and toxic loading, as a result of anthropogenic inputs, found within impacted estuaries such as Chesapeake Bay, will lead to distinct differences in Hg concentration and speciation in the water column and in the sediments. These changes will have a profound affect on Hg uptake at the base of the food chain which will be subsequently transferred up the food chain. While many recent studies have looked at the relationship between water column variables and concentration in fish (e.g. Weiner and Spry, 1991), there has been little study of the accumulation and transfer of Hg compounds lower in the food chain, particularly in the estuarine environment.

MethylHg is one of the most toxic of all the chemicals listed in the Chesapeake Bay Toxics of Concern. There is, however, little reliable information on the concentration and speciation of Hg and methylHg in Chesapeake Bay waters. Plankton concentrations range from 0.06 to 1.65 μg/g dry wt with the highest concentrations being found in Baltimore Harbor (Brinkman et al., 1975). In Chesapeake Bay sediments, Hg ranges from <0.001 μg/g dry wt in the Nanticoke River to >1.0 μg/g dry wt in Curtis Bay and Baltimore Harbor (Wright 1982; 1987; Brinkman et al., 1975). In contrast, in north-central USA lakes, where fish health advisories have been issued, sediment Hg concentrations are typically less than 0.2 μg/g dry wt (Watras el al., 1991): phytoplankton concentrations below 0.5 μg/g dry wt; zooplankton $<1\mu$g/g dry wt and water column concentrations 10 pM. In light of the elevated Hg levels in the Chesapeake relative to those of the lakes, reports of Hg concentrations above the US FDA action level of 1 μg/g dry wt., measured in shellfish from Chesapeake Bay and its source rivers (Wright 1982, 1987), and fish concentrations exceeding 0.5 μg/g dry wt, the action level in Canada and the north-central USA states, are expected.

The Chesapeake Bay water column and sediments have been significantly impacted by man's activities leading to changes in the community structure, primarily as a consequence of increased nutrient inputs. The consequent increase in phytoplankton productivity has, in recent years, resulted in anoxia in the bottom waters for significant periods of the year (Tuttle et al. 1987). The resultant increased sedimentation and burial of organic matter in the sediments and the changed sediment chemistry has altered the availability of sediment-bound trace metals.

Mercury concentrations in sediment pore waters are an order of magnitude higher than those of the water column (Gobeil et al., 199?; Watras et al., 1991). Similarly, Lindqvist et al. (1991) found no correlation between Hg in pike and sediment Hg for Swedish lakes while others (Jackson, 1991; Parkman, 1993) found no correlation between Hg in benthic detritivores and sediment methylHg. Since methylHg usually constitutes less than 1% of total Hg in sediments (Campbell et al. 1988) it seems reasonable to suppose that other forms of Hg could be important in the accumulation of Hg by sediment-dwelling animals. Accumulation by benthic organisms will depend on the bioavailability of the Hg and methylHg in sediments. Trace metal fluxes to and from the sediment are directly related to the oxygen concentration of the overlying water (Riedel et al., 1994) with release of metals such as Cd and Cu occurring when the water column is oxic, while others, such as As and Mn, are released only when bottom waters become anoxic. Iron and Mn oxides dissolve and release bound metals at the initiation of anoxia while anoxic conditions cause the removal of metals that bind strongly to sulfide. Di Toro et al. (1992) have shown that the bioavailability of metals in sediments is related to their affinity for sulphide relative to iron oxide phases, and to the relative abundance of reactive sulphide (the acid volatile sulfide fraction) in the sediment. On this basis it would be expected that Hg would be more available to benthic organisms during oxic conditions while anoxia would lead to complexation of Hg by sulfide, although Hg solubility is enhanced by the formation of other sulfide-containing complexes (e.g. $Hg(HS)_2$, HgS_2^-, polysulfides). Anoxia, which increases Hg methylation by sulfate-reducing bacteria (in both freshwater and marine environments), will stimulate the production of methylHg (Furutani and Rudd 1980; Craig and Moreton 1986). MethylHg is less strongly bound to sulfide than Hg, Cu or Cd (Dyrssen and Wedborg 1988; 1991) and thus it is likely that it would be released into the water column during anoxia, as found in Wisconsin lakes (Bloom et al., 1991) and the Pettaquamseutt estuary (Mason et al., 1993). Methylation is also pH related (Gilmour and Henry 1991). Thus, the availability and uptake of Hg and methylHg to both phytoplankton and benthic algae is likely to be linked to the seasonal cycle of the water column of the Chesapeake Bay.

A compilation of a number of studies have demonstrated that fish Hg concentration is primarily a function of diet; >80% of the accumulation in freshwater fish is attributed to food (Lindqvist et al., 1991). There have been few studies, however, comparing the source and uptake of Hg compounds by benthic and pelagic organisms and the role of the respective food chains in the transfer of Hg and methylHg to fish. It is not clear what factors control the accumulation at the base of the food chain (water to microorganisms to zooplankton/zoobenthos), what controls the availability of Hg and methylHg to these organisms and what fraction of the Hg and methylHg is assimilated at each trophic level. For the pelagic food chain, the bioaccumulation factor is highest between the water and phytoplankton (Watras and Bloom,

1991) and there is a direct correlation between Hg in zooplankton and Hg in perch and between Hg in perch and Hg in pike in Swedish lakes (Lindqvist et al., 1991). The biomagnification factor between zooplankton and predator fish is estimated by these authors to be around 25, compared with the 10^4 - 10^5 bioconcentration between water and phytoplankton. This suggests that differences in the fish Hg concentration - as found, for example, between lakes exposed to similar Hg inputs but having different water column characteristics - is primarily controlled by accumulation of Hg at the base of the food chain (water to phytoplankton).

Our initial lab studies on the toxicity and the accumulation of inorganic Hg and methylHg by phytoplankton (Mason and Morel, 1992; 1993; submitted) have demonstrated this. We have found that the toxicity of inorganic Hg and methylHg is not dependent on the total Hg exposure concentration nor on the free metal ion concentration. Rather toxicity is dependent on the concentration of neutral complexes (as shown for inorganic Hg in Fig. 1), as expected if passive diffusion across the cell membranes of the neutral Hg complexes ($HgCl_2$ and CH_3HgCl) is the main accumulation route. Other investigators have suggested this based on studies with artificial membranes (Gutkneckt, 1981; Bienvenue et al., 1984). Hg and methylHg accumulation is therefore different from that of other trace metals, such as Fe, Zn and Cd, where uptake involves facilitated transport of the metal across the cell membrane (Morel et al., 1991; Sunda, 1991). As a consequence, uptake of Hg and methylHg is not controlled *a priori* by the free metal ion concentration, as is the case with facilitated transport, but is a function of the concentration and form of the neutral, lipid soluble complexes (e.g. $Hg(Cl)_2$, CH_3HgCl, $Hg(OH)_2$, CH_3HgOH) present in solution. Further, as the neutral chloride complexes are significantly more lipid soluble and membrane permeable than the corresponding hydroxide complexes (Mason and Morel, submitted, Table 1), accumulation is influenced by factors such as pH and salinity. Thus, water column characteristics that alter Hg and methylHg speciation - such as pH, chloride concentration, the presence of complexing ligands, sulfide and DOC - dramatically alter the rate of passive uptake of both inorganic and methylHg. We hypothesize that, because environmental conditions and water column speciation do not influence trophic transfer between primary producers and higher trophic (Watras and Bloom, 1992), the final concentration of Hg in fish is directly related to the environmental factors controlling speciation in the water, and, by direct consequence, the accumulation by the microorganisms at the base of the food chain.

In seawater, methylHg is present almost exclusively as CH_3HgCl while only about 3% of the inorganic Hg is present as $HgCl_2$. Thus, if the permeability of the two neutral chloride complexes are the same (as our results indicate; Mason and Morel, submitted) then passive uptake should result in methylHg accumulation being about 30 times higher than inorganic Hg accumulation. Similarly, based on total exposure levels, methylHg should be about 30 times more toxic than inorganic Hg, if toxicity is a direct function of uptake rate. Our preliminary results have demonstrated this (Mason and Morel, submitted). For example, at 35‰, the growth rate of the diatom, *Thalassiosira weisflogii* was 60-70% of the control after exposure to 5 nM Hg, while 150pM methylHg caused the same decrease in growth rate. This difference should also be apparent for direct accumulation by other aquatic organisms exposed to Hg and methylHg if uptake from solution involves passive diffusion of neutral complexes across gill

membranes and/or other surfaces. Riisgard and Famme (1986) demonstrated this: they found a factor of 30 difference between the rate of direct accumulation of Hg and methylHg by the shrimp *Crangon crangon*. Furthermore, Gottofrey (1990) showed that both inorganic Hg and methylHg formed lipophilic complexes with chelating agents, which were accumulated by fish more readily than uncomplexed forms.

While water speciation controls the amount of Hg taken up by microorganisms, food chain transfer leads to discrimination between Hg and methylHg. Our experiments on the food chain transfer of Hg and methylHg between marine diatoms and copepods (Mason and Morel, submitted) indicate that methylHg is more readily transferred to zooplankton fed Hg-contaminated diatoms. We have further shown that, for these copepods, this enhanced bioaccumulation results from differences in the site of sequestration of the Hg compounds in the phytoplankton, rather than due to the increased lipid solubility of methylHg. Our results show that inorganic Hg is primarily bound within the cell membrane (91%) while 64 of the methylHg accumulates in cell cytoplasm. This difference accounts for the differences in zooplankton assimilation found (15% of the inorganic Hg, 63% of the methylHg, Fig. 2a), as found for other elements by Reinfelder and Fisher (1991) i.e. cell membrane bound constituents are poorly assimilated by copepods. Similarly, Boudou and Ribeyre (1981) found that 6% of the inorganic Hg and 58% of the methylHg was assimilated by *Daphnia* fed contaminated phytoplankton (*Chlorella vulgaris*). Riisgard and Famme (1986) found that 75% of the methylHg and 4% of the inorganic Hg was assimilated by shrimp fed Hg-contaminated mussel tissue.

Discrimination also occurs further up the food chain. Laboratory studies in freshwater by Boudou and Ribeyre (1983) showed that methylHg was more efficiently taken up by fish (*Gambusia affinis*) feeding on *Daphnia* (41% of the Hg; 92% of the methylHg), and by trout (*Salmo gairdneri*) feeding on *Gambusia* (54% of the Hg; 95% of the methylHg), although the difference is less marked than that found lower in the food chain. Whether differences in the site of sequestration could account for the preferential accumulation of methylHg in these studies is now known. Results from the studies in Wisconsin lakes concur with the suggestion of preferential accumulation of methylHg between zooplankton and fish (Watras and Bloom, 1992). Furthermore, Reinfelder and Fisher (in prep.) (Fig. 2b) have shown that there is a correlation of Zn, Cd, Co, Se, C, S and P between the percentage of an element that is associated with the soft (non-exoskeleton) tissue of copepods and the assimilation by silversides. Metals which were primarily associated with the skeletal tissue were poorly assimilated. We hope to extend these studies to Hg and methylHg by investigating the sites of sequestration of Hg compounds in zooplankton/zoobenthos fed Hg-contaminated algae and the influence this has on trophic transfer to fish.

Boudou and Ribeyre (1983) found that after 30 days of exposure to contaminated *Gambusia, Salmo gairdnerii* accumulated 36% of the inorganic Hg in the intestine and 21% in muscle tissue while methylHg was mostly accumulated in the muscle (59%). For inorganic Hg, soft tissue (liver, brain, gills, muscle, intestine, kidney, spleen and blood) accounted for 59% of the total Hg in the fish; for methylHg, soft tissue accounted for 77%. Extending the Reinfelder and Fisher accumulation model (i.e. that elements in soft tissue/cytoplasm are readily

assimilated) to fish, and assuming a similar distribution of inorganic Hg and methylHg in exposed *Gambusia*, we argue that the correspondence between the observed assimilation found on feeding *Gambusia* to *Salmo* (54% of the inorganic Hg, 85% of the methylHg) and the projected body distribution (59% of the Hg in soft tissue, and 77% of the methylHg) is a further extension of the hypothesis.

Studies of large benthic detritivores also indicate a difference in the assimilation of inorganic and methylHg (Wright et al., 1991). Furthermore, food source is obviously important for benthic organisms. Fisher and Wente (1993) show that elements that are primarily membrane bound in algae are more slowly released by dead or dying phytoplankton. Thus, it is likely that the relative concentration and availability of Hg and menthylHg in detrital particles derived from phytoplankton will be different from that of phytoplankton, and food source will therefore influence the amount of Hg and methylHg taken up by zoobenthos. The relative amount of organic Hg in a carnivorous shrimp (73%) taken from a polluted region was higher than that in mussels collected from the same area (Riisgard and Famme, 1986). While these differences could have a physiological basis, it is plausible that differences in food source - the shrimp's food comes from higher in the food chain, while the mussels consume primarily algae and detritus - could account for this difference. Filter feeders can accumulate significant amounts of phytoplankton from the water column and therefore might have a Hg and methylHg distribution that does not directly reflect their sediment environment.

In a study of "food chain organisms (including small fish) in Clay Lake, Ontario, detritivores and organisms feeding on botton-dwelling organisms contained much higher Hg concentrations than those found in both herbivorous organisms and in organisms feeding on zooplankton (Armstrong & Hamilton, 1973). This is supported by Parkman and Meili (1993) who found that detrivorous chironomid larvae from Swedish forest lakes occasionally and >10 times higher Hg concentrations than other benthic invertebrates.

We hypothesize therefore that trophic transfer is a function of the site of sequestration of the Hg or methylHg in the food source. Further, we suggest that differentiation between Hg and methylHg inside organisms likely reflects the differences in the reactivity of inorganic Hg and methylHg, particularly toward thiol groups - inorganic Hg forms a stronger thiol complex. We have proposed that a similar difference in reactivity controls the site of sequestration in the marine diatom (Mason and Morel, submitted) with inorganic Hg being membrane bound because it forms strong complexes with membrane thiol groups.

OBJECTIVES AND HYPOTHESIS

Hypothesis

In view of the fact that biomagnification factors for Hg at the primary producer/consumer level are very much larger than higher in the food chain, we hypothesize that this step will be of primary importance in dictating Hg levels reaching top predators.

Environmental conditions (water and sediment chemistry) which control the availability of Hg and methylHg will have the largest impact at the base of the food chain.

Food chain transfer controls the speciation of Hg in higher trophic levels and assimilation is a function of the site of sequestration of Hg and methylHg within the food source.

Even within a particular (e.g. benthic) habitat, Hg accumulation will be dictated by feeding habit, e.g. detritivore, herbivore.

Objectives

Our principal objectives are:

(1) to investigate the bioconcentration of Hg and methylHg in organisms representative of Chesapeake Bay from both the pelagic and benthic food chains;
(2) to ascertain the relative assimilation efficiencies of inorganic and methylHg by algae (pelagic diatoms and benthic diatoms or dinoflagellates) and by zooplankton and zoobenthos;
(3) to elucidate the water column variables and sediment chemistry controlling uptake by microorganisms and the factors controlling assimilation by zooplankton/zoobenthos;
(4) to assess the transfer of Hg and methylHg between primary consumers and fish.

Year One Objectives

Year One laboratory experiments will look at the accumulation of Hg and methylHg by a representative phytoplankton, *Thalassiosira* or *Isocrysis*, to investigate the influence of environmental conditions on Hg uptake. Current studies, which have demonstrated the effect of salinity and pH on accumulation by *Thalassiosira weisflogii*, will be extended to look at the role of dissolved organic matter (as represented by humic substances and model thiol compounds such as cysteine) and sulphide - the principal additional factors controlling Hg and methylHg speciation in estuarine waters - on accumulation by the diatoms. Similar experiments will investigate the role of the water column and sediment porewaters and the water chemistry in supplying Hg and methylHg to the benthic unicellular algae. Specifically, the role of factors such as sediment organic content, redox potential, acid volatile sulfide content and Hg and methylHg speciation in porewaters will be investigated.

After the establishment of environmental conditions which result in highly significant differences in mercury accumulation by diatoms two crustacean species will be added to the system: the copepod *Eurytemora affinis*, currently cultured at CBL on both pelagic algal species, and the benthic amphipod *Leptocheirus plumulosus*, a common mesohaline inhabitant of the Chesapeake Bay (alto in culture at CBL). Experiments with both animal species will be performed at different food (algal) concentrations in order to quantify the role of food in Hg transfer in both food chains.

Year Two Objectives

In the second year, benthic experiments will be extended to the benthic amphipod *Hyalella azteca* (in culture at CBL) and the chironomid larva *Chironomus dorsalis* which is abundant during the spring and summer months in the creeks around Solomons. Both these species are more detritivorous than *L. plumulosus* which is more of a filter feeder. A comparison of Hg accumulation by these species will provide further insights regarding the route of Hg uptake. Amphipods will be dissected so that the principal site of sequestration of inorganic Hg and methylHg can be evaluated.

A small number of experiments will look at transfer between zooplankton/zoobenthos and fish (*Cyprinodon variegatus*).

METHODOLOGY

General

For Hg, the study of food chain processes in the laboratory at environmentally relevant concentrations has historically been hindered by the lack of suitable analytical techniques and contamination problems. Most investigators have resorted to radiotracer techniques which typically cannot be performed at low concentrations due to the low activity of the available ^{203}Hg-radiolabelled Hg solutions. A recent report by Stordal and Gill (1984), however, has suggested that a higher specific activity radiotracer is available and experiments using radiotracers is possible at the ng/L level. If the radiotracer is available, then radiotracer methods will be used where appropriate in the investigation. However, direct measurement of the samples will also be employed and is the preferred approach. The investigations that have been completed by Mason while at MIT have all relied on direct measurement (Mason and Morel, 1992, 1993; submitted). The analytical techniques developed at the University of Connecticit (e.g. Bloom, 1989; Mason and Fitzgerald, 1991), have allowed the development of the protocols required to measure natural level concentrations of Hg and methylHg in environmental samples. Radiotracer techniques will rely on well-tested methods that need to be adapted to the measurement of low concentrations of Hg. Biota will be exposed on low nM=pM concentrations of Hg in the water, concentrations that are only 10-50 times that of impacted estuaries (Mason, 1991; Mason et al., 1994), such as the Chesapeake Bay.

Experimental

All species to be used in this project, with the exception of *Chironomus dorsalis* are currently in culture at the Chesapeake Biological Laboratory.

Thallasiosira and *Isochrysis* will be exposed in batch culture to low levels of both inorganic and methyl mercury. Cell numbers will be checked using a standard counting cell, and cell sizes and densities will be noted and compared with control (Hg-free) cultures. The diatom *Phaeodectylum tricornutum* will also be cultured in Hg-free and low level Hg media to

be used as food for benthic organisms, although we will also conduct some experiments with Hg-dosed Tetramin which we regularly use as a dietary supplement in our *Hyalella* cultures.

Analytical

Strict adherence to trace metal free techniques (clean techniques) will ensure the success of the investigation. The Chesapeake Biological Laboratory has recently acquired a new laboratory building in which one lab and attached clean room will be dedicated to the analysis of mercury samples only. Mercury analyses will rely on atomic fluorescence quantification techniques (Bloom and Fitzgerald, 1988). All samples are converted to a volatile Hg species so that they can be purged from solution and trapped on a solid support: gold coated sand for total and reactive Hg measurements; Carbotrap, a carbon absorbent, for methylHg measurement (Mason et al., 1993). This purge and trap procedure allows sample preconcentration, improving detection limits. For total and reactive Hg Measurement, Hg is reduced to elementalHg (Hg^m) prior to sparging; for methylHg, conversion is to methylethylHg, which is volatile. For the determination of reactive (inorganic) Hg in water, 250 mL of unfiltered water is added directly to a glass bubbler fitted with a glass sparger. One mL of a 10% acidic $SnCl_2$ solution is added and the sample is immediately sparged to strip the reduced species from solution and trap them on a gold column. The sample will be acidified just prior to analysis so that Hg is readily released from inorganic complexes, but not from strong organic-Hg associations. This method provides a suitable measure of the reactive Hg concentration (Mason et al., 1993). For the determination of total Hg, samples (water or filters) are oxidized using bromine monochloride solution and excess oxidant neutralized with hydroxylamine (Bloom and Crecelius, 1983), prior to tin chloride reduction, purge and trap collection on gold. One Ml of a 0.2 M BrCl solution is added to a 150-250 mL aliquot of the water samples. For particulate Hg, 10 mL of distilled-deionized water (Q water) and one mL of BrCl is added to each filter, contained in a Teflon bottle. After 30 minutes, excess oxidant is neutralized with hydroxylamine solution prior to $SnCl_2$ reduction. Samples trapped on gold are released by thermal heating and swept into the cold vapor atomic fluorescence analyzer for quantification. The analyzer has an instrument detection limit of about 1 pg. but sample detection is typically limited by sample size and analytical blanks. Typically, for a 500 mL sample, the detection limit is 0.4 pM (80 pg/L) or less for reactive Hg, 0.8 pM for total Hg.

MethylHg is determined by aqueous phase ethylation of water samples or alkaline digestates of filters, followed by cryogenic gas chromatography with atomic fluorescence detection (Bloom 1989; Mason and Fitzgerald 1991). A 400 mL aliquot of filtered water is acidified and extracted by hand shaking for 5 minutes with two 40 mL aliquots of methylate chloride. Extraction isolates MethylHg from chloride ions that interfere with the ethylation procedure (Bloom 1989). The methylHg is back extracted into the aqueous phase, derivitized to methylethyl mercury with tetracthylborate, and purged from solution and trapped on a Carbotrap column. The overall procedural blank, determined by a re-extraction of the water, is typically 5 pg for a 400 mL sample. The detection limit is 50 fM. For particulate methylHg, two mL of a 25% KOH solution is added to the 0.8 μm quartz filter to decompose the particulate matter. After 24 hours digestion at room temperature, 100 mL of Q water is added

followed by two mL of glacial acetic acid, to neutralize the solution. A 30-40 mL aliquot of the solution is then analyzed by the ethylation technique. This digestion method provides a quantitative recovery for fish tissue (Bloom 1989) and gave a greater than 80% recovery for spiked samples in a previous study (Mason et al., 1993). The detection limit was estimated at 50 fM.

Expected Results

Few details exist on the bioaccumulation of Hg at the base of the food chain, particularly in the estuarine environment. This currently represents a significant deficiency in recent attempts to understand why this metal reaches high concentrations in fish and other vertebrates high in the food chain. Because of analytical problems which have been resolved only recently, we are just beginning to establish a meaningful quantitative relationship between the Hg Action Levels established for food by U. S. and Canadian federal agencies and a Maximal Acceptable Toxic Concentration for the aquatic environment. Results from this project will provide valuable input data for these models as well as highlighting likely differences between Hg cycling in benthic and pelagic food chains. Sediment quality criteria for Hg may require adjustment in light of this information.

Dissemination of Results

The Principal Investigator and co-Principal Investigator will each give at least one paper per year at international conferences on mercury in the environment. Results will also be presented at one regional workshop during each year of the study and reports will be made available to state agencies in the Chesapeake Region.

Relationship to ongoing work in CBEES program

This work interfaces with several other components of the Chesapeake Bay Environmental Effects Program, including work by Sanders (metal/metalloid uptake by phytoplankton), Riedel (particulate/metal uptake by filter feeders) and ongoing studies of methylation/demethylation processes by Gilmour. Data from zooplankton studies should be compatible with food chain investigations by Chu, Faisal and co-workers and with decomposition/settlement studies by Baker et al.

TABLE 1. Measured permeabilities ($x10^{-4}$ cm s^{-1}) for inorganic and methylmercury compounds derived from both long-term and short-term laboratory uptake experiments with the marine diatom, *Thalssiosira weisflogii*. Taken from Mason and Morel (submitted).

COMPOUND	LONG-TERM EXP. ESTIMATE OF P	SHORT-TERM EXP. ESTIMATE OF P
$HgCl_2$	9.4	8.4
$Hg(OH)_2$	-	0.48
CH_3HgCl	6.7	7.6
CH_3HgOH		1.11

* All permeabilities are in units of 10^{-4} cm s^{-1}

LIST OF FIGURES

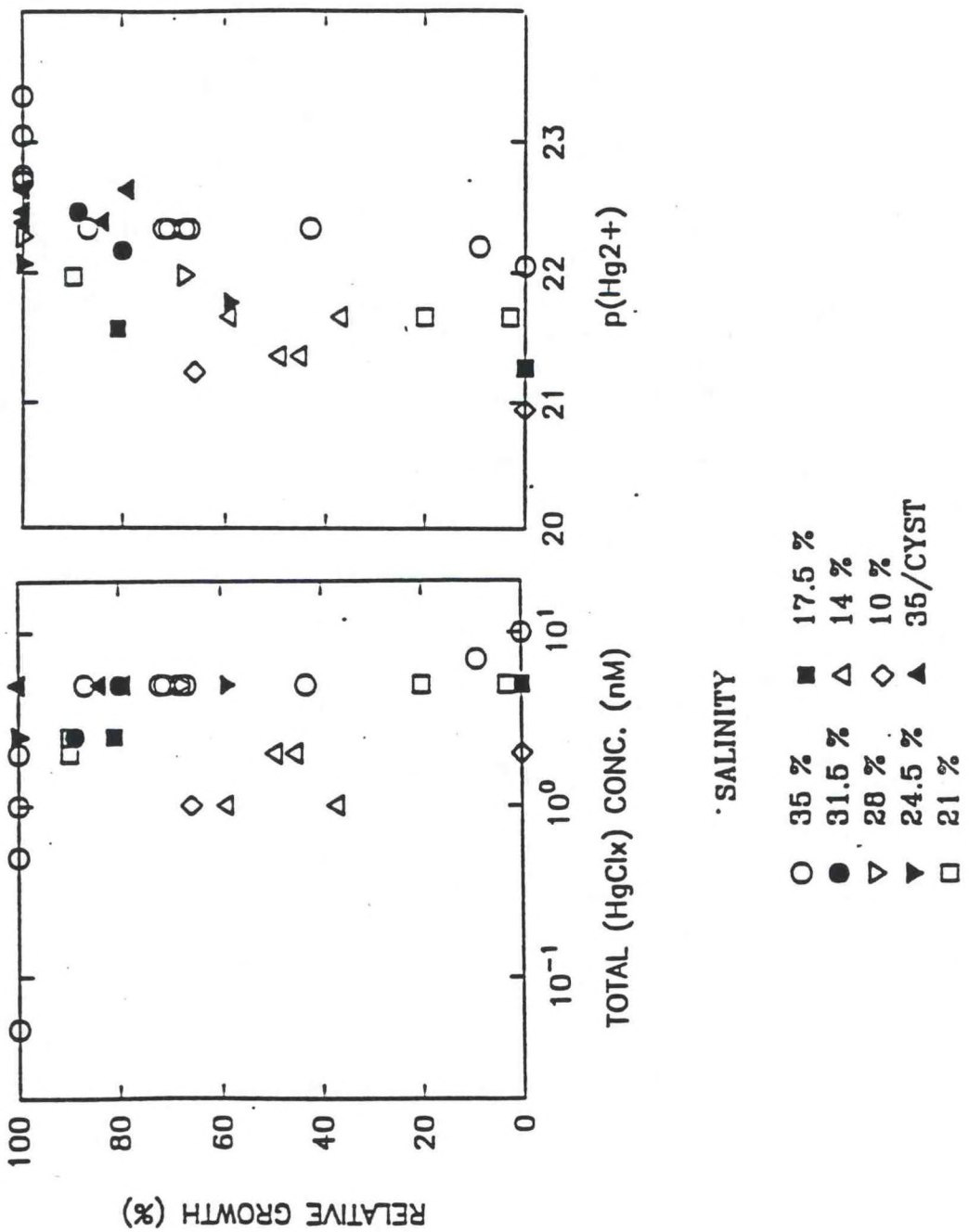

(a)
(b)
RELATIVE GROWTH (%)
100
80
60
40
20
0
10^{-1}
10^{0}
10^{1}
TOTAL (HgClx) CONC. (nM)
20
21
22
23
p(Hg2+)
SALINITY
35 %
31.5 %
28 %
24.5 %
21 %
17.5 %
14 %
10 %
35/CYST

FIGURE 1

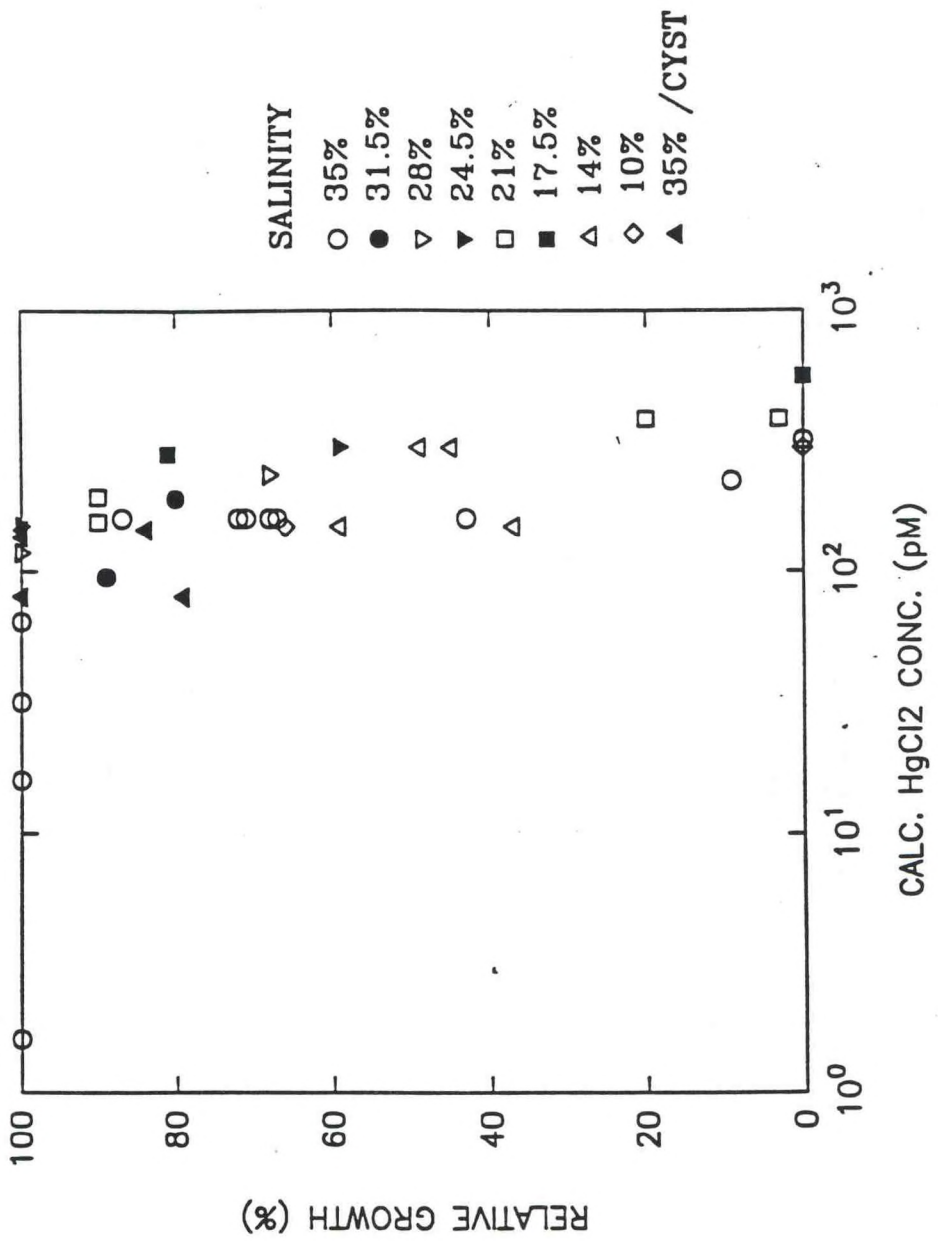

SALINITY
35%
31.5%
28%
24.5%
21%
17.5%
14%
10%
35% /CYST
RELATIVE GROWTH (%)
CALC. HgCl2 CONC. (pM)
0
20
40
60
80
100
10^0
10^1
10^2
10^3

FIGURE 1c

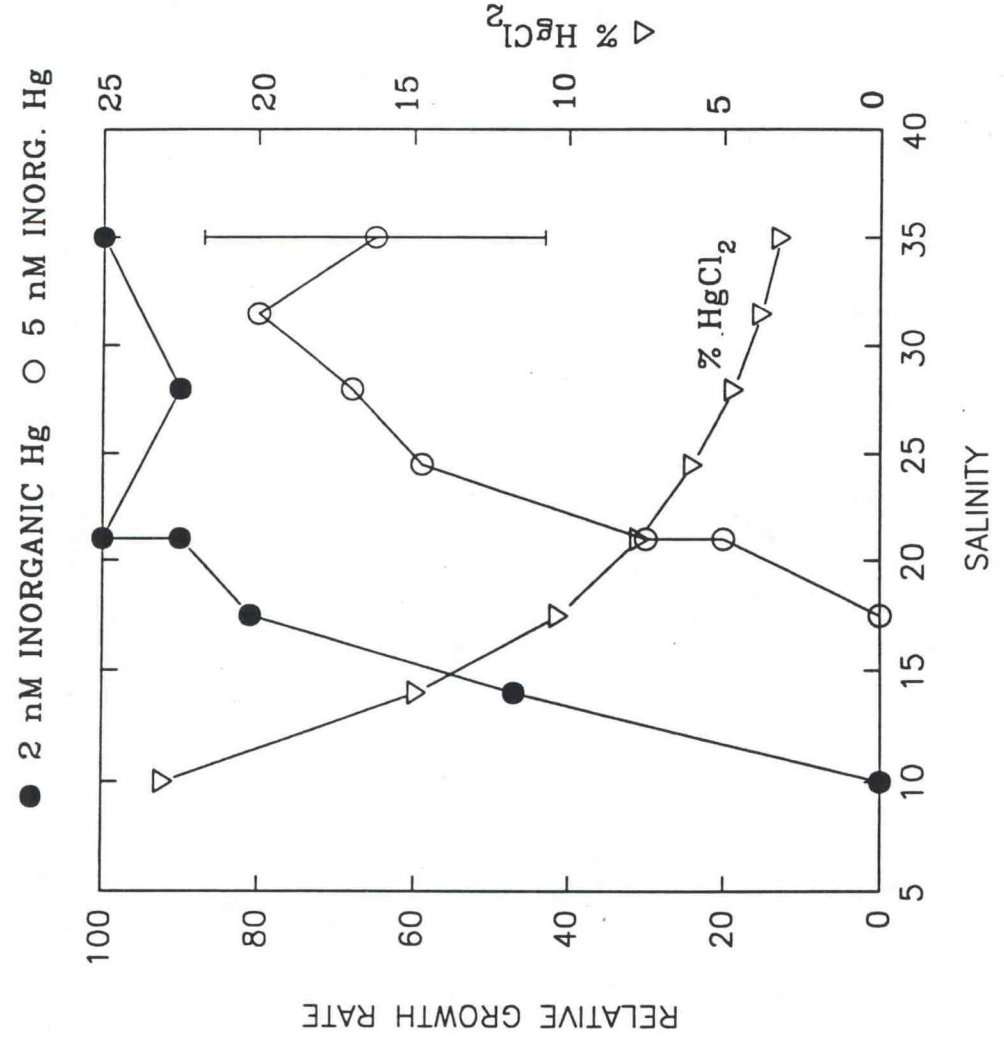

● 2 nM INORGANIC Hg ○ 5 nM INORG. Hg
△ % $HgCl_2$
% $HgCl_2$
RELATIVE GROWTH RATE
SALINITY
0
20
40
60
80
100
0
5
10
15
20
25
5
10
15
20
25
30
35
40

FIGURE 1d

FIGURE 2a

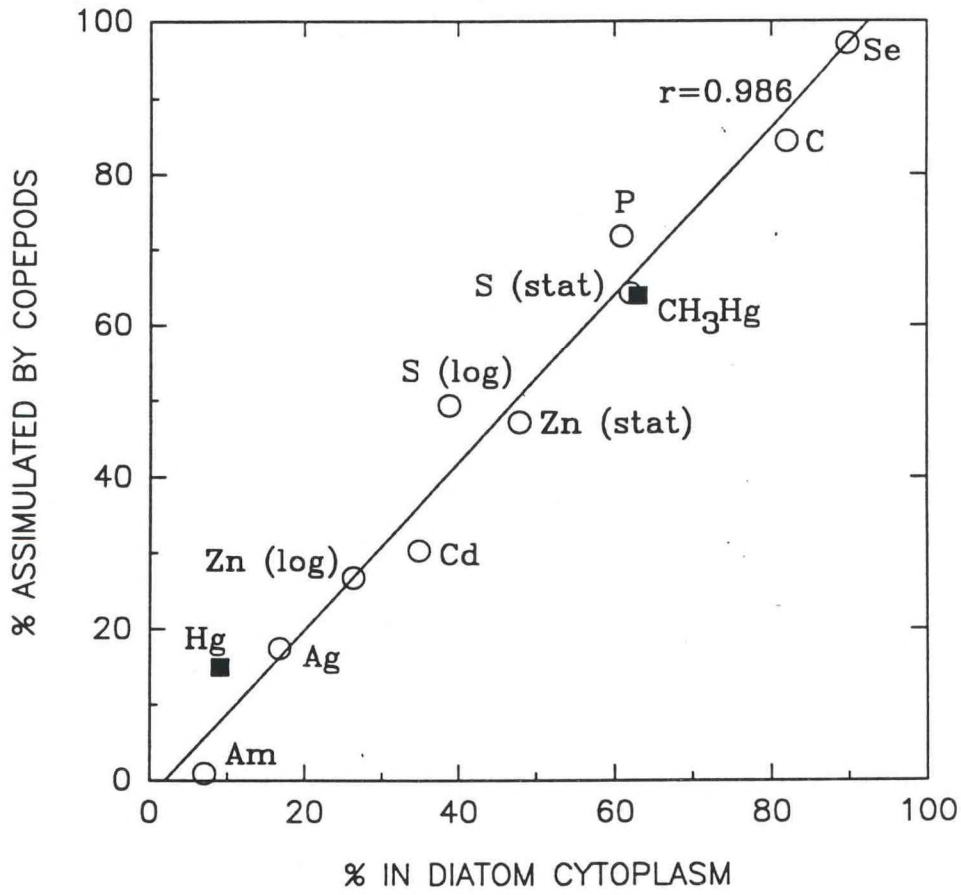
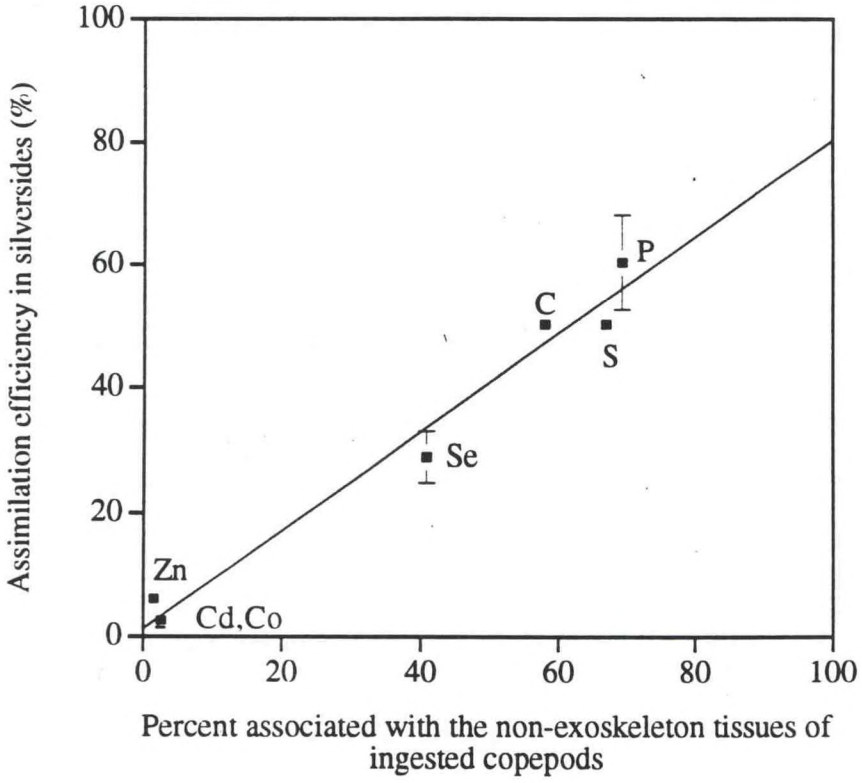

100
80
60
40
20
0
0
20
40
60
80
100
% ASSIMULATED BY COPEPODS
% IN DIATOM CYTOPLASM
r=0.986
Se
C
P
S (stat)
CH3Hg
S (log)
Zn (stat)
Cd
Zn (log)
Hg
Ag
Am

FIGURE 2b


Assimilation efficiency in silversides (%)
100
80
60
40
20
0
0
20
40
60
80
100
P
C
S
Se
Zn
Cd,Co
Percent associated with the non-exoskeleton tissues of ingested copepods

Budget Justification for: **Factors controlling the food chain accumulation and transfer of inorganic and methylmercury in Chesapeake Bay organisms** by R.P. Mason & D. A. Wright (Ref. [UMCEES]CBL-93-151a

Throughout this project, Dr. Wright will have responsibility for the design and supervision of food chain transfer experiments and Dr. Mason will oversee sample preparation and analytical procedures. 8% and 10% of their respective salaries are requested for these purposes. In year one, funds are requested to purchase a new recorder for the mercury analyzer to replace the old (> 15 year) one which is broken. Travel funds are requested to partially cover attendance of the 1995 SETAC Conference in Vancouver, Canada by the P.I. and Co-P.I. Year two travel funds are requested to offset expenses for P.I. and Co-P.I. to attend 1996 SETAC Conference.

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PROJECT NUMBER: _____

PROJECTED TIME SCHEDULE

PROJECTED ACTIVITY	YEARS 1995	1996		
• Testing and calibration of analytical equipment. Acid washing of experimental containers. Culture of diatom and crustacean species for feeding experiments. Preparation and equilibration of experimental sediment samples.	JAN-MAR			
• Experiments on effect of humics, thiol compounds and sulfide on Hg accumulation (inorganic & methyl Hg) by pelagic diatoms.	MAR-MAY			
• Experiments on relationship between sediment organic carbon content, redox potential, AVS and Hg uptake by benthic diatoms and by benthic amphipods.	MAY-AUG			
• Experiments on the effect of water chemistry and food concentration on inorganic and methylHg uptake by copepods.	JULY-NOV			
• Preparation of first year report.	DEC -	JAN		
Investigationof role of food concentration on Hg uptake by *Hyalella*		JAN-MAR		
Collection of chironomid larvae from surrounding creeks. Hg of these and other benthic biota.		APR		
• Experiments on relationship between sediment organic carbon content, redox potential. AVS and Hg uptake by chironomid larvae.		JUNE-AUG		
• Dissection of amphipods to determine principal site of Hg sequestration from inorganic and organic Hg source.		JUNE-AUG		
• Hg uptake studies on *Cyprinodon variegatus* exposed to different diets (chironomids vs. amphipods) and different aquatic OC conditions		AUG-OCT		
• Preparation of final report.		NOV-DEC		
FEDERAL PROJECTED BUDGET: MATCH	$ 60,008.	$ 65,061.		

SEA GRANT PROJECT RECORD FORM

SG-SID No._____
Specialist_____
SG Class_____

I. PROJECT SUMMARY INFORMATION

INSTITUTION: Virginia Graduate Marine Science Consortium
ICODE: 5100
TITLE: Role of benthic macrofauna in trophic transfer of organic contaminants (PAHs, PCBs) to demersal predators

PROJECT NUMBER: R/CBT-28
PROJECT STATUS: 1
SUB PROGRAM: Toxics/CBEEC

REVISION DATE: 06/15/94
INITIATION DATE: 01/01/95
COMPLETION DATE: 12/31/96

PRINCIPAL INVESTIGATOR: Linda C. Schaffner
AFFILIATION: Virginia Institute of Marine Science
ASSOCIATE INVESTIGATOR: Rebecca M. Dickhut
AFFILIATION: Virginia Institute of Marine Science

EFFORT: as needed
AFFILIATION CODE: 5101
EFFORT: as needed
AFFILIATION CODE: 5101

S.G. FUNDS: 0
LAST YEAR'S SG FUNDS: 0
PASS-THROUGH FUNDS: $105,238

MATCHING FUNDS: 0
LAST YEAR'S MATCH FUNDS: 0
LAST YEAR'S PASS-THROUGH: 0

RELATED PROJECTS: All R/CBT projects
PARENT PROJECTS: R/CBT-15, R/CBT-25
SEA GRANT CLASSIFICATION: Pollution-other-toxics (45)
KEYWORDS: TROPHIC TRANSFER, ORGANIC CONTAMINANTS, PAH, PCB, FISH, CRABS, MACROBENTHOS, BIOACCUMULATION, METABOLISM

OBJECTIVES: We will investigate trophic transfer of toxic organic contaminants (PAHs, PCBs) and metabolites of these compounds, from benthic macrofaunal species to Chesapeake Bay demersal predators. Specifically, we will investigate relationships between organic pollutant physical-chemical properties, contaminant and metabolite distribution in prey species and uptake, accumulation and metabolism of these compounds by demersal predators.

METHODOLOGY: A laboratory mesocosm approach will be used to evaluate trophic transfer of organic contaminants and metabolites from benthic macrofauna to selected demersal predators (e.g. spot, *Leiostomas xanthurus*; croaker, *Micropogonius undulatus*; flounder, numerous species; blue crab, *Callinectes sapidus*). In Series 1 experiments predator contaminant absorption efficiencies will be assessed as a function of benthic prey species and type of contaminant. Series 2 experiments will address the importance of changes in contaminant partitioning among various pools within a given prey species, as a function of time, on predator initial absorption efficiencies, subsequent partitioning among predator body regions and final contaminant pools within the predator.

RATIONALE: We have documented rapid uptake and bioaccumulation of organic contaminants (selected PAHs and PCBs) by a variety of Chesapeake Bay benthic macrofauna. These organisms vary with respect to contaminant body burdens of both parent and daughter compounds as a function of differing metabolic capabilities, the relative resistance of some compounds to metabolic breakdown and, perhaps, the ability of each species to eliminate metabolites. The work proposed here is needed to 'make the link' between macrofaunal uptake, accumulation and metabolism processes and the potential for trophic transfer of parent and daughter compounds to demersal predators. Quantitative assessment of organic contaminant transfer between estuarine benthic species and demersal predators will facilitate risk assessment for contaminants in estuarine and coastal environments.

**** SEA GRANT BUDGET ****

GRANTEE:
Virginia Graduate Marine Science Consortium

PROJECT NO:
R/CBT-28

PROJECT TITLE:
Role of benthic macrofauna in trophic transfer of organic contaminants (PAHs,PCBs) to demersal predators

PROJECT STATUS:
1

PRINCIPAL INVESTIGATORS:
Schaffner, L.C. & R.M. Dickhut

DURATION:
1/1/95 - 12/31/95

A. SALARIES AND WAGES	No. of Person.	Months	Sea Grant Funds	Grantee Funds
1. Senior Personnel				
a. Prin. Investigator			0	0
b. Associates:			0	0
Sub Total:			0	0
2. Other Personnel				
a. Professionals	1	12.0	30,000	0
b. Research Assoc.			0	0
c. RA Grad. Stud.	1		14,210	0
d. Prof. School Stud.			0	0
e. Pre-Bac. Stud.			0	0
f. Secret./Clerical			0	0
g. Technical/Shop	1		1,469	0
h. Hourly Labor	1		6,500	0
Total Salaries and Wages			52,179	0
B. FRINGE BENEFITS			8,994	0
Total Sal. Wages & Fringe Benefits (A+B)			61,173	0
C. PERMANENT EQUIPMENT			0	0
D. EXPENDABLE SUPPLIES			15,000	0
E. TRAVEL				
1. Domestic - US & Possessions	1.		700	0
2. International	2.		0	0
Total Travel			700	0
F. PUBLICATION AND DOCUMENTATION COSTS			0	0
G. OTHER COSTS				
1. ship/vessel rental			3,000	0
2. tuition			1,500	0
3. gas cyliner rental			200	0
4. photcopying			300	0
5. graphic arts			250	0
6.				
7.				
8.				
9.				
10.				
Total Other Costs			5,250	0
TOTAL DIRECT COSTS (A through G)			82,123	0
INDIRECT COSTS: On Campus: 35.47% of MTDC*			22,404	0
Off Campus: 5% of A2c			711	0
TOTAL INDIRECT COSTS			23,115	0
TOTAL COSTS			105,238	0

* less A2c, G1, G2, G5

R/CBT-28

ROLE OF BENTHIC MACROFAUNA IN TROPHIC TRANSFER OF ORGANIC CONTAMINANTS (PAHS, PCBS) TO DEMERSAL PREDATORS

Linda C. Schaffner, Assistant Professor, Department of Biological Sciences, School of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062

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INTRODUCTION

A variety of organic contaminants (e.g. polychlorinated biphenyls-PCBs, and polycyclic aromatic hydrocarbons-PAHs) can be found in estuarine and coastal environments such as the Chesapeake Bay system (Huggett et al. 1988, O'Connor and Huggett 1988). Widespread awareness by legislative agencies has led to point source reduction of these contaminants, but non-point sources such as atmospheric transport (Macdonald and Metcalfe 1991), groundwater and contaminated sediments still exist (Landrum 1989). Repeatedly, it has been demonstrated that the preferential association of a variety of organic contaminants with particles leads to increased contaminant concentrations and residence times in benthic subsystems (Olsen et al. 1982). Consequently, organisms residing at, or near, the sediment bed risk the highest exposures to contaminants in aquatic systems (McElroy et al. 1989, Connolly et al. 1991).

In Chesapeake Bay and other estuarine and coastal systems around the world, demersal predators are important components of benthic food webs and often are harvested as commercial resources. Benthic macrofauna are a major dietary component for most demersal predators (Sheridan 1974, Hines et al. 1990, Pihl et al. 1992). Demersal predators risk high exposure to contaminants through activities such as feeding on benthic organisms, and resting or seeking shelter at the sediment-water interface. Contaminants may be accumulated via

direct uptake from food (Pizza and O'Connor 1983; Rubinstein et al. 1984; Fisher et al. 1986), sediments (Stein et al. 1987), and the water column (McCarthy and Jimenez 1985). While the relative importance of diet versus sediment versus water sources of contaminant exposure has not been adequately quantified for most predators, the existing body of evidence indicates that food chain transfer of organic contaminants from benthic organism prey is a significant exposure route for benthic predators (McElroy et al. 1989, Cochran 1994).

Bioaccumulation of organic contaminants by benthic macrofaunal organisms has been widely documented (James 1989, McElroy et al. 1989, Weston 1990, Dickhut et al. 1994). Many of these organisms also have the ability to metabolize organic contaminants, especially PAHs (James 1989, McElroy et al. 1989, McElroy 1991, Dickhut et al. 1994). Metabolic degradation of parent compounds to more hydrophilic, conjugated forms can result in increased excretion and, therefore, elimination from the body. But, the process may also lead to the formation of highly reactive intermediates which are more carcinogenic than the parent form (Andersson and Paert 1989). McElroy and colleagues (McElroy and Sisson 1989, McElroy et al. 1991) have investigated the potential for metabolites produced by a benthic organism to be transferred to a predator. They found that benzo[a]pyrene metabolites were transferred from the polychaete *Nereis virens* to the winter flounder, *Pseudopleuronectes americanus*, although bioavailability of naturally occurring metabolites was found to be lower than that of parent compound. Both parent and daughter compounds resulted in the formation of DNA adducts in the predator. Broman et al. (1990) found that metabolizable PAHs showed decreasing concentrations at higher trophic levels, but did not assess the fate of metabolites bound to macromolecules. Contaminant loss due to metabolism, or alternatively, a failure to account for all metabolite pools, may account for the apparent lack of biomagnification of parent forms of PAHs in food webs. Conversely, PCBs, which tend to be resistant to biodegradation, often exhibit biomagnification of the parent form (e.g. Thomann and Connolly 1984, Connolly and Pedersen 1988, Evans et al. 1991). Clearly, rates of metabolite production and contaminant partitioning among various pools within organisms are likely to be highly important factors governing trophic transfer and contaminant fate.

Our recent studies have documented rapid uptake and bioaccumulation of organic contaminants (selected PAHs and PCBs) by a wide variety of Chesapeake Bay benthic macrofaunal taxa (Dickhut et al. 1993, Schaffner et al. 1993). We have shown that taxa vary with respect to contaminant body burdens of both parent and daughter compounds. Furthermore, we have hypothesized that differing metabolic capabilities of each species and the relative resistance of some compounds to metabolic breakdown are important factors governing the rates of contaminant uptake, accumulation and elimination (Dickhut and Schaffner 1994). Thus, we are evaluating the rates of metabolism, and distribution of organic contaminants (selected PAHs and PCBs) among various pools (i.e. parent compounds, phase I (nonpolar metabolites), phase II (conjugated, polar) metabolites, and bound residues (e.g. DNA adducts) within selected macrofaunal species. Further work, such as is proposed here, is needed to 'make the link' between macrofaunal uptake, accumulation and metabolism processes and the potential for trophic transfer of parent and daughter compounds to demersal predators.

The proposed research will investigate the relative importance of trophic transfer of toxic organic contaminants (PAHs, PCBs) and metabolites of these compounds from benthic macrofaunal species to demersal predators of Chesapeake Bay. Specifically, we will investigate relationships between organic pollutant physical-chemical properties, contaminant and metabolite distribution in prey species and uptake, accumulation and metabolism of these compounds by demersal predators. This research will be highly applicable to other estuarine and coastal systems where the benthic food web is important.

Relevance to the Problem

Biological uptake of organic contaminants by aquatic organisms has important implications for ecosystem health and utilization by humans or other living resources. Biomagnification, that is increased contaminant concentrations with trophic level, of some compounds (e.g. DDT and PCBs), are of particular concern. If movement of organic contaminants between various phases is via equilibrium partitioning processes, then the concentration of

contaminant relative to the lipid phase of all animals should be the same, regardless of trophic level, when the system attains equilibrium (Connell 1989). However, contaminant concentrations increase above that predicted by equilibrium distribution as trophic level increases, due to suspected uptake from food sources (Connolly and Pedersen 1988; Clark et al. 1990). For example, Goerke et al. (1979) found more than a three-fold increase in PCB concentration in a demersal predator (flatfish, *Solea solea*) relative to deposit and filter-feeding benthic invertebrates in a German estuary. Other field studies have reported four to twelve-fold increases in contaminant concentrations at higher trophic levels (Thomann and Connolly 1984; van der Oost and Opperhuizen 1988; Rasmussen et al. 1990; Evans et al.1991).

Our ability to predict trophic transfer and biomagnification of organic contaminants remains inaccurate due to a lack of evaluation and incorporation of biological processes (e.g. food chain transfer) into contaminant ecosystem models (Clark et al.1990). Quantitative assessment of organic contaminant transfer between estuarine benthic species and demersal predators will facilitate risk assessment for contaminants in estuarine and coastal environments.

HYPOTHESES AND OBJECTIVES

The major hypotheses for the proposed study are:

(1) macrofaunal uptake, accumulation and metabolism processes influence rates and forms of contaminants transferred to predators;

(2) physical-chemical properties of contaminants (structure, octanol-water partitioning, desorption rates) affect efficiencies and rates of contaminant uptake and transformation by macrofauna and, therefore, the bioavailability of contaminants to predators.

Overall Objectives

A laboratory mesocosm approach will be used to evaluate the relative importance of food chain transfer, and investigate the pathways and rates organic contaminant and metabolite transfer from benthic macrofauna to selected demersal predators (e.g. spot, *Leiostomas xanthurus*; croaker, *Micropogonius undulatus*; flounder, numerous species; blue crab, *Callinectes sapidus*). Contaminant transfer to predators from different prey species will be quantified and related to contaminant distribution in prey and chemical properties.

1995 Objectives

During 1995 we will conduct experiments to assess the importance of trophic transfer, measured as absorption efficiency of contaminants by demersal predators, via consumption of different benthic prey species and as a function of type of contaminant (Series 1 Experiments).

1996 Objectives

During 1996 we will address the importance of contaminant partitioning among various pools within a given prey species, as a function of time and contaminant type, on uptake, accumulation and metabolism of these compounds in demersal predators (Series 2 Experiments).

METHODOLOGY

Experimental Organisms

Laboratory experiments will be conducted using two or more demersal predators and selected invertebrate macrofauna species. Predator species will be chosen based upon commercial importance, functional importance and availability. Macrofaunal species to be used as prey

will be selected based upon available information on importance as prey items and evidence regarding metabolism capabilities. Possible predators include the fish *Leiostomas xanthurus* (spot), *Micropogonius undulatus* (croaker), flatfish (numerous species available), and the blue crab *Callinectes sapidus*. Possible macrofauna taxa for use as prey include the polychaetes *Paraprionospio pinnata*, *Streblospio benedicti*, *Nephtys* spp., *Glycera* spp., the amphipod *Leptocheirus plumulosus* and bivalves of the genus *Macoma*.

Leiostomas xanthurus, *Micropogonius undulatus*, flounder and *Callinectes sapidus* are abundant, commercially important predators in the Chesapeake Bay system. The polychaetes are numerically important in Chesapeake Bay benthic communities and are important prey items for many demersal predators (Pihl et al. 1992). We have demonstrated metabolism of PAHs and lower chlorinated PCB congeners by *Paraprionospio pinnata* in ongoing laboratory experiments. Existing evidence suggests that, as a group, annelids demonstrate moderate to high PAH metabolism capabilities (James 1989). The amphipod *Leptocheirus plumulosus* reaches extremely high densities (i.e. > 10,000 per sq. meter) and is likely to be an important food item for demersal fish in the Chesapeake Bay system. We have not evaluated the metabolic potential for this species, but, in general, crustaceans exhibit high metabolism capabilities for PAHs. Bivalve mollusks of the genus *Macoma* are especially important in the diet of *Callinectes sapidus*. Mollusks have low ability to metabolize PAHs (James 1989).

Organisms will be collected from the York River-Chesapeake Bay system. Whenever possible, they will be maintained in laboratory culture (the polychaete *Streblospio benedicti* has been maintained in culture in our lab in the past; the amphipod *Leptocheirus plumulosus* currently is in culture in a colleague's lab). Predator species (juveniles, young-of-year) will be collected by trawl or trap. For all organisms used, a narrow size range will be selected to minimize possible effects of body size and growth on contaminant 'dilution'. All organisms will be acclimated to experimental conditions (25° C) for 1 or 2 weeks (dependent on temperatures at time of collection) before each experiment.

Laboratory Seawater System

All experiments will be done under controlled laboratory conditions with a flow-through seawater system. Water will be sand and carbon filtered, passed into a head tank, and gravity pipetted to each aquarium allowing for a rate of exchange of at least 6 times daily. Flow rates will be monitored daily. Water parameters (temperature, salinity, dissolved oxygen, pH etc.) will be continuously monitored using an environmental monitoring unit with a self-contained data-logger.

Organic Contaminants

We propose to use a range of organic contaminants such as the polycyclic aromatic hydrocarbons (PAHs) (e.g. pyrene, anthracene, benzo[a]pyrene) and polychlorinated biphenyl (PCBs) congeners in our experiments. PAHs and PCBs tend to be hydrophobic, and hence, associate with sediments in aquatic systems and may be bioaccumulated. Many individual PAHs and PCBs are known to be toxic. Most PAHs are byproducts of fossil fuel combustion and have widespread distribution in coastal and estuarine systems due to both point and nonpoint (e.g. atmospheric deposition) source inputs. PCBs are especially persistent synthetic hydrocarbons that may be biomagnified (van der Oost and Opperhuizen 1988, Swackhammer and Skoglund 1993). Although active production of these compounds has been halted, release from secondary sources (e.g. sediment reservoirs) is a continuing problem.

Organic contaminant distributions in Chesapeake Bay and its tributaries vary widely with area, chemical type and concentrations, however, there is a general decrease of contaminants from north to south and west to east, which may reflect higher inputs from more densely populated, higher industrialized areas (Huggett et al. 1988). Localized high levels of PCBs and related compounds have been reported, with sediment concentrations up to 26,000 ug/kg, and edible food concentrations greater than three times the US Food and Drug Administration (USFDA) action limits (Hale et al. 1990). Sediment polycyclic aromatic

hydrocarbon (PAHs) concentrations range from 9 ug/kg (dry weight) in relatively pristine areas (e.g. York River) to 2200 mg/kg in grossly polluted areas (e.g. Elizabeth River) (Foster and Wright 1988, van Veld et al. 1990, Vogelbein et al. 1990). Vogelbein et al. (1990) have reported up to 93% prevalence of liver cancer in the mummichog (*Fundulus heteroclitus*) exposed to high levels of PAHs in the Elizabeth River.

Test compounds for our studies will be selected based on their hydrophobicity (octanol/water partition coefficient - K_{ow}), anticipated difference in uptake, transformation and elimination rates, and availability as ^{3}H and/or ^{14}C radiolabeled compounds. Several CBEEC sponsored studies of chemical species from these different classes of toxic organic contaminants are ongoing. Research on selected PAHs and PCBs will provide information on pollutant behavior which generalizes to numerous related chemical species.

As a starting point, we intend to expose prey organisms to contaminant concentration levels below those that cause acute toxicity, i.e. at levels similar to those observed in main-stem Chesapeake Bay (Huggett et al. 1988, van Veld et al. 1990). Subsequently, we hope to extend these studies to examining systems reflective of heavily polluted or impacted environments (e.g. Elizabeth River). One way to accomplish this would be to conduct a series of experiments in which prey organisms are exposed to mixtures of radiolabeled contaminants and (diluted) Elizabeth River sediments. We will proceed at this level dependent upon our success with our initial experiments.

Background Concentrations of Organic Contaminants

Background PAH and PCB concentrations in ambient sediments and organisms will be measured utilizing a combination of gas chromatography/mass spectrometry (GC/MS) and gas chromatography/electron capture (GC/ECD) analysis. Briefly, samples are freeze dried (or extracted first with methanol) and subsequently solvent extracted for 48 hr with dichloromethane using a soxhlet apparatus. Clean-up of samples is achieved by gel permeation chromatography for removal of organic polymers and liquid chromatography for

separation of aliphatics and polar compounds (e.g. Bieri et al. 1986). The extract is then concentrated using rotoevaporation, followed by evaporation under purified nitrogen and solvent exchange into hexane, for subsequent GC/MS and GC/ECD analyses.

PAHs will be identified and quantified using GC/MS with selected ion monitoring and isotopic (deuterated) internal standards. Subsequent to PAH analysis, the remaining extract, is fractionated by solid-liquid chromatography using a Florisil column (1.25% water deactivated) eluted with hexane followed by 10% (v/v) diethylether in hexane to separate PCBs and interfering compounds. PCBs are then analyzed by high resolution GC/ECD with noncommercial PCB congeners as the internal standards.

Contaminated Sediment Preparation

The contaminated sediments that will serve as substrate for infaunal prey in the experiments discussed below will be prepared by spiking fresh, sieved, field-collected, surface sediments (collected from the 0 to 1 cm interval) with radiolabeled organic contaminants. Pairs of radiolabeled compounds (e.g. one ^{3}H-labeled and one ^{14}C-labeled) will be added to the sediment by mixing aqueous slurries with organic contaminant spikes (in ca. 1 ml methanol) in 1 L glass jars. The organic contaminant spikes will be added to the system such that initial contaminant concentrations do not exceed one half the aqueous solubility for the chemical. The sediment concentration and amount of radiolabeled compound utilized will be adjusted in order to obtain appropriate trace level spikes (see Landrum 1989 and Weston 1990). All preparative and analytical procedures will be performed under gold fluorescent lights to avoid PAH photodegradation. Sediment spikes are then aged for at least one week and later homogenized before adding to experimental systems.

Series 1 Experiments - Trophic Transfer as a Function of Prey Species

These experiments will assess the importance of trophic transfer of contaminants to demersal predators via consumption of different benthic prey species, and as a function of type of

contaminant. Several types of macrofaunal prey will be established in culture dishes with radiolabeled sediments. Organisms will be maintained in contaminated sediments for one week. This will insure that they have high tissue concentrations and some will have high metabolite production within this time interval (based on our previous results). During this exposure period, water will be changed on a daily basis.

At the end of a week a few prey will be 'harvested' from the culture dishes. Their gut contents will be removed and retained. The animals will be blotted dry, weighed, extracted and analyzed for radiolabeled parent and metabolite compounds as described below. Gut contents and small sediment samples from the culture dishes will be sampled for organic carbon content and contaminant concentrations (as discussed below) and then related to prey concentrations.

The organisms remaining in the culture dishes will be harvested immediately and then fed to predators within one hour. An additional set of prey, subsampled just before the predators are fed, will then be extracted with gut contents intact. Predators will previously have been established individually in small aquaria in the laboratory. They will be starved for 12 hrs prior to feeding with contaminated prey. After a feeding interval (e.g. 4-6 hours, a period of time sufficient for dietary fat digestion and absorption, (Vetter et al. 1985, Van Veld et al. 1990) they will be killed (using MS-222, as described below) and analyzed for total contaminant concentrations, evaluation of partitioning among body regions and contaminant forms present. Detailed methodologies are presented below.

For each predator, trophic transfer will be assessed by determining absorption efficiencies for each contaminant pool (i.e. parent compound or metabolite(s)) from prey (e.g. Tanabe et al. 1982; Fisher et al. 1986) using the equation:

$$AE = \frac{predator\ concentration * mass\ of\ predator}{prey\ concentration * mass\ of\ prey} \times 100$$

where concentration refers to parent compound mass equivalence per unit biomass for each contaminant pool, respectively.

Series 2 Experiments - Trophic Transfer as a Function of Time of Prey Exposure

These experiments will address the importance of changes in contaminant partitioning among various pools within a given prey species, as a function of time, on predator absorption efficiencies, subsequent partitioning among predator body regions and final contaminant pools within the predator. The questions we propose to study with this series of experiments are: 1. Does the predator absorption efficiency for the various contaminant pools change with time or remain constant? and, 2. Do the forms of metabolizable contaminants change with time in the prey species, and influence the overall contaminant bioavailability to predators. With respect to the first question, the absorption efficiency of a contaminant by the predator may change with exposure time if the physiology of the organism is altered by exposure to contaminants or if uptake is not a simple first order rate process. The second question arises from observations from our previous experiments that contaminant forms change within macrofauna as a function of exposure duration (Dickhut et al. 1993). Thus, over time, forms of contaminants will vary within and among macrofaunal organisms depending on each species ability to metabolize organic contaminants and eliminate both parent compounds and metabolites. For example, some species may slowly metabolize contaminants to water soluble forms, accumulating reactive intermediates (e.g. DNA adducts). Other species may rapidly metabolize parent compounds but accumulate water soluble, conjugated compounds due to an inability to eliminate structurally bulky metabolites. Thus, potential changes in bioavailability of metabolizable organic contaminants and predator absorption efficiencies need to be assessed to develop accurate models of food chain transfer of these toxic compounds.

Using procedures similar to those described above, prey species will be exposed to contaminated sediments for a predetermined period of time, e.g. 1 day, 7 days, 21 days. Predators will be randomly assigned to treatment groups and will be maintained in flow-

through aquaria containing only seawater. Predators will be fed a daily ration (10% of body weight, adjusted for growth) of prey exposed to contaminants for specific durations (e.g. 1 day, 7 days, 21 days). Predators will be removed at selected time intervals and sampled as described below. The overall temporal sampling scheme relative to prey and predator exposure durations is shown in Figure 1. Exposure of predators to radiolabeled contaminants in their diet throughout the experiment will be used to assess regions of accumulation within their bodies. Exposure to nonradiolabled compounds followed by radiolabeled chemicals will be used to determine changes in assimilation efficiencies with time.

Organism Sampling Protocols

During each experiment, prey organisms will be 'harvested' from sediments by gentle sieving. In some cases they will be allowed to evacuate their guts prior to further analyses. This will be accomplished by placing them into small culture dishes filled with 'clean' sediments for a time interval sufficient to allow full gut turnover. This time interval will be determined using organisms from 'blank' (no chemical) samples and a fine, particulate fluorescent tracer. We will assume that full gut evacuation of contaminated sediment has occurred when organisms begin producing fecal pellets containing the tracer. We expect this time interval to be less than 2-3 hours based upon previous experience.

On each sampling date, predators will be removed from aquaria and killed by overdose with 500 mg/L ethyl m-aminobenzoate methanesulfonate (MS-222) (Cochran 1994). They will then be blotted dry, weighed and, in some cases, dissected prior to chemical analysis. For whole organism analyses, stomach and intestinal contents will be removed prior to processing for contaminants. Length and weight will be measured for calculating a condition index (CI) and to allow an adjustment of food ration for animals remaining in an experiment:

$$CI = \frac{weight \times 10^5}{length^3}$$

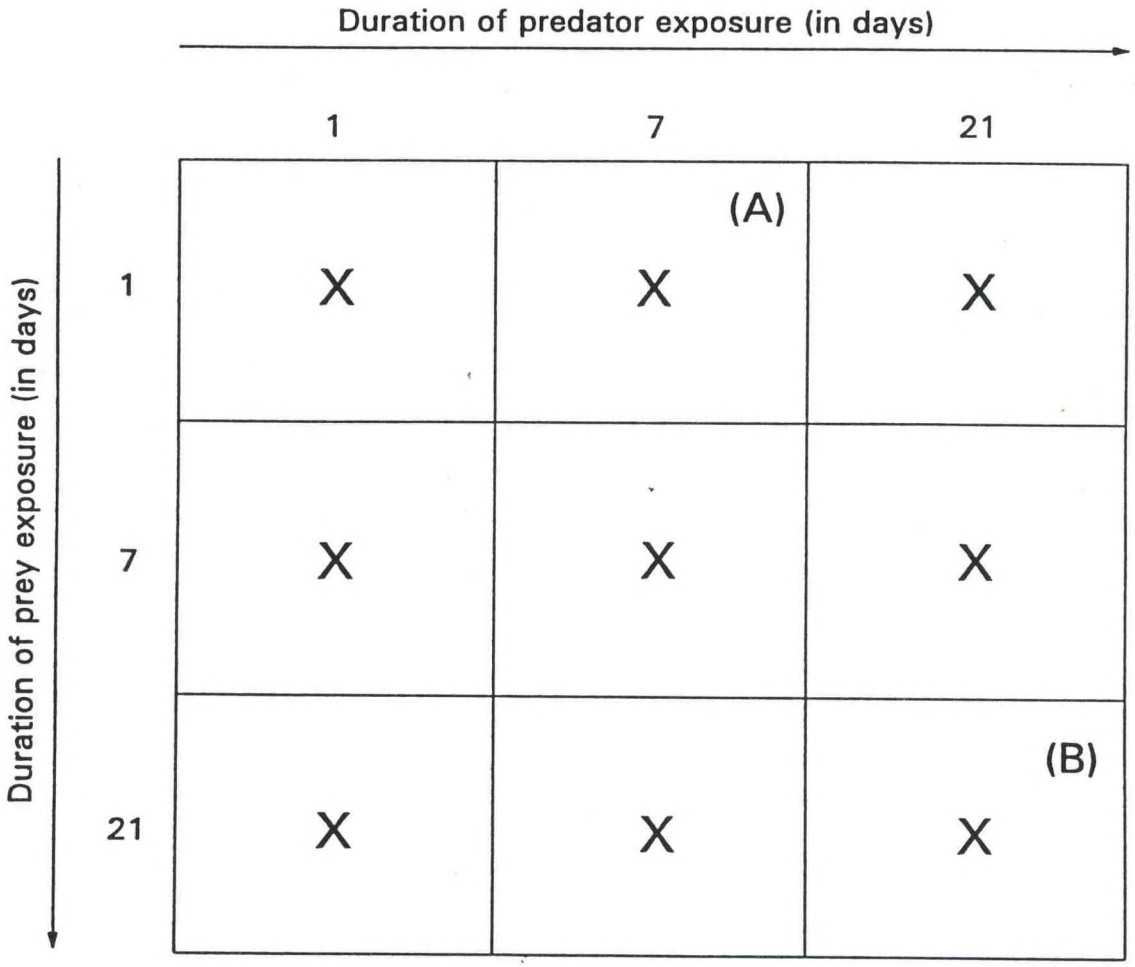

Figure 1: Temporal sampling scheme for Experiment 2. For example, predators sampled as in example A have been fed for 7 days on a diet of prey previously exposed to contaminants for 1 day. In example B, predators have been fed for 21 days on a diet of prey previously exposed for 21 days.

The calculated CI will be compared to values for a natural wild population as a relative indication of predator health (e.g CI of spot = 1.93, Fisher 1985).

Macrofauna and predator tissue samples from various compartments (e.g. body tissue, liver, bile and intestine) will be analyzed for both lipid content and radiolabeled compounds as described below.

Lipid Analysis

Organism lipid content will be determined using a modified Bligh and Dyer method (found in Randall et al. 1991) for each compartment sampled. Briefly, tissues are extracted with a homogenizer in a mixture of chloroform and methanol. After centrifugation and solvent separation, the chloroform extracts are removed by syringe and filtered through a 0.45 μm filter to prevent contamination. The residue remaining after being air- and oven-dried to constant weight are then weighed and reported as percent lipid.

Radiolabeled Contaminant Analysis - Prey and Predators

Organisms and selected tissues will be analyzed for radiolabeled organic chemicals using methods modified from those of McElroy (1991) (Figure 2). Four major classes of compounds are considered in the biota: [1] parent compound, [2] primary metabolites which are more polar than the parent compound but still extractable with organic solvents, [3] secondary, conjugated metabolites which are water soluble, and [4] bound metabolites which are resistent to extraction. Briefly, organic contaminants and degradation products are extracted twice using a combination of methanol and dichloromethane (DCM), and back extracted with Milli-Q water. Subsequently, the sample residue is removed via centrifugation and the aqueous (methanol/water) fraction is reextracted with dichloromethane. Residual tissue is then dried and combusted at 1000°C with evolution of bound radioactivity (e.g. as $^{14}CO_2$) which is trapped in phenethylamine and subsequently quantified by liquid scintillation counting (LSC). The DCM extracts are combined and concentrated by rotoevaporation with

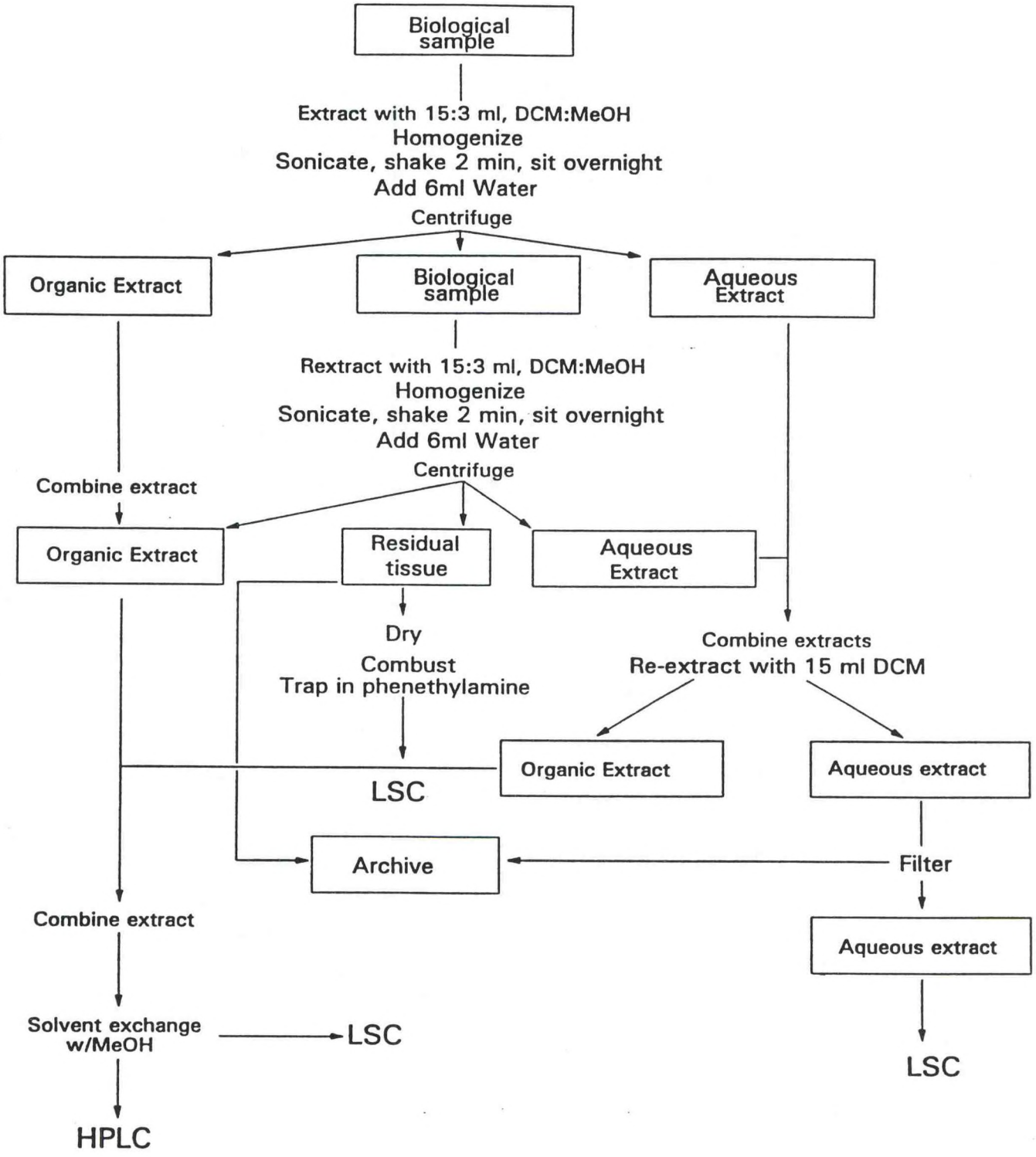

Figure 2: Extraction procedure for biological samples less than 5 grams: LSC, liquid scintillation counting; MeOH, methanol; DCM, dichloromethane; HPLC, high-performance liquid chromatography. Ratios will be adjusted for larger size samples.

solvent exchange to methanol. Subsequently, the samples are examined for total radioactivity using LSC. Parent compounds and polar metabolites are determined via a combination of high performance liquid chromatography (HPLC) with LSC detection. Evaluation of radioactivity by LSC in the aqueous fraction results in quantification of the secondary, conjugated metabolites.

McElroy (1991) cites sample recoveries as 90% using this procedure, and blank levels are low due to use of radiolabeled compounds. Radiolabeled organic chemical activity will be measured for the extract fractions using a Beckman Model LS 5000TD liquid scintillation system and a Waters HPLC with a Packard Radiomatic detector. A Packard (Model OX-500) biological oxidizer will be used for sample combustion.

Radiolabeled Contaminant Analysis - Sediment

Sediment samples will be analyzed for radiolabeled contaminants using a hexane/methanol extraction procedure currently employed in our labs (Figure 3). Our contaminant recoveries with this procedure are >95% as established by replicate extractions. Nonetheless, combustion analysis of the residual sediment will allow confirmation of our extraction efficiencies for each individual sample. In our previous experiments (Schaffner and Dickhut, 1992) aqueous extractable, water soluble compounds in the sediment were at or near background (blank radioactivity) levels.

Elimination rate experiments

To model bioaccumulation the elimination rate constants for contaminant pools in predators will be determined during the course of the experiments outlined above. Animals will be removed from aquaria at selected sampling times, placed into clean (uncontaminated), flowing seawater and elimination followed for approximately 30 days by measuring

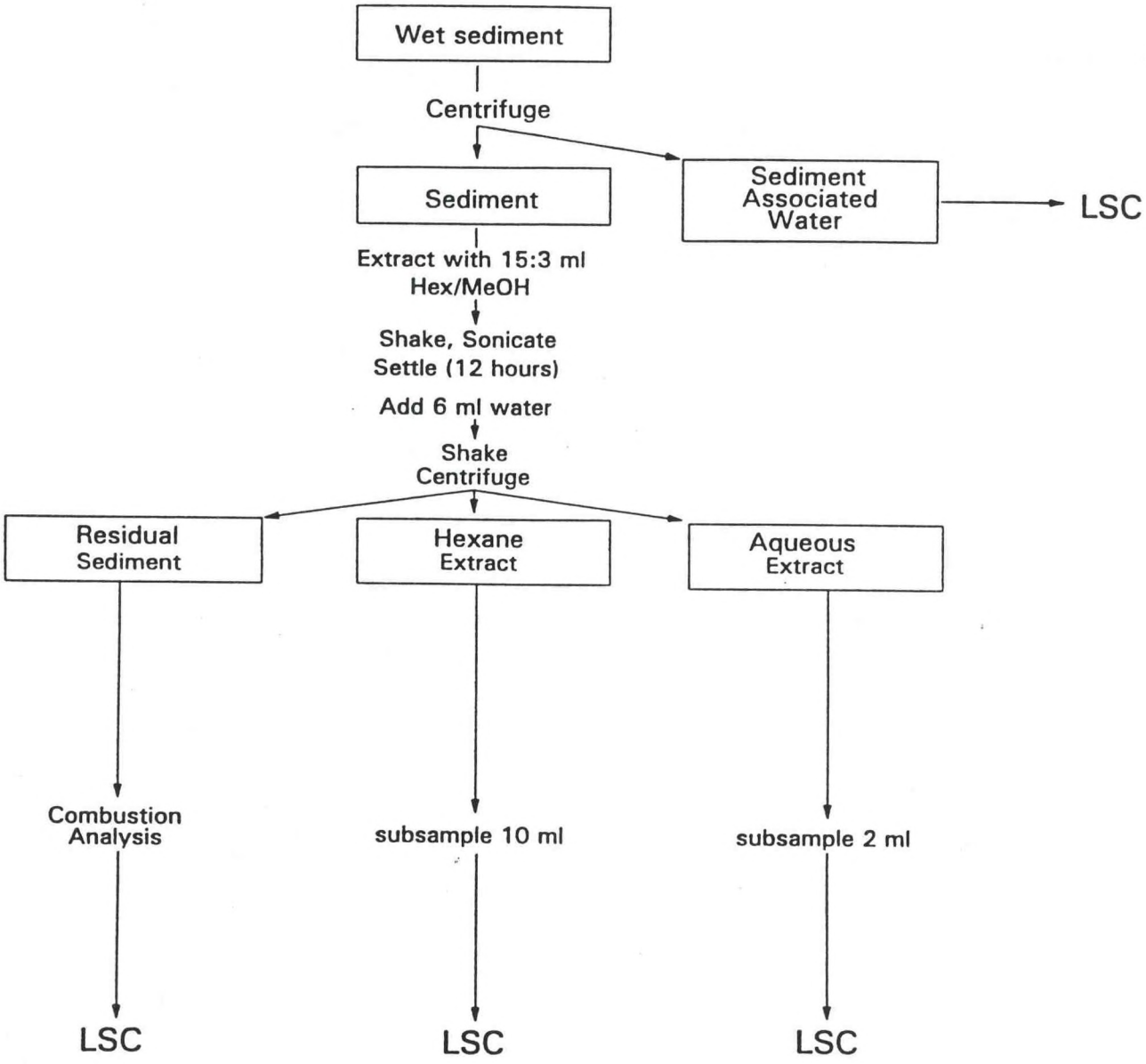

Figure 3: Extraction procedure for less than 5 grams of sediments: LSC, liquid scintillation counting; MeOH, methanol; Hex, hexane. Ratios will be adjusted for larger size samples.

contaminant concentrations over the elimination period. On each sampling day, animals will be sacrificed and sampled for contaminant pools and lipids as already described.

Modelling

MacKay (1982) developed a model for bioaccumulation as a balance between kinetic processes involving first-order rate constants: uptake (k_1) from water, elimination (k_2), and absorption efficiency (k_3) from food. This equation is:

$$\frac{dC_B}{dt} = k_1 C_M - k_2 C_B + k_3 Q$$

where C_M = concentration of contaminant in medium, C_B = concentration of contaminant in biota, Q = the daily dietary dose (weight of food offered x fish weight^{-1} x time^{-1}), and t = time in days.

For fish with constant exposure to contaminated food in the absence of exposure from water, the concentration is given by:

$$C_B(t) = \frac{k_3}{k_2} Q(1 - e^{-k_2 t})$$

(Fisher et al. 1986). Thus, if the processes of dietary uptake and elimination are first order, the food chain transfer of contaminants from benthic macrofauna to demersal predators can be modeled with the coefficients to be generated through our experiments.

EXPECTED RESULTS

The proposed work will identify the potential for trophic transfer of representative organic contaminants (PAHs and PCBs) from benthic macrofaunal prey species to commercially important demersal predators such as spot, croaker, flounder and the blue crab. Specifically, we will assess the bioavailability and fates of various forms of organic contaminants (e.g. parent compound, metabolite pools) to benthic predators. This will allow us to estimate ecologically-relevant rates and modes of exposure to organic contaminants for commercially important demersal predators through the benthic food web in the Chesapeake Bay estuarine system. The data obtained from this study will be useful for modelling contaminant accumulation in commercial fish species and, for developing human health risk assessment models. In general, the information to be generated by the proposed research will aid in developing our understanding of organic contaminant effects on higher trophic level aquatic organisms and overall ecosystem effects. Our studies will have general applicability to other systems where the benthic food web is important.

DISSEMINATION OF RESULTS

We expect that the results of this work will be of significant interest to those involved in making management decisions for the Chesapeake Bay and to the scientific community in general. As with our ongoing work, we will make every attempt to deliver our findings to the scientific and management community. For example, we have reported findings from our ongoing related studies at two CBEES workshops, at a Contaminated Sediments Critical Issue Forum (December 1992) for CBP Toxics Subcommittee members and CBP Scientific and Technical Advisory Committee (STAC) members, at an invited seminar for the NOAA Status and Trends group, at the 1993 Estuarine Research Federation Meeting, and in a number of other forums. We are highly responsive to requests to distribute information to appropriate agencies and individuals. We will continue to present our results at national scientific meetings (e.g. Society of Environmental Toxicology and Chemistry and Estuarine Research Foundation). We now are in the process of manuscript preparation for recently

completed studies. We fully expect to submit future findings for publication in the appropriate peer-reviewed scientific journals.

RELATIONSHIPS TO OTHER WORK

Our work will be among the first studies to make the link between uptake, accumulation and metabolic processes in benthic macrofaunal prey species and uptake, accumulation and metabolism in benthic predators. The proposed project is an extension of our previous work examining the role of benthic communities in sediment-associated toxic organic chemical fate and transport in lower Chesapeake Bay Schaffner and Dickhut 1992), and organic contaminant metabolite production, accumulation and elimination in benthic macrofauna of lower Chesapeake Bay (Dickhut and Schaffner 1994). This research will further our understanding of food chain transfer of organic contaminants between benthic macrofauna and demersal predators in marine systems. We will generate data on the absoprtion efficiencies of various estuarine fish species for organic contaminant forms which are present in their diets after ecosystem exposure to these toxicants. This work will provide valuable information for those evaluating toxics effects, because our results will allow them to determine ecologically-relevant mechanisms and rates of trophic transfer for benthic predators.

BUDGET NOTES

Salary is requested for a post-doctoral associate who will have expertise in analytical biochemistry. The salaries of the principal investigators will serve as voluntary match. Each principal investigator expects to devote at least 20% of their time to this project. We also have requested support for a Ph.D. student (Patrick Lay) who will use some of the proposed research for his dissertation.

During the first year we have requested funds for local (field work - 4 trips @ $50. ea.) and regional (workshops - 2 trips @ $250. ea) travel. During the second year we have included funds for travel to national scientific meetings (3 trips @ $600. each).

Under the supply category, the bulk of the funds ($10,000.) are requested for the purchase of radiolabeled organic contaminants. Other supplies include laboratory glassware and solvents, wet laboratory supplies ($4,500.). Also included in this category is research vessel fuel ($500.).

We have not requested funds for permanent equipment.

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PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	Y E A R			
	1995	1996	1997	1998
Conduct experiments to assess the importance of trophic transfer of organic contaminants by demersal predators via consumption of different benthic prey species and as a function of type of contaminant	———			
Conduct experiments to assess the importance of contaminant partitioning among various pools within a given prey species, as a function of time and contaminant type, on uptake and partitioning in predators		———		
Evaluate data and prepare manuscripts for publication		——		
PROJECTED FEDERAL BUDGET	$105,238	$107,699	$	$
PROJECTED MATCH BUDGET	$ 0	$ 0	$	$

SEA GRANT PROJECT RECORD FORM

SG-SID-No.:_____
Specialist:_____
SG Class: _____

I. PROJECT SUMMARY INFORMATION

INSTITUTION: Virginia Graduate Marine Science Consortium
ICODE: 5100
TITLE: Impacts of CCA pressure-treated wood structures in Chesapeake Bay
PROJECT NUMBER: R/CBT-29 **REVISION DATE:** 06/17/94
PROJECT STATUS: 1 **INITIATION DATE:** 01/01/95
SUB PROGRAM: Toxics/CBEEC **COMPLETION DATE:** 12/31/96

PRINCIPAL INVESTIGATOR: Judith S. Weis **EFFORT:** 4.0 mo
AFFILIATION: Rutgers University
AFFILIATION CODE: 3406
CO-PRINCIPAL INVESTIGATOR: Peddrick Weis **EFFORT:** 2.4 mo
AFFILIATION: University of Medicine and Dentistry of New Jersey
AFFILIATION CODE: 3408
ASSOCIATE INVESTIGATOR: **EFFORT:**
AFFILIATION:
AFFILIATION CODE:

S.G. FUNDS: $ 0 **LAST YEAR'S SG FUNDS:** $ 0
STATE MATCHING FUNDS: $ 0 **LAST YEAR'S MATCH FUNDS:** $ 0
PASS-THROUGH FUNDS: $ 44,935 **LAST YEAR'S PASS-THROUGH:** $0
RELATED PROJECTS: All Toxics/CBEEC
PARENT PROJECTS:
SEA GRANT CLASSIFICATION: 45 Pollution - Other Toxics
KEYWORDS: copper, chromium, arsenic, estuary, wood, benthos, sediment

OBJECTIVES: Leachates from chromated copper arsenate pressure-treated wood bulkheads will be investigated for their spatial extents, bioavailability, effects on benthos, and movement through the food web. Bulkheads of different age and exposure to different tidal flushing regimes will be compared.

METHODOLOGY: Transects will be established perpendicular to newer vs. older bulkheads in both protected coves and in open water, and examined in relation to nearby reference sites. For each site, chemistry (Cu, Cr and As) of macrobenthos and sediments, community structure (species number, species richness, biomass, diversity), will be determined. Trophic transfer will be demonstrated by chemical analysis of resident predators (small fish and crustaceans) living near bulkheads vs. reference areas.

RATIONALE: These metal-leaching structures have been constructed in relatively uncontaminated areas throughout the Chesapeake estuary. We have previously shown that the leachates are found in nearby sediments and benthos, in relation to distance from the structures. Our data will demonstrate if older structures continue to be problematic, and if construction with CCA wood might be permissible in areas with substantial water movement. Such information should be useful to resource managers.

**** SEA GRANT BUDGET ****

GRANTEE:
Virginia Graduate Marine Science Consortium
PROJECT TITLE:
Impacts of CCA pressure-treated wood structures in Chesapeake Bay
PRINCIPAL INVESTIGATORS:
Weis, J.S. and P. Weis

PROJECT NO:
R/CBT-29
PROJECT STATUS:
1

DURATION:
1/1/95 - 12/31/95

A. SALARIES AND WAGES	No. of Person.	Months	Sea Grant Funds	Grantee Funds
1. Senior Personnel				
a. Prin. Investigator	1	2.0	8,000	0
b. Associates:	0	0.00	0	0
Sub Total:			8,000	0
2. Other Personnel				
a. Professionals			0	0
b. Research Assoc.			0	0
c. RA Grad. Stud.			0	0
d. Prof. School Stud.			0	0
e. Pre-Bac. Stud.			0	0
f. Secret./Clerical			0	0
g. Technical/Shop	1		15,000	0
h. Hourly Labor			0	0
Total Salaries and Wages			23,000	0
B. FRINGE BENEFITS			2,408	0
Total Sal. Wages & Fringe Benefits (A+B)			25,408	0
C. PERMANENT EQUIPMENT			0	0
D. EXPENDABLE SUPPLIES			750	0
E. TRAVEL				
1. Domestic - US & Possessions	1.		1,625	0
2. International	2.		0	0
Total Travel			1,625	0
F. PUBLICATION AND DOCUMENTATION COSTS			100	0
G. OTHER COSTS				
1. phone, postage, copying			300	0
2. contractual services - benthic analyses			2,400	0
3. subcontract UMDNJ - chemical analyses			6,400	0
4.				
5.				
6.				
7.				
8.				
9.				
Total Other Costs			9,100	0
TOTAL DIRECT COSTS (A through G)			36,983	0
INDIRECT COSTS: On Campus:			0	0
Off Campus: 26% of MTDC*			7,952	0
TOTAL INDIRECT COSTS			7,952	0
TOTAL COSTS			44,935	0

* less G4

IMPACTS OF CCA PRESSURE-TREATED WOOD STRUCTURES IN CHESAPEAKE BAY

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INTRODUCTION

In the course of development in estuaries, wooden docks, pilings and bulkheads have been installed in relatively pristine areas. To prevent wood decay and destruction by marine borers, wood is treated with chemicals. The favored method of wood preservation at present is pressure treatment of Southern Yellow Pine with 1.5 - 2.5 lb/ft^3 of chromated copper arsenate (CCA). CCA- type C, most often used, is comprised of 47.5% hexavalent chromic oxide, 18.5% cupric oxide, and 34% arsenic pentoxide. This material is in extensive use along coastlines in the US. Its typical green coloration is apparent in newer marinas and waterfront home developments. In 1987, CCA formulations accounted for over 90% of the market for pressure-treated wood, which was estimated at 6.5 billion board feet (Merkle, 1990). The metal oxides are "fixed" to the cellulose of the cells of the wood by the pressure treatment, and leaching is supposed to be minimal when wood is properly fixed (which is not always the case).

Some leaching of Cu, Cr and As does in fact occur. The percentage of leaching may be small, but because of the large quantities of metals in the wood (1.5 or 2.5 lbs per cubic foot), this small percentage may result in environmental levels that can be toxic to nearby aquatic biota (which are sensitive to parts-per-million or less), despite the fact that the concentrations found remaining in the wood itself (measured in lbs per cubic foot) may not appear to be reduced.

Cherian et al. (1979) and Hegarty and Curran (1986) reported significant leaching from treated wood in marine water. Sanders et al. (1994) has found leaching rates of 14.8 μg Cu/cm^2, 0.65 μg Cr/cm^2, and 28.4 μg As/cm^2. Warner and Solomon (1990) demonstrated that all three metals leach from treated wood placed in fresh water and that leaching was greatest at low pHs. All three elements can accumulate in sediments and biota, and all have had extensive study of their toxicity to a variety of marine and estuarine organisms (Luoma and Carter, 1991). All three are EPA "priority pollutants," placing in the top ten of inorganic pollutants.

The degree of toxicity of the three chemicals is dependent on their form, or speciation (particulate, acid soluble, complexed, etc.). Copper is used as an algicide and molluscicide. It is particularly toxic as the free ion, but it is mostly bound to organic material in the aquatic environment (Newell & Sanders 1986). Cr (VI), the predominant species in seawater, is readily taken up by phytoplankton and is more toxic and carcinogenic than Cr (III) (Sanders & Riedel, 1987). Under reduced oxygen conditions it may be reduced to Cr(III) which is substantially less harmful. Arsenic is toxic as well as carcinogenic, mutagenic, and teratogenic. It is readily taken up by phytoplankton to enter food webs. Of the two common oxidation states, As(III) is more toxic and less prevalent than As(V) (Sanders & Windom, 1980). Because of chemical similarity to phosphate, arsenate is readily taken up by phytoplankton and thus enters the food web. During algal blooms, with their associated oxygen depletion, As in Chesapeake Bay was as much as 80% in reduced and methylated forms (Sanders, 1986).

Our previous work has demonstrated leaching in the laboratory of metals from CCA-treated wood and subsequent toxicity to several species of estuarine organisms, including mummichog (*Fundulus heteroclitus*) embryos, fiddler crabs (*Uca pugilator*), green algae (*Ulva lactuca*), mud snails (*Nassarius obsoletus*), and sea urchin (*Arbacia punctulata*) gametes. (Weis et al., 1991, 1992). Since residential and commercial construction is occurring in previously undisturbed areas of every coastal state, this potential problem has national implications. These studies also demonstrated that the rates of leaching decreased over time, and that copper leached to the greatest extent.

The above studies were done in confined aquaria in which chemicals could build up to higher concentrations than would be seen in an estuary in which water movements can dilute the leachates considerably. The actual impact of CCA wood in an estuary depends of the amount of chemicals leached (dependent on surface area of leaching wood and on leaching rate), metal speciation, rate of uptake by the epibiota which grow directly on the wood (determined by the particular species and their density), the degree of dilution and dispersion by water movements, adsorption by sediments (determined primarily by particle sizes), uptake by benthos from the sediments, and trophic transfer from benthos or epibiota to grazers and predators. Our previous field studies have shown elevated levels of the metals in epibiota living on the wood (algae, barnacles, etc.), decreased diversity of this community on CCA wood, and trophic transfer of contaminants from these organisms to their predators (Weis and Weis 1992a, Weis and Weis 1992b).

A significant proportion of the chemicals leaching from wood would be expected to be adsorbed onto nearby sediment particles. Fine-grained sediments (silts and clays) adsorb contaminants to a much greater degree than sand and provide an important sink and source of metals (Luoma and Davis, 1983). Since contaminants tend to be associated with small particles, sediment samples with higher proportions of silts and clays tend to exhibit higher contaminant levels. We have examined whether chemicals released from CCA wood bulkheads can accumulate in adjacent sediments (Weis et al, 1993). Sediments immediately adjacent to the several bulkheads we examined in NJ and NY estuaries were sandy and had very low percentages of the fine-grained particle fraction (probably because of the hydrographic regime) but very high concentrations of the three metals associated with the fine particles. The Cu concentration was typically twice that of Cr or As. Sediments further away from the bulkheads (up to 10 m away, and in deeper water) had higher percentages of silts and clays, but lower concentrations of the toxicants in this fraction. This indicates that Cu, Cr, and As leached from the wood accumulate in fine-grained sediments nearby. Their bioavailability would be determined by a complex set of geochemical and biological factors including metal speciation, presence of organic ligands in the sediments, levels of sulfides, salinity, etc. (Luoma, 1989).

While we have found bioaccumulation of the metals in intertidal sand-dwelling fiddler crabs living near CCA wood structures (Weis and Weis, 1992b) it is not known whether subtidal organisms living immediately adjacent to the bulkheads, which are exposed to scarce but highly contaminated fine sediments are at greater or lesser risk than organisms living further away, which are exposed to more abundant but less contaminated fine particles in the sediments.

The organic content of the sediments, and the amount of acid-volatile sulfide are also associated with the metal-binding capacity of sediments (Chapman, 1989; DiToro et al, 1991). One might predict that the sandy sediments immediately adjacent to bulkheads would have less organic matter and sulfides than the siltier deeper sediments further away, and would therefore have more metal in the pore waters available for uptake by the biota, but this remains to be determined. In a study of a bulkhead in Pensacola Beach FL (Weis and Weis, 1994), we found that polychaete worms living immediately adjacent to the bulkhead had higher body burdens of the metals than worms living further away. However, the sediments in this area were different from those in the Atlantic estuaries in that they remained $< 1\%$ silt/clay at all stations up to 10 m and did not have the gradient of increasing fine particles. Since the proportion of silt and clay did not increase with distance from the bulkhead as it generally does in the Atlantic estuaries, we cannot extrapolate from the findings in Florida to the Chesapeake Bay system.

It is important to note that different species of benthic animals could be exposed primarily by different routes - suspension feeders by exposure to metals in the pore water and/or water column, and deposit feeders by directly ingesting the contaminated sediment particles. Cairns et al (1984) have shown that the soluble forms of Cd and Cu were primarily responsible for toxicity to exposed filter-feeding invertebrates, and that sediment-associated metals played little role in the responses. However, Nalepa and Landrum (1988) found that benthic deposit feeders which ingest sediments acquire their body burden primarily through that route. Thus, proximity to the bulkhead may have differential effects on species with different feeding modes.

Another factor is the effects of the benthic animals themselves on the distribution of metals in the sediments. Through normal feeding and burrowing activities (bioturbation) animals may redistribute the metals in the sediments. There is clearly much more to learn about bioavail-

ability of contaminants from sediments; thus, the results of our study will have applicability to sediment quality beyond the specific issue of effects of CCA wood on the environment.

While metal uptake is an unambiguous indication of bioavailability, the relationship between bioaccumulation and toxicity is complex, and bioaccumulation does not necessarily mean that any functional impairment has occurred. Functional impairment can occur at many levels of organization. Effects of toxicants can be seen on biochemical parameters, cell function, physiological processes, and on the whole organism, as manifested by changes in growth and survival. Above the level of the individual organism, toxic effects may be seen at the population, community and ecosystem levels.

Structural changes in communities often occur in response to contaminant inputs. These changes can be seen by studying the composition, number, and abundance of taxa, and diversity index, which includes a component of "evenness" of the species in the community. These parameters can be modified by impacts of contaminants on the species that inhabit the ecosystem. In general, changes in community structure are more sensitive than ecosystem function (Pratt, 1990). Metal-sensitive species are the most likely to decrease in response to toxic metal contamination, causing a shift in the overall community composition to include representation of more metal resistant species or a reduction in the total number of species. Species richness was the most sensitive indicator of effects of a metal gradient on community structure (Winner et al, 1980). When contamination is more severe, more species are affected. A correlation between benthic diversity and sediment copper concentration was seen by Rygg (1985) in a study of Norwegian fjord sediments. Community diversity immediately adjacent to the bulkhead we studied in Pensacola FL was far lower than at reference sites (Weis and Weis, 1994). We did not investigate how far away from the bulkhead effects on community structure could be found, however.

We have previously demonstrated that CCA-treated wood leaches Cu, Cr, and As, and that CCA bulkheads have metal-enriched sediments and organisms nearby. However, it is not known whether organisms living directly next to bulkheads - which are subjected to the most contaminated but scarce fine particles - are at greater or lesser risk than the more distant

organisms living in sediments with greater amounts of fine particles which are less contaminated. Furthermore, the influence of water movements (i.e., tidal exchange) has not been determined.

Relevance to the Problem:

While toxics are not a severe system-wide problem in Chesapeake Bay, in some locations toxics problems have been observed. CCA wood, which is used extensively in residential areas which are generally considered fairly uncontaminated, may be a significant source of metal contamination in these areas. Our research will examine the effects of leachates from this wood on benthic biota at the community level of organization and examine trophic transfer.

Fine-grained sediments (silts and clays) act as a reservoir for contaminants in aquatic ecosystems. By adsorbing contaminants from water, the "fines" serve as a sink, but then benthic organisms can remove the contaminants from the particulates and transfer them into the food web. We have previously demonstrated a steep decreasing gradient of Cu, Cr, and As in the fine fraction of sediments leading out from treated wood bulkheads, and an increasing gradient of percent fines. It is not known, however, whether benthic organisms inhabiting these sediments are at greater risk if they are close to the bulkhead, i.e., in sediment with a lower percentage of highly contaminated fines, or further away, i.e. in sediments with a higher percent of less contaminated fines. The steep gradients found in relation to treated wood bulkheads provide a convenient and useful model for this problem because complicating environmental factors are minimized within the narrow range, and reference sites can be found nearby.

OBJECTIVES

Overall Objectives:

The objectives of this project are to investigate the spatial extents, bioavailability, and effects on benthos of sediment Cu, Cr, and As from chromated copper arsenate (CCA) wood bulkheads. We will look at bulkheads of different ages and in environments with different degrees of tidal flushing. This should give a clear understanding of the spatial extent and severity of effects of

leachate from treated wood on the nearby benthic community in estuaries with different sediment characteristics and water movements. This will shed light on the degree of deleterious effects of this product in Chesapeake Bay.

1995 Objectives:

In 1995 we will investigate a relatively new (2 year old) bulkhead in a cove with relatively little water circulation, and another in an area with greater water movement.

1996 Objectives:

In 1996, we will investigate an older bulkheads. The exact sites will be determined during our 1995 studies, but will include at least one with little water movement and one with greater movement.

METHODOLOGY

We shall select CCA bulkheads of different ages (in areas where background metal pollution is low) in relatively protected areas and in areas with strong tidal exchange. A four-pronged approach will be used to analyze benthic impacts in these areas: measurement of sediment contaminant levels, chemical analyses of metals in the benthic biota, community analyses, and investigation of movement of the metals through the food web (trophic transfer).

1. Sediment analyses: for particle size distribution, organic carbon, and total Cu, Cr and As concentrations in the fine-grained portion (< 63 μm) and in whole sediments.

2. Contaminants in biota: Sediments from the same sites will be sieved to collect resident macrobenthic organisms. These will be analyzed for Cu, Cr and As.

3. Community analyses: sediments will be sieved to collect resident macrobenthos. Organisms will be identified and samples analyzed for species richness, numbers of organisms, biomass, and Shannon-Wiener diversity index.

4. Trophic Transfer: Non-migratory species of finfish and crustacea that eat benthic worms will be collected in the immediate vicinity of bulkheads and from reference areas for measurement of Cu, Cr, and As in their tissues.

These approaches should give a clear understanding of the spatial extent of the impacts of leachates of CCA wood on the nearby benthic community, and its severity. We are aware that field observations of altered biological processes are often difficult to attribute to specific metal contamination, since other contaminants and natural stressors (such as food supply, temperature, etc) can often contribute to these responses. In most contaminated estuarine situations, metal distributions are patchy and irregular, due to many individual inputs. Our study will simplify and greatly reduce some of these common confounding factors. By studying bulkheads in "unpolluted" residential areas we will be better able to attribute any effects we may find to the leachates from the wood, rather than other contaminants. The finding of elevated levels of all 3 metals serves as a "fingerprint." In addition, we will be studying benthos within the restricted steep metal gradient in the sediments from 0 to 10 M from the bulkheads; within this very limited spatial area other environmental influences (such as variations in water quality and temperature) should not be of consequence.

Details of Methods:

We shall select CCA bulkheads of different ages (and, thus, different lengths of leaching time) in areas where background metal pollution is low. We shall choose sites in relatively protected areas, including poorly flushed residential canals, and in areas with strong tidal exchange. It is expected that there will be different sediment characteristics among these different sites, with different amounts of organic material, different particle size distributions, etc. The first site we have identified will be in Osborn Cove, which is off St. Leonard Creek, a tributary of the Patuxent River in Lusby MD. A 100-ft. bulkhead was built in the summer of 1993 by a neighbor of Kent and Nancy Mountford, who have agreed to cooperate with us (see letter, enclosed). The Mountfords have studied the ecology of this cove (Mountford, 1980) and Nancy's "Cove Corporation," which specializes in sorting and identification of benthic organisms, is located

there. Other sites with contrasting characteristics (older bulkheads, different water flow) will be selected in Solomons MD.

Unbulkheaded reference sites will be carefully chosen in each estuary to have comparable sediment characteristics to the bulkheaded area. The reference sites will be located near the bulkheaded sites, so that other sources of contamination (e.g. stormwater runoff, septic systems, roads) will be equivalent at the bulkheaded and reference sites. The sites will be residential areas with houses but no industry. Some homeowners have constructed bulkheads and some, like the Mountfords, have not; thus the sites are similar in other respects. A four-pronged approach will be used to analyze impacts in these areas:

1. Sediment analyses:

Sediments will be collected by grab sampler adjacent to the bulkheads and at 1, 3, and 10 m away (with replicates at each site), as well as at control sites. They will be stored in plastic jars at 4 C until analyses are performed. Split samples will be analyzed for particle size distribution by settling/decantation rather than by sieving in order for the Cr and Cu analysis not to be compromised by the use of metallic sieves. The decanted fine-grained material will be collected by filtration through fiberglass filters, dried, weighed, and extracted with 3:1 nitric acid/perchloric acid (Baker reagents "for trace metal analysis") at 70 C for 24 hours. The extracts of both the fine fractions ($< 63\ \mu m$) and the whole sediments will be analyzed for Cr, Cu, and As by atomic absorption spectrophotometry, Cu and Cr by flame aspiration and As by hydride generation, in a Perkin-Elmer Model 603 instrument. Quality assurance will be demonstrated with simultaneous analysis of N.I.S.T. Standard Reference Material Estuarine Sediment (SRM 1646). Organic carbon in the sediments will be analyzed after drying sediments at 105 C for 24 hours, weighing, and analysis in a C,H,N, analyzer. Data will be analyzed by one-way ANOVA followed by Bonferroni t-tests, and correlation coefficient.

2. Contaminants in biota:

Sediments from the same sites (adjacent to the bulkheads, 1, 3, and 10 m away, and reference sites for each estuary, with 3 replicates at each site) will be collected and sieved through a 1 mm

sieve to collect resident macrobenthic organisms, (which will be primarily polychaete worms); these will be analyzed for Cu, Cr and As. We expect that there will be dominant species that are present in large enough numbers at all stations at a given site; these organisms will be chosen for chemical analysis, but we will attempt to sample a number of different species at each station. After 24 hours in the laboratory for evacuation of gut contents, tissues will be digested in 3:1 $HNO_3/HClO_4$ and the metals analyzed by atomic absorption spectrophotometry, as above. Split samples will be used for As analysis. Tissues of marine organisms, unlike sediments, have the majority of their As in organic form. Since organo-arsenicals are resistant to mineralization by the $HNO_3/HClO_4$ digestion, the split samples will be digested in HNO_3/HCl, roasted with $Mg(NO_3)_2$, re-dissolved and boiled in dilute HCl and analyzed for As by hydride generation (J. Sanders, personal commun.). Data will be analyzed by one-way ANOVA. Different chemical concentrations in different species collected at the same time from the same site will be correlated to different feeding modes and related to sediment metal concentrations at the different stations along the gradient.

Quality Assurance: N.I.S.T. Standard Reference Material SRM 1566a (oyster tissue) will be analyzed along with the organisms. For the sediment analyses, S.R.M. 1646, estuarine sediment, will be used. An aliquot of the standard reference material will be included every 10-15 samples. Quality control is assured by achieving measurements within 95% confidence limits provided by N.I.S.T.

3. Community analyses:

Standardized amounts of sediments collected by grab sample from the same sites will be sieved to collect resident macrobenthos. Four replicates will be taken at each station. When time permits, animals will be identified when alive. Otherwise, samples will be preserved in buffered formalin with Rose Bengal, then cleared and stored in 70% alcohol until identifications can be performed. Organisms will be identified to the lowest taxon possible using keys and consulting with experts if necessary. Data will then be analyzed for number of species, numbers of organisms, biomass (wet weight), and Shannon-Wiener diversity index at each station. ANOVA will be used to compare species numbers, biomass, and total numbers at the different stations,

and non-parametric tests such as Kruskall-Wallis will be used to compare the diversity index at the different stations. Community similarity indices and ordination analysis will be done to further analyze community composition and similarity. A multiple regression can show the relationships of the various community parameters (dependent variables) with the metal levels in organisms and sediments (independent variables).

We are aware of the possibility that community diversity may change with distance from the bulkhead due to changing depth or sediment types (more fine particles) rather than because of decreased bulkhead-derived contaminants. This possibility will be investigated by examining community characteristics in reference sediments from corresponding depths and corresponding physically to the sediments at 0, 1, 3, and 10 M from the bulkhead at each estuary.

4. Trophic Transfer

Fish and crustacea which are associated with the benthos will be collected with a beach seine from the immediate vicinity of the bulkheads and from reference sites in each estuary. Species that are non-migratory and relatively numerous at the bulkhead and reference sites will be selected and will be analyzed for body burdens of Cu, Cr, and As. Species we are likely to find include the mummichog, *Fundulus heteroclitus*, and the grass shrimp, *Palaemonetes pugio*. We shall make every effort to use animals of comparable sizes and ages at a given site for the chemical analysis. Levels will be compared by t-tests.

Data Management: Data on metal concentrations in sediments and in biota, and community parameters will be collected and entered into computerized data analysis system. Statistical methods for each aspect of the project are discussed above, and significance will be set at the $p = 0.05$ level.

EXPECTED RESULTS:

We expect that the contaminants found in the organisms closest to the wood will be highest, despite the fact that overall higher levels in bulk sediments will probably occur further away.

We also expect that the effects on the community will be greatest right next to the wood, but that effects will also be seen further away where the percent of fine particles in the sediments is

higher. However, we expect that the spatial extent of the effects will be greatest in areas with the newest wood and the least tidal flux.

Resource managers might wish to restrict the use of this product to areas with substantial water exchange, if the data support this, or to encourage the use of alternative construction materials.

DISSEMINATION OF RESULTS:

Results will be published in peer-reviewed journals and presented at scientific meetings. We will also present our findings to Sea Grant extension agents, relevant state and federal agencies and other groups concerned with the environment of the Chesapeake Bay. We will write articles for the general public in newsletters that deal with Chesapeake Bay issues.

RELATIONSHIPS TO OTHER WORK

a. Work by others: We are in touch with Jim Sanders of the Benedict Estuarine Lab who has done some CCA leaching work in Chesapeake Bay, but is not currently looking at CCA wood effects, although copper and arsenic continue to be of interest to his group.

b. Work by ourselves: We have a proposal under consideration at NOAA's National Estuarine Research Reserve Program to do similar research in some of the National Estuarine Research Reserves. However, the Chesapeake Bay NERRs are not among those in which we have proposed to examine effects of CCA wood.

BUDGET JUSTIFICATION

A. Salaries and Wages.

1. The Principal Investigator will devote two months to this project, one during the academic year (offered as matching funds) and the other during the summer, when she is eligible for summer salary.

2. A geochemist, Mr. Theodore Proctor, will work half-time on this project.

Both (1) and (2) will receive a 5% increment in the second year.

B. Fringe Benefits.

The standard rates at Rutgers University are applied - 33% for full-time employees, 16.05% for part-time employees, and none for summer salaries.

C. Permanent Equipment.

No equipment is requested.

D. Expendable Equipment and Supplies.

Our estimate is based on prior experience with this type of effort, and includes glassware, sieves, and chemicals including preservatives, as well as other miscellaneous items.

E. Travel.

- Field Sampling. We shall use our private car to reach the field sites; this is approximately 300 mi each way at $0.26/mi., plus $9 tolls, plus per diem for three persons. estimated $625.00.
- Mileage, lodging, and meals for PI to participate in CBEEC workshop. Estimated $300.
- Travel to a professional meeting (Estuarine Research Federation) by the PI. Estimated $700.

F. Publication and Documentation Costs.

The cost of graphics, reprints and page charges are included for one publication.

G. Other Costs.

2. Office Expenses include telephone, FAX and photocopying.

3. A fee for services will be paid to Cove Corporation for analysis of the benthic collections. Each year, a total of 40 samples will be analyzed @ $60. Cove personnel are familiar with the Chesapeake fauna.

4. Subcontract. The CoPI will affiliate with the project by contractual agreement between Rutgers and UMDNJ. His function is to provide chemical analysis, with the assistance of Mr. Proctor. The expenses are mostly involved with atomic absorption spectrophotometry - analytical standards, standard reference materials, gases, reagent-grade acids, glassware, and a share of the maintenance contract for the Perkin-Elmer 603. Funds for attending a professional meeting (Soc. Environ. Toxicol. Chem.) and for office expenses are also requested. Separate budget pages for the subcontract are provided for each year and for the total project.

Indirect Costs.

The rates applied are those most recently negotiated between both Rutgers and UMDNJ and the Dept. of Health and Human Services. Rutgers' off-campus rate can be used as noted because the majority of the expenses involve activity at the field sites and Mr. Proctor's work at UMDNJ.

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SEA GRANT PROJECT RECORD FORM
I. PROJECT SUMMARY INFORMATION

SG-SID-No.:_____
Specialist:_____
SG Class: _____

INSTITUTION: Virginia Graduate Marine Science Consortium
ICODE: 5100
TITLE: Chesapeake Bay Toxics Research Program: New Project Initiatives

PROJECT NUMBER: M/PD-CBT-1
PROJECT STATUS: 2
SUB PROGRAM: Program Admin.

REVISION DATE: 07/01/94
INITIATION DATE: 09/01/88
COMPLETION DATE: 12/31/95

PRINCIPAL INVESTIGATOR: William L. Rickards **EFFORT:** as needed
AFFILIATION: Virginia Graduate Marine Science Consortium
AFFILIATION CODE: 5100
CO-PRINCIPAL INVESTIGATOR: Gail B. Mackiernan **EFFORT:** as needed
AFFILIATION: Maryland Sea Grant College Program
AFFILIATION CODE:

S.G. FUNDS: 000
LAST YEAR'S SG FUNDS: 000
PASS-THROUGH FUNDS: $ 182,217

MATCHING FUNDS: 000
LAST YEAR'S MATCH FUNDS: 000
LAST YEAR'S PASS-THROUGH: $199,218

RELATED PROJECTS: all
PARENT PROJECTS:
SEA GRANT CLASSIFICATION: Program Development (81)
KEYWORDS: DEVELOPMENT

OBJECTIVES: To provide the capability to develop new studies related to the impacts of ecological processes and water quality conditions upon the living resources of the Chesapeake Bay. Previous CBEEC research has focused on the study of Bay hypoxia, however current CBEEC research is being focused on the role of toxics and their effects on ecological processes in Chesapeake Bay.

METHODOLOGY: Proposals for studies related to the impacts of environmental conditions upon organisms and/or populations will be received and evaluated for submission to the National Sea Grant College Program. The Directors of both the Virginia and Maryland Sea Grant College Programs will participate in the evaluation process.

RATIONALE: Studies of the transport, fate and effects of toxic contaminants on the biota of the Bay, and on the relevant ecosystem-level processes and pathways, are necessary for determining how these impact the populations of organisms of commercial and ecological importance in the Chesapeake Bay.

NOAA FORM 90-4 (ADAPTED) NOAA, U.S. DEPT OF COMMERCE

SEA GRANT BUDGET

GRANTEE:
Virginia Graduate Marine Science Consortium

GRANT/PRO. NO:
M/PD-CBT-1

PRINCIPAL INVESTIGATORS:
W. Rickards and G. Mackiernan

DURATION:
1/1-12/31/95

A. Salaries and Wages 1. Senior Personnel	No.	Man-Mo.	Sea Grant Funds	Grantee Funds
a. Prin. Investigator				
b. Associates:			0	0
Sub Total:			0	0
2. Other Personnel				
a. Professionals	1		20000	
b. Research Assoc.				
c. RA Grad. Stud.				
d. Prof. School Stud.				
e. Pre-Bac. Stud.				
f. Secret./Clerical				
g. Technical/Shop				
h. Hourly Labor				0
Total Salaries and Wages			20000	0
B. Fringe Benefits			5000	0
Total Sal. Wages & Fringe Benefits			25000	0
C. Permanent Equipment			0	0
D. Expendable Supplies			2000	0
E. Travel				
1. Domestic - US & Possessions		1.	2000	
2. International		2.	0	0
Total Travel			2000	0
F. Pub. and Documentation Costs			3217	0
G. Other Costs				
1. R/CBT-26 and 27 renewals			150000	
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
Total Other Costs			150000	0
TOTAL DIRECT COSTS (A through G)			182217	0
Indirect Costs: On Campus: **				
Off Campus: **				
Total Indirect Costs			0	0
TOTAL COSTS			182217	0

** see budget notes

CHESAPEAKE BAY TOXICS RESEARCH PROGRAM: NEW PROJECT INITIATIVES

William L. Rickards, Director
Virginia Sea Grant College Program

Gail B. Mackiernan, Acting Director
Maryland Sea Grant College Program

BACKGROUND

Each year since initiation of the Toxics Research Program, funds have been set aside under a "New Project Initiatives" project to support the initiation and/or development of research projects, workshops or other efforts related to ongoing studies which are relevant to the Program's emphasis on the effects of toxics upon the Chesapeake Bay ecosystem.

As the Toxics Research Program continues to evolve and mature, the Environmental Effects Committee continues the process of developing research which will provide the management agencies and Chesapeake Bay Program Executive Committee with information needed to make decisions regarding restoration efforts in the Bay. Having the capacity for relatively quick response to emerging resource problems or the need for educational efforts related to the environmental effects program continues to be a worthwhile and increasingly integral part of the overall Toxics Research Program.

PROGRESS TO DATE

As of this date, program development funds have been used to: 1) support the initiation of two projects (R/CBT-26 Chu and Hale; R/CBT-27 Sanders et al.) which were submitted for review as full proposals but were found to require preliminary studies to determine their feasibility; 2) provide support for an expanded work plan for R/CBT-22 in the form of necessary chemical analyses in support of biological results being generated; and 3) provide scientific expertise to assist CBEEC in its interactions with the Chesapeake Bay Program committees and in improving the focus and direction of the overall Toxics Research Program.

FUTURE DIRECTIONS

Since the initial Congressional appropriation, the Chesapeake Bay Environmental Effects Studies program has followed a progression from investigating the processes and mechanisms responsible for the creation and maintenance of the low dissolved oxygen phenomenon, to investigating the effects of the low dissolved oxygen conditions on the biota in the Bay. For the past four years, emphasis has been placed on the transport, fate and effects of toxics upon the Bay ecosystem.

Throughout this program's duration, efforts have been made to disseminate and distribute the information gained to various audiences for their use. Such activity will continue to be supported, through the application of program development funds, during the coming year. The CBEEC is currently developing plans for a series of workshops which will facilitate interaction between its researchers and all of the resource management entities involved in the Chesapeake Bay Program. These workshops will serve to educate the managers regarding research results being generated, and the researchers, in turn, will gain a better understanding of the questions

that the resource managers must resolve in the near future. Thus, the toxics research program will become increasingly linked to assisting the Bay Program in its effort to determine the actual extent of impacts on the living resources.

As the present research package was reviewed and assembled, projects R/CBT-26 and R/CBT-27 (noted above) were in the midst of their preliminary studies. CBEEC anticipates awarding continuation funds for these projects if the preliminary results are satisfactory. This will be judged on the basis of reports to be submitted late in the present grant period (i.e. around November, 1994). If both projects are approved as expected, they will require approximately $150,000. Revised proposals will be processed through the National Office of Sea Grant for final approval before funds are awarded to the investigators.

It has become increasingly evident over the past two years, that the Environmental Effects Committee needs assistance and input from a person who is scientifically credible, well-versed in matters related to toxics research, and familiar with the Bay Program's management structure. This assistance is especially critical during the preparation of the Request for Proposals as well as during the year when technical interaction occurs with such groups as the Toxics Subcommittee and/or the research community. CBEEC intends to allocate approximately $25,000 during 1995 in order to continue acquiring such assistance. A proposal for this activity will require final approval from the National Office of Sea Grant before a final agreement is reached with the person to be involved.

The balance of the New Project Initiatives funding will be used to develop reports and information transfer mechanisms which will convey research project results to the Chesapeake Bay resource management community. As with other initiatives, approval of the National Office of Sea Grant will be sought, as required, prior to allocation of the funds.

APPROACH

Access to the new project initiation funds will be possible for all researchers in Virginia and Maryland. In fact, the CBEEC encourages the development of inter-institutional, interstate or regional investigations.

We anticipate that specific proposals will be developed and submitted to the local Sea Grant College Programs for initial evaluation. Following this evaluation, the proposals will be submitted to the National Sea Grant College Program for final review and evaluation. Proposals submitted could either be fully developed research activities or "seed" efforts which would be more fully developed through subsequent funding. Successful projects will be incorporated into the Chesapeake Bay Toxics Research Program, and they will be monitored accordingly for research and fiscal accountability.

Approval of this funding request will permit the development of research which the Chesapeake Bay Environmental Effects Committee has encouraged for studies in the Chesapeake Bay and will be applicable to issues identified as important by the Chesapeake Bay Stock Assessment Committee, the Chesapeake Bay Program's Scientific and Technical Advisory Committee, and the Bay Program's Toxics Subcommittee.

BUDGET NOTES

A. SUPPLIES: $2,000 expected to be allotted for expendables needed for projects initiated; items likely to be included are sample containers, vials, plasticware, reagents, and computer discs.

B. TRAVEL: $2,000 expected to be allotted to cover mileage and lodging for investigators from laboratory/base to field sampling locations.

C. INDIRECT COSTS: indirect costs will be allotted for each project which is funded through New Project Initiation at the rate provided by that institution's Negotiated Indirect Cost Agreement. Since the direct cost distribution is not yet known, the indirect costs can not be identified. (see NOTE below).

NOTE: all cost items noted in the budget are best available estimates. Project budgets can not be finalized until proposals are submitted and reviewed by CBEEC and the National Office of Sea Grant (NOSG), and this occurs during the period of performance, i.e. in mid-grant. Final approval of all budgets is by NOSG prior to submission to GMD for processing.

Curriculum Vitae

ANDERSON, ROBERT S.

Professor
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P.O. Box 38
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Phone: 410-326-7247
FAX: 410-326-7210

EDUCATION

B.S., Drexel University, Philadelphia, PA, 1961
M.S., Hahnemann Medical University, Philadelphia, PA, 1968
Ph.D., University of Delaware, Newark, DE, 1971

EXPERIENCE

1986-Present	Professor, Chesapeake Biological Laboratory, UMCEES, Solomons, MD
1982-1986	Research Biologist/Immunologist, U.S. Army Aberdeen Proving Ground, MD
1973-1982	Section Head, Sloan-Kettering Institute of Cancer Research, Rye, NY
1970-1973	Postdoctoral Fellow. Department of Pathology, University of Minnesota

PUBLICATIONS (Relevant to this proposal; >70 total publications to date):

Anderson, R.S. 1987. Immunocompetence in invertebrates. pp. 93-110 In: C.S. Giam and L.E. Ray (eds.) Pollutant Studies in Marine Animals. CRC Press, Inc., Boca Raton, FL.

Anderson, R.S. 1988. Effects of anthropogenic agents on bivalve cellular and humoral defense mechanisms. In: W.S. Fisher (ed.) Disease Processes in Marine Bivalve Molluscs, American Fisheries Society. Special Publication 18:238-242.

Anderson, R.S., L.M. Oliver, and D. Jacobs. 1992. Immunotoxicity of cadmium for the Eastern oyster (*Crassostrea virginica* [Gmelin, 1791]): effects on hemocyte chemiluminescence. *J. Shellfish Res.* 11:31-35.

Anderson, R.S., K.T. Paynter and E.M. Burreson. 1992. Increased reactive oxygen intermediate production by hemocytes withdrawn from *Crassostrea virginica* infected with *Perkinsus marinus*. *Biol. Bull., 183:476-481.*

Anderson, R.S., L.M. Oliver, and L.L. Brubacher. 1992. Superoxide anion generation by *Crassostrea virginica* hemocytes as measured by nitroblue tetrazolium reduction. *J. Invertebr. Pathol. 59:303-307.*

Anderson, R.S. and L.L. Brubacher. 1992. In vitro inhibition of medaka phagocyte chemiluminescence by pentachlorophenol. *Fish Shellfish Immunol. 2:299-310.*

Anderson, R.S. 1993. Modulation of nonspecific immunity by environmental stressors. pp. 483-510 In: J.A. Couch and J.W. Fournie (eds.). Patholbiology of Marine and Estuarine Organisms. CRC Press, Inc., Boca Raton, FL.

Anderson, R.S. 1994. Modulation of blood cell-mediated oxyradical production in aquatic species: implications and applications. pp. 241-265 In: D.C. Malins and G.K. Ostrander (eds.) Aquatic Toxicology: Molecular, Biochemical, and Cellular Perspectives. Lewis Publishers, Boca Raton, FL.

Name: Joel Eric Baker **Title: Assistant Professor**
Date of birth: 17 July 1959 **Social Security No:** 286-62-9375

Education

1981 B.S., SUNY College of Environmental Science & Forestry, Chemistry
1985 M.S., University of Minnesota, Civil and Mineral Engineering
1988 Ph.D., University of Minnesota, Civil and Mineral Engineering

Professional Background

1988-present Assistant Professor, Research Associate, Chesapeake Biological Laboratory, Center for Environmental and Estuarine Studies, The Univ. of Maryland System, Solomons, MD
1982-1988 Research Assistant, Environmental Engineering Sciences, University of Minnesota Minneapolis, MN

Areas of Professional Expertise

Modeling contaminant transport and fate in natural waters; atmospheric deposition of contaminants; aqueous physical chemistry of hydrophobic chemicals; organic carbon dynamics in marine, estuarine, and lacustrine systems; environmental analytical chemistry.

Relevant Publications

Baker, J.E.; P.D. Capel; S.J. Eisenreich (1986) Influence of colloids on the sediment-water partition coefficients of polychlorobiphenyl congeners in natural waters. Environ. Sci. Technol., 20, 1136-1143.

Baker, J.E. and S.J. Eisenreich (1989) PCBs and PAHs as tracers of particle dynamics in large lakes. J. Great Lakes Research.15, 84-103.

Baker, J.E. and S.J. Eisenreich (1990) Concentrations and fluxes of polycyclic aromatic hydrocarbons and polychlorinated biphenyls across the air-water interface of Lake Superior. Environ. Sci. Technol., 24, 342-352.

Baker, J.E.; S.J. Eisenreich and B.J. Eadie (1991) Sediment trap fluxes and benthic recycling of organic carbon, polycyclic aromatic hydrocarbons, and polychlorobiphenyl congeners in Lake Superior. Environ. Sci. Technol., 25, 500-509.

Kuwabara, J.S. and J.E. Baker (1993) Trace contaminants and nutrients in estuaries: the importance of process interdependence. Estuaries, 16, 383-384.

Leister, D.L. and J.E. Baker (1993) Atmospheric deposition of organic contaminants to the Chesapeake Bay. Atmos. Environ., in press.

NAME: Eugene M. Burreson **TITLE:** Professor

DATE OF BIRTH: 7-25-44 **SSN:** 540-50-8595

EDUCATION AND EXPERIENCE:

B.S. Eastern Oregon State College, LaGrande, OR, 1966
M.S. Oregon State University, Corvallis, OR, 1973
Ph.D. Oregon State University, Corvallis, OR. 1975.

PUBLICATIONS:

Burreson, E. M., M. E. Robinson and A. Villalba. 1988. A comparison of paraffin histology and hemolymph analysis for the diagnosis of *Haplosporidium nelsoni* (MSX) in *Crassostrea virginica* (Gmelin). J. Shellf. Res. 7: 19-23.

Burreson, E. M. and J. D. Andrews. 1988. Unusual intensification of Chesapeake Bay oyster diseases during recent drought conditions. Oceans 88 Proc. Vol. 3: 799-802.

Burreson, E. M. 1991. Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*: I. Susceptibility of native and MSX-resistant stocks. J. Shellfish Res. 10(2): 417-424.

Paynter, K. T., Jr. and E. M. Burreson. 1991. Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*: II. Disease development and impact on growth rate at different salinities. J. Shellf. Res. 10(2): 425-432.

Meyers, J. A., E. M. Burreson, B. J. Barber and R. Mann. 1991. Susceptibility of diploid and triploid Pacific oysters, *Crassostrea gigas*, and eastern oysters, *Crassostrea virginica*, to *Perkinsus marinus*. J. Shellfish Res. 10(2): 433-438.

Ragone, L. M. and E. M. Burreson. 1993. Effect of salinity on infection progression and pathogenicity of *Perkinsus marinus* in the eastern oyster, *Crassostrea virginica* (Gmelin). J. Shellfish Res. 12(1): 1-7.

La Peyre, J. F., M. Faisal and E. M. Burreson. 1993. In Vitro propagation of the protozoan *Perkinsus marinus*, a pathogen of the eastern oyster, *Crassostrea virginica*. J. Eukaryotic Microbiol. 40(3): 304-310.

Ragone Calvo, L. M. and E. M. Burreson. 1994. Characterization of overwintering infections of *Perkinsus marinus* (Apicomplexa) in Chesapeake Bay oysters. J. Shellf. Res. 13(1): In Press.

Burreson, E. M., V. Vidal-Martinez, R. Sima-Alvarez and L. Aguirre-Macedo. 1994. *Perkinsus marinus* (Apicomplexa) as a potential source of oyster (*Crassostrea virginica*) mortality in coastal lagoons of Tabasco, México. Diseases of Aquatic Organisms. (In Press).

Burreson, E. M. L. M. Ragone Calvo, J. F. LaPeyre, F. Counts and K. T. Paynter, Jr. 1994. Acute osmotic tolerance of cultured cells of the oyster pathogen *Perkinsus marinus* (Apicomplexa: Perkinsida). Comparative Biochemistry and Physiology, Part A. (In Press).

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Education:
B.Sc. 1971 Honors, Chemical Oceanography - University of Liverpool
Ph.D. 1974 Chemical Oceanography - University of Liverpool

Professional Experience:

1985-Present Associate Professor, UMCEES, Chesapeake Biological Laboratory, Solomons, MD
1983-1985 Assistant Secretary. Intergovernmental Oceanographic Commission, UNESCO, Paris
1982-1983 Consultant. Marine Pollution Research and Monitoring, IOC/UNESCO
1974-1983 Section Leader. Marine Organic Chemistry, Sonderforschungsbereich 95, University of Kiel

Pertinent Publications: (selected from >60)

Dawson, R. and G. Liebezeit. 1983. Determination of amino acids and carbohydrates. Pages 319-340 In: K. Grasshoff, M. Ehrhardt and K. Kremling (eds.), Methods of Seawater Analysis, Second Edition. Verlag Chemie, Weinheim.

Mopper, K. and R. Dawson. 1986. Determination of amino acids in seawater - Recent chromatographic developments and future directions. Science of the Total Environ. 49: 115-131.

Smucker, R.A. and R. Dawson. 1986. Products of photosynthesis by marine phytoplankton: Chitin in TCA 'protein' precipitates. J. Exp. Mar. Biol. Ecol. 104: 143-152.

Dawson, R., T.S. Bianchi, P. Sawangwong and C.E.F. Orano-Dawson. 1990. Production flux and fate of photosynthetic pigments in estuaries. Pages 824-844 In: Y. Guohui, J-M. Martin, Z. Jiayi, H. Windom and R. Dawson (eds.), Biogeochemical Studies of the Changjiang Estuary. China Ocean Press, Beijing.

Ehrhardt, M., G. Wattayakorn, and R. Dawson. 1990. GC/MS based analyses of individual constituents of Chao Phrya River water and estimated discharge rates into the Upper Gulf of Thailand. Estuarine Coastal Shelf Sci. 30:439-451.

Carpenter, E.J., B. Bergman, R. Dawson, P.J.A. Siddiqui, E. Soederbac and D.G. Capone. 1992. Glutamine synthetase and nitrogen cycling in the marine diazotrophic cynabacteria Trichodesmium ssp. Appl. and Environ. Microbiol. 3122-3129.

Mopper, K., C.A. Schultz, L. Chevolot, C. Germain, R. Revuelta and R. Dawson. 1992. Determination of sugars in unconcentrated seawater and other natural waters by liquid chromatography and pulsed amperometric detection. Environ. Sci. Tech. 26:133-138.

Bianchi, T.S., S. Findlay and R. Dawson. 1993. Organic Mattter Sources in the Water Column and Sediments of the Hudson River Estuary: the Use of Plant Pigments and Tracers. Estuarine, Coastal and Shelf Science 36:359-376.

Carpenter, E.J., J.M. O'Neil, R. Dawson, D.G. Capone, P.J.A. Siddiqui, T. Roenneberg and B. Bergman. 1993. The tropical diazotrophic phytoplankter *Trichodesmium*: biological characteristics of two common species. Mar. Ecol. Prog. Ser. 95:295-304.

NAME: Rebecca Marie Dickhut **TITLE:** Assistant Professor

BORN: July 31, 1960 **SSN:** 388-76-2801

EDUCATION AND EXPERIENCE:

The College of William and Mary - Virginia Institute of Marine Science - Assistant Professor - 1989 to present

University of Wisconsin - Madison - Ph.D. - 1989; M.S. - 1985
Major: Water Chemistry; Minor: Chemical Engineering
Wisconsin Sea Grant Research Assistant 1983-1988
Teaching Assistant - Water Analysis 1983

Water Analyst - Univ. of Wisconsin Environmental Health and Safety Department 1982-1983

St. Norbert College - De Pere, Wisconsin - B.S. - 1982
Major: Natural Sciences and Mathematics
Teaching Assistant - Mathematics 1981-1982

PUBLICATIONS

Dickhut, R.M., A.W. Andren, D.E. Armstrong. 1986. "Aqueous Solubilities of Six Polychlorinated Biphenyl Congeners at Four Temperatures." *Environ. Sci. Technol.* 20:807-810.

Andren, A.W., W.J. Doucette, R.M. Dickhut. 1987. "Methods for Estimating Solubilities of Hydrophobic Organic Compounds: Environmental Modeling Efforts." In Sources and Fates of Aquatic Pollutants, R.A. Hites and S.J. Eisenreich (eds.). Advances in Chemistry Series 216, American Chemical Society: Washington, D.C., Chapter 1.

Andren, A.W., R.M. Dickhut, W.J. Doucette, L.P. Burkhard. 1987. "Chemical Property Estimation Techniques for Environmental Modeling." In Oceans '87 Proceedings: The Ocean - An International Workplace. The Institute of Electrical and Electronics Engineers; IEEE Catalogue No. 87-CH2498-4. pp. 1761-1764.

Dickhut, R.M., A.W. Andren, D.E. Armstrong. 1989. "Naphthalene Solubility in Selected Organic Solvent/Water Mixtures." *J. Chem. Eng. Data* 34:438-443.

Dickhut, R.M., D.E. Armstrong, A.W. Andren. 1991. "The Solubility of Hydrophobic Aromatic Chemicals in Organic Solvent/Water Mixtures: Evaluation of Four Mixed Solvent Solubility Estimation Methods." *Environ. Toxicol. & Chem.* 10:881-889.

Gustafson, K.E. and R.M. Dickhut. 1994. "Molecular Diffusivity of Polycyclic Aromatic Hydrocarbons in Aqueous Solution." *J. Chem. Eng. Data* 39:281-285.

Gustafson, K.E. and R.M. Dickhut. 1994. "Molecular Diffusivity of Polycyclic Aromatic Hydrocarbons in Air." *J. Chem. Eng. Data* 39:286-288.

Dickhut, R.M., K.E. Miller and A.W. Andren. 1994. "Evaluation of Total Molecular Surface Area for Predicting Air-Water Partitioning Properties of Hydrophobic Aromatic Chemicals." *Chemosphere* In press.

Liu, K. and R.M. Dickhut. 1994. "Vapor Pressures and Thermodynamic Properties of Selected Chlorinated Benzenes at Environmental Temperatures" *Chemosphere* In press.

Dickhut, R.M. and K.E. Gustafson. 1994. "Atmospheric Inputs of Organic Contaminants to Southern Chesapeake Bay." Submitted to *Marine Pollution Bulletin*.

CURRICULUM VITAE

NAME: Jonathan G. Kramer **TITLE:** Research Assistant Professor

BORN: May 17, 1957 **SOCIAL SECURITY NO.:** 016-40-4843

EDUCATION:

1988	Ph.D.	Marine Estuarine Environmental Sciences The University of Maryland, College Park, Maryland
1982	M.S.	Marine Environmental Sciences The State University of New York, Stony Brook, New York
1979	B.S.	Environmental Sciences The University of Massachusetts, Amherst, Massachusetts

EXPERIENCE:

1993	Research Assistant Professor; Center of Marine Biotechnology
1988-1992	Faculty Research Associate/Postdoctoral Fellow; Center of Marine Biotechnology
1987-1988	Visiting Scientist; Univ. of Warwick, Coventry, U.K. (With Prof. N.G. Carr)
1983-1988	Grad. Research Assist; Horn Point Environ. Laboratory, U. MD. (With Ian Morris)
1983	Research Associate; Marine Sciences Research Center, SUNY Stony Brook
1979-1982	Graduate Research Assistant; Marine Sciences Research Center, SUNY Stony Brook

PUBLICATIONS:

Kramer, J.G. 1990. The Effects of Irradiance and Specific Inhibitors on Protein and Nucleic Acid Synthesis in the Marine Cyanobacterium *Synechococcus* sp. WH 7803. Arch. Microbiol 154:280-285.

Kramer, J.G. and I. Morris. 1990. Macromolecular Bases of Growth Regulation in Irradiance Limited Marine *Synechococcus* spp. WH 7803. Arch. Microbiol. 154:286-293.

Kramer, J.G. and F.L. Singleton. 1992. Variations in rRNA Content of Marine *Vibrio* spp. during Starvation-Survival and Recovery. Appl. Environ. Microbiol. 58:201-207.

Kramer, J. G. and F.L. Singleton. (1993) Measurement of rRNA Variations in Natural Communities of Microorganisms on the Southeastern U.S. Continental Shelf. Appl. Environ. Microbiol. 59:2430-2436.

Kramer, J. G. and H.J. Schreier (In prep.) Cloning and Sequence Analysis of the Structural Gene Encoding Glutamine Synthetase (*glnA*) in Marine *Synechococcus* spp.

Wyman, M., **J.G. Kramer**, J.P. Zehr and D.G. Capone (In prep.) Diel Variability in Transcription of the Structural Gene for Glutamine Synthetase (*glnA*) in Natural Populations of the Marine Cyanobacterium *Trichodesmium thiebautii*.

Name: John Robert Kucklick **Title:** Research Assistant Scientist

Date of Birth: December 25, 1960 **Social Security Number:** 293-70-8826

Education:

1983	B.Sc., Miami University, Miami Ohio. Zoology.
1988	M.S., University of North Carolina at Wilmington. Marine Biology.
1992	Ph.D., University of South Carolina, Columbia. Marine Science.

Professional Background

1992-present Postdoctoral Research Assistant, Chesapeake Biological Laboratory, University of Maryland Center for Environmental and Estuarine Studies (Solomons, Maryland).

1990-1992 Research Assistant, University of South Carolina, Columbia, South Carolina

1987-1988 Research Fellow, North Carolina Department of Natural Resources and Community Development (Wrightsville Beach, North Carolina).

1983-1986 Staff Biologist, Mote Marine Laboratory (Sarasota, Florida).

Areas of Professional Expertise: Environmental, analytical and marine chemistry, fate and transport of hydrophobic organic pollutants in aquatic ecosystems, coastal oceanography, limnology, nutrient cycling in aquatic ecosystems.

Relevant Publications

Kucklick, J.R., T.F. Bidleman, L.M. McConnell, G.P. Ivanov and M.D. Walla. 1993. Toxaphene contamination in Lake Baikal's water and foodweb. *Chemosphere* 27: 2017-2026.

McConnell, L.L., J.R. Kucklick, T.F. Bidleman, G.P. Ivanov and M.D. Walla. 1993. Long-range transport of toxaphene to Lake Baikal. *Chemosphere* 27: 2027-2036.

Kucklick, J.R. and T.F. Bidleman. 1994. Organic contaminants in Winyah Bay, South Carolina: Pesticides and polycyclic aromatic hydrocarbons in subsurface and microlayer waters. *Marine Environ. Res.* 37: 63-78.

Kucklick, J.R. and T.F. Bidleman. 1994. Organic contaminants in Winyah Bay, South Carolina: Using natural fluorescence to follow atrazine levels and river mixing. *Marine Environ. Res.* 37: 79-91.

Kucklick, J.R., T.F. Bidleman, L.M. McConnell, G.P. Ivanov, M.D. Walla. 1994. Organochlorines in the water and biota of Lake Baikal, Siberia. *Environmental Science and Technology*. 28: 31-37.

MACKIERNAN, GAIL B.

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EDUCATION:

B.S., American University, Washington, D.C., 1964
M.A., College of William and Mary, VIMS, Gloucester Pt., VA, 1968
Ph.D. candidate, Johns Hopkins University, 1970-1975

EXPERIENCE:

Faculty Research Assistant, Chesapeake Biological Laboratory, Solomons, MD, 1976-1978.
Marine Ecologist, Western Ecosystems Technologies, Laurel, MD, 1979-1982.
Environmental Scientist, U.S. EPA Chesapeake Bay Program, Annapolis, MD, 1982-1985.
Oceanographer, U.S. Army Corps of Engineers, Baltimore District, Baltimore, MD, 1985-1986.
Assistant Director, University of Maryland Sea Grant College, College Park, MD, 1986 - 1994.
Interim Director, University of Maryland Sea Grant College, College Park, MD, 1994-present.

PROFESSIONAL ACTIVITIES:

American Geophysical Union
American Society of Limnology and Oceanography
Estuarine Research Federation
Oceanography Society

PUBLICATIONS (recent/relevant):

D'Elia, L.W. Harding, Jr., M. Leffler and G.B. Mackiernan. 1992. The role and control of nutrients in Chesapeake Bay. Water Sci. Tech. 26:2635-2644.

Smith, D.E., M. Leffler, and G. B. Mackiernan, eds. 1992. Oygen Dynamics in the Chesapeake Bay: A Synthesis of Recent Research. Maryland Sea Grant College, College Park, MD, 234 pp.

Mackiernan, G.B. 1991. Where freshwater and saltwater meet: The Chesapeake Bay estuary. Atlantic Nat. 41:7-23.

Mackiernan, G.B. 1990. State of the Chesapeake Bay. Water Environ. Tech. 2(9): 60-67.

Mackiernan, G.B., editor. 1987. Dissolved Oxygen in the Chesapeake Bay: Processes and Effects. Maryland Sea Grant College, College Park, MD, 177 pp.

Flemer, D.A., V.K. Tippie, G.B. Mackiernan, R.S. Biggs, W. Nehlsen, and K.S. Price. 1987. Characterizing the Chesapeake Bay Ecosystem and Lessons Learned. In: Estuarine and Coastal Management - Tools of the Trade. The Coastal Society. Bethesda, Maryland.

Mountford, K. and G.B. Mackiernan, 1987. A multi-decade trend monitoring program for Chesapeake Bay, a temperate east coast estuary. In: T.P. Boyle, (ed.), New Approaches to Monitoring Aquatic Ecosystems, pp. 91-106. ASTM Special Technical Publication: 940, Philadelphia, PA.

Name: Christopher J. Madden
Title: Faculty Research Associate
Date of Birth: 29 July, 1957
Social Security #: 166-48-0335

Education:

1992	Ph.D. Louisiana State University: Oceanography and Coastal Science
1986	M.S. Louisiana State University: Marine Sciences
1980	B.A. Cornell University: Biological Sciences/Ecology

Professional Background:

1992-present	Faculty Research Associate, Horn Point Environmental Laboratory, Ctr for Estuarine and Environmental Studies, Univ. of Maryland
1992-present	Visiting professor, Universidad Autonoma de Campeche, México/National University of Mexico
1980-1991	Research Associate II, III, IV, Coastal Ecology Institute, Center for Wetland Resources, Louisiana State University
1984	Visiting Research Associate, Instituto de Ciencias del Mar y Limnologia, Universidad Nacional Autonoma de México
1979-1980	Research Assistant, Cornell University Biol. Field Station, Ithaca, NY

Areas of professional expertise:
Estuarine ecology, ecological modeling, computer programming, primary productivity, nutrient chemistry, laboratory information systems, data acquisition systems, wetland-water column interactions, systems analysis

Relevant Publications:

Madden, C. J. and J. W. Day. 1992. Induced turbulence in rotating bottles affects phytoplankton productivity measurements in turbid waters. Journal of Plankton Research 14(8):1171-1191.

Madden, C. J., and J. W. Day. 1992. An instrument system for high speed mapping of chlorophyll and physico-chemical parameters in surface waters. Estuaries 15(3):421-427.

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Madden, C. J., and J. Day. Aquatic primary productivity in Fourleague Bay. In: J. Day (ed.). Oceanography of a shallow deltaic system: The Atchafalaya Delta Region. Springer-Verlag. submitted.

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1984-87	Research Scientist, Sea Fisheries Research Institute, South Africa
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Pertinent Publications:

1) Mason, R.P. and Fitzgerald, W.F. (1993). The distribution and biogeochemical cycling of mercury in the equatorial Pacific Ocean. Deep-Sea Research **40** 1987-1924.
2) Mason, R.P., Fitzgerald, W.F., Hurley, J.P., Hanson, A.K., Jr., Donaghay, P.L. and Sieburth, J.M. (1993). Mercury biogeochemical cycling in a stratified estuary. Limnology and Oceanography **38** 1227-1241.
3) Mason, R.P., Morel, F.M.M. and Hemond, H.F. (1993). The role of microorganisms in elemental mercury formation in natural waters. In Heavy Metals in the Environment, Allan & Nriagu (Eds.), CEP Consultants, Edinburgh pp 293-296.
4) Mason, R.P. and Morel, F.M.M. (1992). The accumulation of mercury and methylmercury by microorganisms. EOS Transactions **73** (43) 041F-7.
5) Mason, R.P., Fitzgerald, W.F. and Vandal, G.M. (1992). The sources and composition of mercury in Pacific Ocean rain. J. Atmos. Chem. **14** 489-500.
6) Mason, R.P. and Fitzgerald, W.F. (1991). Mercury speciation in open ocean waters. Water, Air and Soil Pollution **56** 779-789.
7) Fitzgerald, W.F., Mason, R.P. and Vandal, G.M. (1991). Atmospheric cycling and air-water exchange of mercury over mid-continental lacustrine regions. Water, Air and Soil Pollution **56** 791-803.
8) Mason, R.P. and Fitzgerald, W.F. (1990). Alkylmercury species in the equatorial Pacific. Nature **347** 457-459.

Papers in Press:

9) Mason, R.P., Fitzgerald, W.F. and Morel, F.M.M. (1994). The biogeochemical cycling of elemental mercury: Anthropogenic influences. Geochimica et Cosmochimica Acta (accepted)
10) Mason, R.P., O'Donnel, J. and Fitzgerald, W.F. (1994). Elemental mercury cycling within the mixed layer of the equatorial Pacific Ocean. In Mercury as a Global Pollutant, Watras & Huckabee (Eds.), Lewis Publishers (in press).
11) Cossa, D., Mason, R.P. and Fitzgerald, W.F. (1993). Chemical speciation of mercury in a meromictic lake. In Mercury as a Global Pollutant, Watras & Huckabee (Eds.), Lewis Publishers (in press).

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1981 - Director, Virginia Graduate Marine Science Consortium - University of Virginia
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PUBLICATIONS:

Copeland, B. J., W. L. Rickards and D. R. Berg. 1975 N. C. Coastal Resources and Short Term Needs: a view of priorities. University of North Carolina Sea Grant Pub. UNC-SAG-75-14, 45pp.

Rickards, W. L., J. E. Foster and W. R. Jones. 1978. A feeding tray for use in eel farming. University of North Carolina, Sea Grant Pub. UNC-SG 78-04, 11 pp.

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Rickards, W. L. 1981. A discussion of aquaculture candidate species for eastern North Carolina. UNC Sea Grant Working Paper, UNC-SG-WP-81-2. 10 pp.

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Virginia Institute of Marine Science, Marine Scientist, 1981 to 1987

RELEVANT PUBLICATIONS:

Schaffner, L. C., R. J. Diaz, C. R. Olsen, and I. L. Larsen. 1987. Faunal characteristics and sediment accumulation processes in the James River Estuary, Virginia. Estuarine, Coastal and Shelf Science 25:211-226.

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Nichols, M. N., R. J. Diaz and **L. C. Schaffner**. 1990. Effects of dredging and sediment dispersion. Environmental Geology and Water Sci. 15:31-43.

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Mayer, M. M., **L. C. Schaffner** and W. M. Kemp. Nitrification activity of macrofaunal tubes and burrows: effects of environmental conditions and animal behavior. (in review).

NAME: Michael A. Unger **TITLE:** Assistant Professor

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EDUCATION AND EXPERIENCE:

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1990-1993	Assistant Research Scientist, Head of Analytical Chemistry Section, Virginia Institute of Marine Science, the College of William and Mary
1988-1990	Environmental Chemist, Applied Physics Laboratory, Johns Hopkins, Shady Side, Md.

SELECTED RELATED PUBLICATIONS:

Unger, M. A., W. G. MacIntyre, J. Greaves and R. J. Huggett. 1986. GC Determination of Butyltins in Natural Waters by Flame Photometric Detection of Hexyl Derivatives with Mass Spectrometric Confirmation. Chemosphere 15(4):461-470.

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Visiting Scientist	EPA Laboratory, Gulf Breeze FL	1992

PUBLICATIONS:

Weis, J.S. and P. Weis 1989. Tolerance and stress in a polluted environment: the case of the mummichog. BioScience 39: 89-96

Weis, J.S. and P. Weis 1989. Effects of environmental pollutants on early fish development. Reviews in Aquatic Sci. 1:45-74.

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SELECTED PUBLICATIONS

WEIS, P. Ultrastructural changes induced by low concentrations of DDT in the liver of the zebrafish and guppy. Chem.-Biol. Interact. 8:25-30 (1974).

WEIS, P., and J.S. WEIS. DDT causes changes in activity and schooling behavior in goldfish. Environ. Res. 7:68-74 (1974).

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ESPINA, N.G., and **WEIS, P.** DNA repair in fish from polluted estuaries. Mar. Environ. Res. (in press).

SMITH, G.M., KHAN, A.T., WEIS, J.S., and **WEIS, P.** Behavior and brain chemistry correlates in mummichogs (*Fundulus heteroclitus*) from polluted and unpolluted environments. Mar. Environ. Res. (in press).

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Pertinent Publications:

Wright, D.A. and P.M. Welbourn. 1993. Disturbance of ionic regulation in the crayfish *Orconectes propinquus* exposed to mercury. Environ. Pollut. (in press)

Wright, D.A. and P.M. Welbourn. 1991. Effect of mercury on unidirectional sodium and calcium influx in *Asellus aquaticus*. Arch. Environ. Contam. Toxicol. **21**: 567-570.

Wright, D.A., P.M. Welbourn and A.V.M. Martin. 1991. Inorganic and organic mercury uptake and loss by the crayfish *(Oreonectes propinquus)*. Water Air Soil Pollut. **56**: 697-707.

Wright, D.A. and C.D. Zamuda. 1991. Copper contamination in the Patuxent River, Maryland. Hydrobiologia **215**:31-41.

Wright, D.A., P.M. Stokes and M. Allard. 1990. Mercury uptake and effects on ionic regulation in freshwater crustaceans. Proc. Int. Conf. "Mercury as an Environmental Pollutant:. Gavle, Sweden, 1990. p.29.

Pinkney, A.E., D.A. Wright, M.A. Jepson and D.W. Towle. 1989. Effects of tributyltin compounds on ionic regulation and gill ATPase activity in estuarine fish. Comp. Biochem. Physiol. **19**:425-431.

Sinex, S.A. and D.A. Wright. 1988. The distribution of trace metals in the sediments and biota of Chesapeake Bay. Mar. Pollut. Bull. **19**:425-431.

Wright, D.A. 1988. Dose-related toxicity of copper and cadmium in striped bass larvae from the Chesapeake Bay: Field considerations. Water Sci. Tech. **20**:39-48.

Wright, D.A. and C.D. Zamuda. 1987. Copper accumulation by two bivalves: salinity effect is independent of cupric ion activity. Mar. Environ. Res. **23**:1-14.

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Phelps, H.L. D.A. Wright and J.A. Mihursky. 1985. Factors affecting trace metal accumulation by estuarine oysters *Crassostrea virginica*. Mar. Ecol. Prog. Ser. **22**:187-197.

Zamuda, D.C., D.A. Wright and R.A. Smucker. 1985. The importance of dissolved organic compounds in the accumulation of copper by the American oyster, *Crassostrea virginica*. Mar. Environ. Res. **16**:1-12.