U.S. ENVIRONMENTAL PROTECTION AGENCY

SEMI-ANNUAL REPORT

JULY - DECEMBER 1975

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE ENVIRONMENTAL RESEARCH LABORATORY
GULF BREEZE, FLORIDA 32561

This report describes the activities of the Environmental Research Laboratory, Gulf Breeze, during the period July - December, 1975. This period was an exciting one for the laboratory - a time of change in laboratory organization, diversification of research programs, and initiation of construction of a major new laboratory facility. Throughout these six months the laboratory staff has been involved in many activities, including research, conferences, public and adjudicatory hearings, technical assistance to states and regions and interaction with Federal and local agencies. All of these activities have been entered into with a spirit of dedication to the mission of the Envionmental Protection Agency and that of the Environmental Research Laboratory, Gulf Breeze. This dedication is reflected in the increasing dependence of the Agency on the expertise within this laboratory as EPA develops and promulgates effective environmental management and control programs. As a result, ERL,GB is playing an ever greater role in the formulation of pollution control measures developed by the Agency for the protection and improvement of our Nation's estuarine and coastal environments.

The outlook is for more hard work ahead but the probability of success for accomplishing the ERL, GB mission has been dramatically increased during the past six months as a result of the changes made during this time. For example, the recent reorganization of the Office of Research and Development has resulted in a redefined laboratory branch structure. Each branch conducts research directed at particular aspects of estuarine pollution problems. The synthesis of the information generated by all the branches produces a total definition of potential environmental impact which ranges in focus from microbial populations to higher trophic levels such as fish and man and which varies in complexity from single species through populations and communities to total estuarine ecosystems. More detailed descriptions of the branch in-house and extramural research programs are presented in Section I of this report.

As part of the reorganization, Dr. Tudor Davies has assumed the position of Deputy Laboratory Director. In another organizational change, three Associate Director positions were established to provide for greater management capability of our in-house, extramural and Technical Assistance programs, a need which became evident as the laboratory programs expanded in size and diversity.

Historically, the research programs of ERL, GB have concentrated on the effects of pesticides on estuarine and marine organisms. In addition to this research, several new program areas have been initiated at the

laboratory during the past six months. The first is a program aimed at determining the health and environmental effects of pollutants associated with energy extraction, conversion, transmission and use. The specific objectives of this program are to determine the health and ecological effects of energy related pollutants which enter these systems during off-shore drilling activities and from the increased use of new fuels derived from shale oil. This information is essential to the integration of potential environmental impacts into the achievement of energy self-sufficiency for the Nation.

The second major effort initiated during the past six months is concerned with determining the environmental acceptability of pesticide manufacturing wastes treated by advanced industrial waste treatment techniques. The information from these experiments will be used to establish guidelines for the treatment and disposal of such complex wastes.

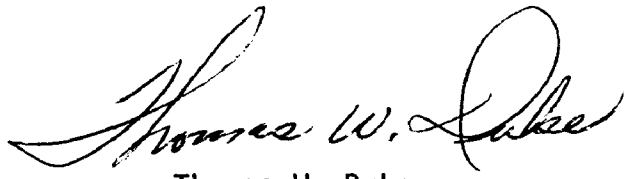
A third new program, dealing with the environmental impact of off-shore oil drilling, has been initiated in response to a need of the Bureau of Land Management, U.S. Department of the Interior.

Of major import to the successful operation and completion of our programs is the recently initiated construction of a new laboratory facility. When completed in the spring of 1977 at a cost of approximately $1 million, this building will house an analytical chemistry section and a flowing seawater bioassay laboratory. This new facility will greatly increase our ability to respond to both short and long term Agency needs with respect to the effects of specific pollutants on estuarine and marine organisms.

In the Technical Assistance area, the past six months have seen our staff members involved in problems of National importance. As you know, the occurrence of PCB's in the aquatic environment recently came to the forefront. As a result of experience and knowledge gained while conducting research on these compounds over the past few years, our staff was asked by the State of New York to provide expert testimony at Judicial hearings concerning the introduction of PCB's into the Hudson River. The data and information presented will enable the State of New York to more adequately control the disposal of this dangerous class of compounds in the future.

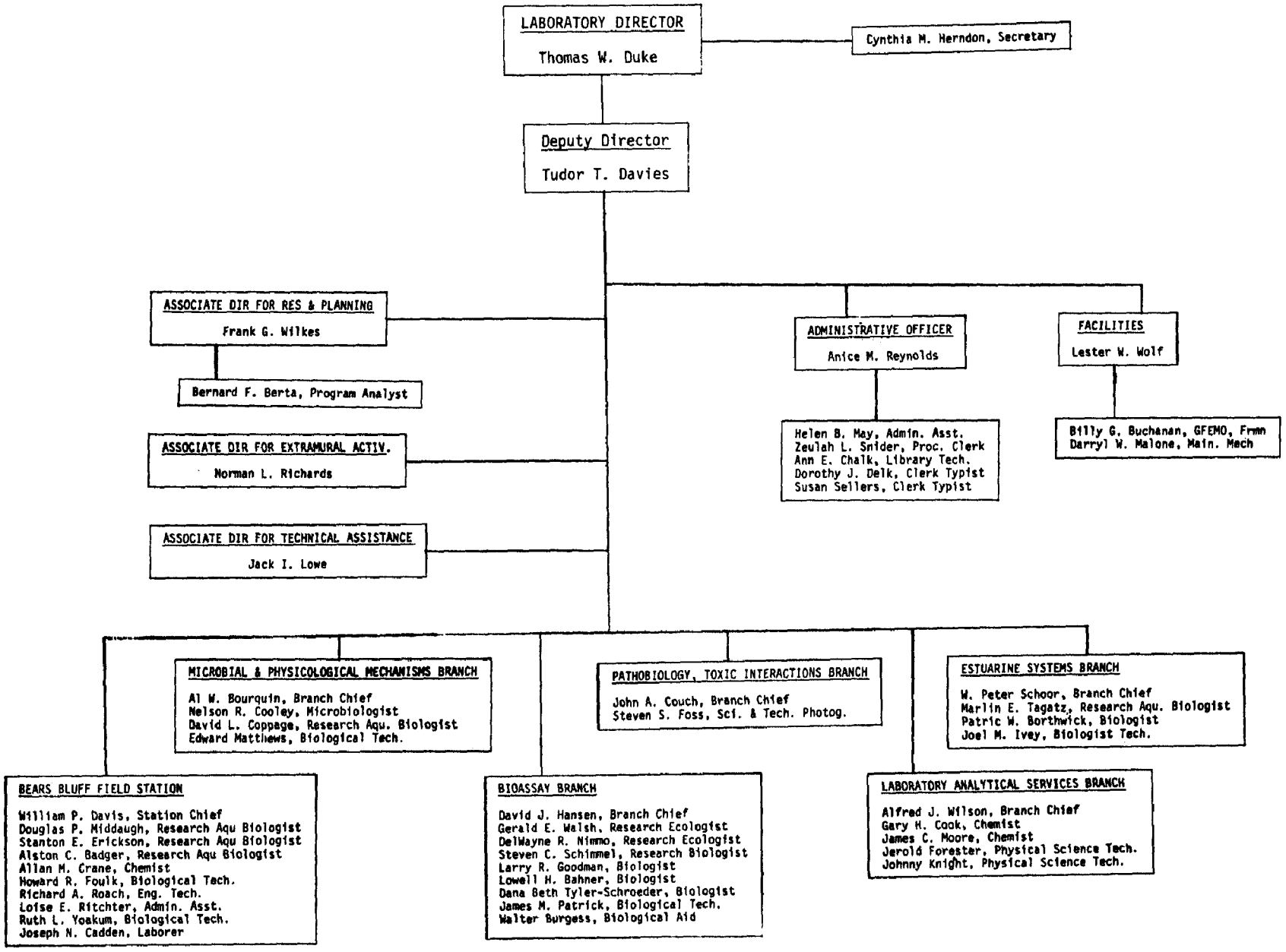
Another recent development in which we have been active is the investigation of contamination by Kepone of the James River and estuary below Hopewell, Virginia, the site of a Kepone manufacturing plant. A program has been initiated to analyze Kepone levels in James River oysters, determine the accumulation and depuration rates involved, and determine the effects of Kepone on individual species and the estuarine ecosystem. This work is being conducted in conjunction with the State of Virginia, U.S. Corps of Engineers, and other EPA offices and Federal Agencies. Our data will be used to aid in the assessment of the problem and in developing effective corrective action. Details of these and other areas of Technical Assistance are presented in Section II of this report.

In summary, the past six months have been extremely productive and we look forward with great anticipation to continuing in the months to come our various activities and laboratory programs, both old and new. Each of you is invited to visit us or, if that is not possible, write or telephone us if there are any problems we can help you with.



Thomas W. Duke
Laboratory Director

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL RESEARCH LABORATORY
GULF BREEZE, FLORIDA 32561



MISSION

The Environmental Research Laboratory, Gulf Breeze, is responsible for the conduct and management of research on ecological systems with emphasis on the determination of exposure-effects relationships in marine, coastal and estuarine ecosystems of hazardous organic and inorganic pollutants. This information is required by the EPA pesticide registration and control program and for the development of water quality criteria designed to protect man and aquatic life in marine, coastal and estuarine environments. Research is also conducted to determine the health and ecological impact of pollutants, both singly and in combination, derived from energy extraction, conversion, transmission and use. Baseline information and technical methodologies are developed in order to assess the potential effects of energy resource development. This information is essential to the integration of potential environmental impacts into the achievement of energy self-sufficiency for the Nation.

I. BRANCH ACTIVITIES

BIOASSAY BRANCH

Objectives

The Bioassay Branch of the Environmental Research Laboratory, Gulf Breeze, conducts research on effects of pesticides and other synthetic organics on individual species and communities of organisms. Studies include bioassays on phytoplankton, vascular aquatic plants, arthropods, mollusks, fishes and communities of animals from several phyla. Duration of bioassays ranges from a few days to several months and tests may include an entire life-cycle of the test animal. Bioassays provide data on effects of chemicals on survival, growth, reproduction, pathogenesis, and accumulation of chemicals and localization within organisms. Data provide a basis for water quality criteria, effluent standards, pesticide registration, and ocean dumping permits. Methods developed and used are incorporated into manuals for use by government and private laboratories for producing data for pesticide registration and testing toxicity of waste materials.

Status of Projects

Chronic Bioassays: Hansen, Goodman and Burgess

Research in the chronic bioassay task is conducted to determine effects of long-term exposures of sensitive life stages of fishes to pollutants. Projects in progress or completed during the first six months of the fiscal year include bioassays on effects of toxaphene on embryos and fry of the sheepshead minnow (Cyprinodon variegatus) and effects of heptachlor and diazinon on its reproduction.

Embryo-fry Bioassays: The effects of toxaphene on sheepshead minnows exposed for 28-days to five concentrations ranging from 0.2 to 2.5 µg/ℓ were determined. Newly hatched fry were affected by, but survived exposure to, 1.1 µg/ℓ. Fry in 2.5 µg/ℓ died. Number of days before embryos hatched and growth rates were apparently unaffected by 0.2-2.5 µg/ℓ of toxaphene, Concentrations of toxaphene in fry averaged 9,800 (range 6,100 to 14,000) times the concentration measured in the water.

Life-cycle Bioassays: Effects of heptachlor and diazinon on the life cycle of sheepshead minnows are being studied. Results of 96-hr bioassays will be compared to results from continuing exposures of juvenile fish through maturation, spawning and subsequent survival of progeny. Application factors that can be used with data from acute tests on sheepshead minnows and other fishes to develop water quality criteria will be calculated from these data.

Heptachlor: Acute 96-hr bioassays to determine effects of heptachlor on various life-stages were investigated. The 96-hr LC50, (measured concentration) for fry was 3.6 µg/ℓ, juveniles 10.5 µg/ℓ, and adults 16.3 µg/ℓ. Tentative analysis of data from the partial life-cycle tests indicates that the application factor is between 0.18 and 0.27.

Diazinon: Results from acute bioassays in which juvenile sheepshead minnows were exposed to between 10 and 500 µg/ℓ of diazinon indicate that the 96-hr LC50 is greater than 500 µg/ℓ.

A partial life-cycle bioassay similar to that with heptachlor was begun. Five exposure concentrations range from 0.6 to 10 µg diazinon/ℓ . cholinesterase concentrations in fishes from all five concentrations were inhibited by 17 to 77% of normal on day 7 of the exposure; acetylcholinesterase levels decreased with increasing diazinon concentration. Chemical analyses of water and fish tissue samples have not been completed.

CHRONIC AND ACUTE TOXICITY OF METHOXYCHLOR, MALATHION AND CARBOFURAN TO SHEEPSHEAD MINNOWS (Cyprinodon variegatus). Contract 68-03-264. Bionomics, EG&G, Inc. Principal Investigator: P. R. Parrish. Project Officer: David J. Hansen.

A final report on this contract is being prepaired following the completion of exposures of sheepshead minnows to methoxychlor and malathion. Exposures began with juvenile fish and lasted through maturation and spawning of adult fish. Exposures ended after progeny from exposed adults were exposed for one month. Data on survival, growth and reproduction were obtained. Chemical analyses of tissue and water samples have not been completed.

CHRONIC AND ACUTE TOXICITIES OF CHLORDANE, PENTACHLOROPHENOL, TRIFLURALIN AND 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN TO SHEEPSHEAD MINNOWS. Contract 68-03-2069. Bionomics, EG&G, Inc. Principal Investigator: P. R. Parrish. Project Officer: David J. Hansen.

Sheepshead minnows are being exposed throughout their entire life-cycle to chlordane, pentachlorophenol and trifluralin. The fish exposed to chlordane have hatched from eggs and grown to maturity. Reproductive effects are now being assessed. The fish exposed to pentachlorophenol have hatched from eggs and are approximately one month old. Acute toxicity tests with trifluralin are complete and life cycle texts will begin next month.

INSECTICIDE EFFECTS ON LARVAL DEVELOPMENT OF BLUE CRAB. Grant R 801128. Duke University Marine Laboratory. Principal Investigator: C. G. Bookhout. Project Officer: Jack I. Lowe.

The objective of this project is to determine the effects of different concentrations of Mirex, Methoxychlor, and Malathion on the development of the blue crab Callinectes sapidus, from the time of hatching to the first crab stage. No effect, sublethal and lethal concentrations will be determined. Effects of various pesticide concentrations on zoel and megelopal development, larval behavior and occurance of structural abnormalities will be determined. The most sensitive larval development stage will be determined and related to pesticide exposure concentration.

The final report of this project is in press.

EFFECTS OF INSECT GROWTH REGULATORS AND JUVENILE HORMONE MIMICS ON CRUSTACEAN DEVELOPMENT. Grant R 803838. Duke University Marine Laboratory. Principal Investigator: John Costlow, Jr. Project Officer: Thomas W. Duke.

Chemicals which mimic juvenile hormones are under development for potential use as alternatives to synthetic organic pesticides. The effects of these compounds on non-target, but closely related, organisms is unknown. The objectives of this research are to determine: (1) the effects of sublethal concentrations of juvenile hormone mimics and insect growth regulators on the development of marine crustacea, from hatching to juvenile stages; (2) the effects of sublethal levels of these compounds on larval stage behavior; and (3) the effects of sublethal concentrations of these compounds on the long-term mutagenesis of small marine copepods. The information produced by this research will be used to develop water quality criteria applicable to juvenile hormone mimics and guidelines for their registration and application.

During the reporting period, research concentrated on the deterioration of Altosid (ZR-515) in aqueous solution, the effects of the insect growth regulator TH-6040 on the larvel development of the estuarine crabs Rhithropanopeus harrisii and Sesarma reticulatum, and the mutagenic effects of ZR-515 on the harpacticoid copepod Tisbe holothuriae.

Acute Bioassays: Schimmel and Patrick

Studies were conducted to determine effects of the insecticides BHC and Lindane (-isomer of BHC) on several estuarine animals in 96-hr flow-through bioassays. Results of these studies are listed in Table 1. Edible tissues from animals that survived the 96-hr tests are being analysed for pesticide content.

Two, separate, 28-day bioaccumulation studies on BHC were conducted with the American oyster (Crassostrea virginica) and the pinfish (Lagadon rhomboides). Each test was followed by a 28-day depuration period to determine the rate of loss of each of BHC's four isomers. Maximum concentration factors (the concentration of the various isomers of BHC in tissues divided by the concentration measured in exposure water) were 218 in oyster meat and 137 in pinfish muscle. After seven days depuration, no BHC was detected in oysters or pinfish.

A 96-hr flow-through bioassay was conducted to determine the LC50 of DDT to juvenile brown shrimp (Penaeus aztecus). These data were required to better evaluate the environmental effects of proposed DDT applications to crops in Louisiana. The measured LC50 for DDT in this test was 0.14µg/ℓ (95% C.I. = 0.10 to 0.24 µg/ℓ).

An apparatus was constructed for the purpose of investigating the bioconcentration of pesticides by estuarine animals. The device delivers one liter of water each cycle (400 cycles/day) to two control and four treated aquaria. One control aquarium provides seawater with carrier (if necessary) and the other seawater without carrier. The treated aquaria receive concentrations of carrier identical to the control. Two

Table 1. 96-hour toxicity of the insecticides lindane and BHC to several estuarine animals in flow-through bioassays. The 95% confidence intervals are in parentheses. Test animal size is rostrum-to-telson length for shrimps and standard length for fishes.

SPECIES	INSECTICIDE	96-HOUR LC50 (µg/ℓ)	SIZE (mm)	TEMPERATURE (°C)	SALINITY (°/oo)
Penaeus duorarum	Lindane	0.13 (0.10-0.17)	30-52	24.0-26.0	20-22
P. duorarum	BHC	0.34 (0.21-0.44)	---	21-27	18-22
Palaemonetes pugio	Lindane	4.44 (3.44-6.08)	---	23-27	18-22
Cyprinodon variegatus	Lindane	103.9 (91.8-130.8)	17-21	24-28	10-18
Lagodon rhomboides	Lindane	31.12 (29.20-33.36)	42-61	22.5-25.0	21.0-23
L. rhomboides	BHC	86.43 (80.77-96.70)	55-89	22.0-26.0	20-23
Mysidopsis bahia	Lindane	6.28 (3.81-14.08)	8-9.5	23.0-25.0	15-22

concentrations of a toxicant are possible, with two replicates for each concentration. The apparatus uses Lamda pumps with a predetermined counter which is capable of delivering 0.01 ml to 100 ml of toxicant stock solution each cycle.

Studies are continuing to evaluate the pinfish as a fish suitable for bioassays involving the embryo and larval stages. Although techniques have not yet been devised to culture larvae beyond 11 days after hatching, static 96-hr bioassays using newly-hatched pinfish are possible.

Special bioassays are now under way to determine the uptake and depuration rates of the insecticide Kepone by American oysters. Two studies are anticipated, each lasting two months. Results of these tests may be helpful in estimating the recovery of oysters in the James River, Virginia, system from recent Kepone contamination.

Estuarine Productivity: Walsh

Effects of Pollutants on Marine Unicellular Algae

Estuarine algae are subjected to daily variations in salinity and nutrient composition of the water and to combinations of pollutants. This task is designed to study growth responses of estuarine unicellular algae to heavy metal ions, organometals, and other pollutants in relation to salinity and nutrient status of growth media. The findings will be used to relate water quality criteria for these pollutants to the composition of individual bodies of water.

Growth of algae in the presence of lead (Pb): Concentrations up to 100 ppb Pb (in $PbCl_2$) had no effect on growth of Dunaliella tertiolecta or Isochrysis galbana[2] in 30 ppt salinity and three nutrient concentrations of trace metals, minor salts and vitamins. Concentrations up to 100 ppb had no effect on growth of Chlorococcum sp. at 30 ppt salinity and high nutrient concentration. Also, toxicity of herbicide was not enhanced when D. tertiolecta and I. galbana were grown in the presence of 100 ppb Pb and the EC50 concentrations of atrazine.

Growth of algae in the presence of cadmium (Cd): Dunaliella tertiolecta was grown in the presence of 100 and 500 ppb Cd (in $CdCl_2$ and cadmium acetate) in four nutrient concentrations. In first tests, Cd had no effect on growth in the three highest concentrations. Cadmium appeared to stimulate growth at the lowest nutrient concentration.

The role of chaelators in toxicity of heavy metals to algae in our tests is being studied and will be described in the next report.

Effects of chlorinated naphthalenes on growth of algae: Studies on growth of algae in the presence of chlorinated naphthalenes began near the end of the reporting period. No effect of the compounds at 50, 100 and 500 ppb were found in preliminary tests, but this must be confirmed in future tests.

Effects of Heavy Metals on Development of Red Mangrove Seedlings

Mangrove stands are subjected to pollutants that occur inshore, including heavy metals from dumping of sewage. Tests were performed to determine effects, if any, of lead, cadmium and mercury on seedlings. Data were collected to determine safe concentrations of the metals in estuarine soils that contain mangroves.

Seedings of the red mangrove (Rhizophora mangle) were treated twice with up to 500 ppm of lead, cadmium or mercury. No effects of the first treatment were found on rate of growth, weights of hypocotyls, stems, leaves or roots, and total productivity. Second treatments with cadmium or mercury caused chlorosis of leaves followed by death of the plants. Microscope slides are now being prepared for pathology associated with treatment, and plant parts are being analyzed for tissue concentrations of the three heavy metals.

WATER QUALITY AND MANGROVE ECOSYSTEM DYNAMICS, Grant R 803340. University of Florida, Gainesville, Florida. Principal Investigator: Samuel C. Snedaker. Project Officer: Gerald E. Walsh.

Progress during this past quarter has focused on the nutrient and heavy metal concentrations present in the major functional compartments of intertidal mangroves and in the construction of simulation models at Harvard University using the facilities of the Department of Engineering and Applied Physics. Evaluations made thus far suggest that mangroves do not concentrate heavy metals above levels commonly found in other types of halophytes. However, variations in metal concentrations (and the major and minor plant nutrients) are relatable to the type of mangrove forest relative to local topography, intensity of tidal flushing and proximity to sources of metals. Compartment turnover times for these forest types are known and it is now possible to estimate the maximum loadings relative to the incoming load of metals.

Entrained metals in litterfall may be released during in situ decomposition or be exported in the detrital flow to the downstream bay or estuary. Work is underway to begin an intensive monitoring of the rates of detrital export (ergo export of metals) for the various forest types and their ultimate fate in the downstream water body. The successful completion of this task should allow us to define the intertidal mangrove ecosystem as either a sink (with time dimensions) or as a conduit for each of the metals of interest. Also possible is speculation on the role of mangroves in concentrating metals in heterotrophic consumers by virtue of the detrital flux recognized now as a major food source for estuarine organisms.

HERBICIDE TOXICITY IN MANGROVES. Grant R 801178. University of Miami, Coral Gables, Florida. Principal Investigator: Howard J. Teas. Project Officer: Gerald E. Walsh.

The amine salts of 2,4-D and picloram were applied to the Florida species of mangroves: red mangrove (Rhizophora mangle), white mangrove (Laguncularia racemosa and black mangrove (Avicennia germinans). Treatments

were to soil or water, by aerial spray and to single leaves as droplets. The effects on radiochloride uptake and on localization of radiocarbon-labelled picloram after leaf application were studied in red mangrove.

Lethal application rates of picloram for young seedlings were 2.7 kg/ha for white mangrove, 13 kg/ha for red and 13 kg/ha for black; for mature plants they were 2.7, 13 and greater than 53 kg/ha respectively. Rates of application tolerated by young seedlings were 0.01, 5.3 and 5.3 kg/ha; for mature plants they were 0.5, 5.3 and 53 kg/ha. "No effect doses" for seedlings were less than 0.01 kg/ha for all species; for mature plants they were less than 0.1, 0.5 and 2.7 kg/ha.

Spray applications of 6.3 - 12.2 kg/ha of a commercial mixture of 2, 4-D, and picloram to the canopy of a mixed-species forest caused partial defoliation within three weeks. Within 16 months it killed all of the white, 78 - 100% of the mature red, but none of the mature black mangroves.

Radiocarbon-labelled picloram concentrated in dormant buds of red mangrove and it is concluded that the tree is killed by the mixture because of its effects on them.

The final report for this project has been received.

EFFECTS AND FATE OF SELECTED WASTE-WATER CHLORINATION PRODUCTS AND PESTICIDES IN MARINE ALGAE. Grant R 803943. Syracuse University Research Corporation, Syracuse, N. Y. Principal Investigator: Harish C. Sikka. Project Officer: Gerald E. Walsh.

Effects of 4-chlororesorcinol, 3-chlorophenol, 3-chlorobenzoic acid and 5-chlorouracil on growth of the marine unicellular algae Dunaliella tertiolecta and Skeletonema costatum were studied.

Skeletonema was more sensitive to all compounds than was Dunaliella. Growth of Dunaliella was not altered significantly by any of the four compounds at concentrations of 1 and 10 ppm. Growth of Skeletonema was completely inhibited by 10 ppm of 4-chlororesorcinol and 3-chlorophenol and reduced 15-20% by the same concentration of 3-chlorobenzoic acid. 5-chlorouracil had no effect on growth of Skeletonema at 1 and 10 ppm.

STUDY OF EFFECTS OF PESTICIDES ON DEVELOPMENT OF ALGAE. Grant R 802388. Virginia State College. Principal Investigator: Bernard R. Woodson. Project Officer: Gerald E. Walsh.

The purpose of this study is to investigate effects of selected pesticides on growth, respiration, photosynthesis, cellular organization and motility on freshwater algae. The pesticides are dephenamid, Baygon, atrazine, diuron, malathion, seven and simizine. The study also includes determination of uptake of pesticides by selected algal species.

UPTAKE AND EFFECTS OF MIREX, METHOXYCHLOR AND 2,4-D ON ULVA SP., ENTEROMORPHA SP. AND RHODYMENIA SP. Syracuse University Research

Corporation. Principal Investigator: Harish C. Sikka. Project Officer: Gerald E. Walsh.

This was a study concerning effects, uptake, and metabolism of mirex, methoxychlor, and 2,4-D in the seaweeds Ulva sp., Enteromorpha sp. and Rhodymenia sp. None of the pesticides, at concentrations corresponding to their maximum solubility in seawater, had any significant effect on photosynthesis, protein, carbohydrate, lipid, chlorophyll, carotenoid or trace metal content of the algae. All three algae removed substantial amounts of mirex and methoxychlor from the medium, but uptake of 2,4-D was extremely low. The rate of uptake of methoxychlor was considerably greater than that of mirex. Enteromorpha accumulated considerably more mirex and methoxychlor than Ulva or Rhodymenia. Both Ulva and Enteromorpha failed to metabolize either mirex or 2,4-D. Enteromorpha metabolized methoxychlor to a limited extent. After 7 days of incubation with carbon labelled methoxychlor, a major portion of the label in the tissue and medium was present in unchanged methoxychlor. A small amount of radioactive metabolite, 2,2-bis (p-methoxyphenyl)-1,1-dichloroethylene, was detected in both the tissue and medium. In addition, medium contained 2,2-bis (p-hydroxyphenyl)-1,1,1-trichloroethane and four unidentified minor radioactive metabolites. Unlike Enteromorpha, Ulva did not metabolize methoxychlor.

The final report for this project has been received.

DYNAMICS OF AN ESTUARY AS A NATURAL ECOSYSTEM. Grant R 802928. University of South Carolina. Principal Investigatory: F. John Vernberg. Project Officer: Gerald E. Walsh.

This study consisted of two separate but interrelated substudies: 1) a macroecosystem study and 2) a microecosystem study.

The macroecosystem study, designed to continue work on the dynamics of a relatively undistrubed marsh-estuarine ecosystem, had two objectives: 1) to establish baseline data on an undisturbed estuary to provide a scientific basis for comparative studies on the effects of various pollutants, and 2) to develop models of an estuarine ecosystem that would predict the probable effects of environmental perturbation. The final report includes sections on the data retrieval system, radiation balance, hydrography, microbiology, composition and seasonality of the zooplankton, vertical migration of crustacean larvae, metabolism of crustacean larvae, and abundance, diversity and respiration of the macrobenthic fauna.

The objectives of the microecosystem study were: 1) to develop and test replicate experimental salt marsh units at the microecosystem level as diagnostic tools for the assessment of both long and short term pollution effects on the Spartina alterniflora salt marsh community, and 2) to utilize these simulated marshes to test and to provide data to the overall ecosystem model being constructed. The final report describes community metabolism and benthic community analyses in the system.

The final report for this project has been received.

Physiology: Nimmo and Bahner

These studies are divided into four categories: (1) effects of toxicant combinations on shrimp, (2) methods development for assessing the uptake of toxicants from sediments by marine organism, (3) methods development of bioassay procedures, and (4) effects of cadmium on shrimp.

Species used for bioassay in the laboratory are usually exposed to toxicants singly, but they are often exposed to combinations of toxicants under varying environmental regimes. Consequently, an experimental flowing-water bioassay system was developed that controls salinity and temperature while testing toxicants either singly or in combination. Obvious advantages of this control are that rates of toxicant accumulation, translocation, and loss, and acute and chronic toxicty to animals can be better assessed and repeated. Our bioassays were conducted with pink shrimp (Penaeus duorarum) exposed to the following toxicant combinations: cadmium-malathion, cadmium-methoxychlor, cadmium-methoxychlor-Aroclor^{R} 1254, and a complex industrial waste which contained both inorganic and organic constituents. The toxicities of the pesticide-metal combinations, when compared to that of each constituent singly, appeared to be independent or additive, and varied with the method(s) of bioassay.

Accumulation of cadmium from natural (contaminated) sediments was tested using 5 genera from 3 phyla. Sediments containing less than 100 ppm Cd were collected from Corpus Christi Bay, Texas and placed to a depth of 4 cm in 30 glass aquaria. Seawater, from Santa Rosa Sound at ambient temperature and salinity flowed (60 l/hr) through each aquarium for 60 days. About 30 animals of each species, Palaemonetes vulgaris, Penaeus duorarum, Crassostrea virginica, Uca pugilator and Cyprinodon variegatus were placed in each aquarium. Concentrations of cadmium in water, sediment and tissues were determined at regular intervals using atomic absorption spectrophotometry.

A chronic bioassay that induced exposure of the entire life-cycle of an estuarine/marine crustacean has been completed. In flowing seawater, the mysid Mysidopsis bahia was affected by exposure to 10 µg/ℓ cadmium. The 16-17 day life-cycle exposure showed that survival as well as length of time to formation of brood pouch and length of time to brood release all appear as indicators of deleterious effects. This mysid has optimum growth at 20 o/oo salinity, 25°C, and can complete a life cycle in static or flowing water. Mr. Bill Peltier (EPA Region IV) has recently completed a successful flow-through bioassay with this species using dilutions of an industrial effluent.

Cadmium was more toxic to marine decapod crustaceans in 96-hr bioassays than to marine gastropods, bivalves and teleosts (Eisler, R. 1971. J. Fish. Res. Bd. Canada 28:1225-1234). Therefore, some of the more subtle effects which might be apparent in long-term bioassays (up to 30 days) were investigated using estuarine and marine shrimps. Studies included (1) rates of accumulation and localization in various tissues, (2) histological effects of acute (96-hr) and chronic (24-days) exposures, and (3) a

comparison of accumulation of cadmium from food with cadmium administered in water.

Cadmium was accumulated by shrimp from water that contained as little as 8 µg/ℓ. All tissues had detectable concentrations, but muscle cadmium concentration increased after shrimp were transferred to cadmium-free water. Background concentrations of the metal were eliminated by holding feral animals in flowing water in the laboratory.

Shrimp exposed to cadmium consistently developed blackened foci or lamellae in the branchia. Occasional blackened cuticular lesions on the appendages and body surfaces were also observed. Often whole branchial processes were necrotic and congested with hemocytes. Shrimp that survived these exposures sloughed the blackened portions of the branchial processes and appeared normal within 14-days after transfer to cadmium-free water.

Accumulation of cadmium from food by Palemonetes pugio appears to be negligible compared to accumulation from water. In 30-day bioassays, the toxicity of cadmium to grass shrimp, P. vulgaris, was approximately an order of magnitude lower than the toxicity to pink shrimp, P. duorarum.

Bioassay-Crustaceans: Tyler-Schroeder

The past six months were spent gaining experience in handling grass shrimp Palaemonetes pugio larvae, in a flow-through exposure apparatus using salinity-controlled, temperature-controlled, filtered seawater.

In 96-hr acute tests using this system with Altosid, a synthetic juvenile hormone, observations were made on mortality and molting of grass shrimp exposed to concentrations from 400 to 1000 µg/ℓ . Rostrum-telson lengths of surviving larvae showed growth of larvae exposed to 400 µg/ℓ of the chemical was significantly less than larvae reared in the seawater control (X=0.05). Lengths of larvae from higher concentrations were not measured.

A 21-day test with Altosid was performed on P. pugio larvae to determine if the chemical accelerated or retarded the onset of metmorphosis to the postlarval stage. Exposure to concentrations of 400 to 1000 µg/ℓ of the chemical was begun on the 12th day of larval development. Salinity was 15-20 o/oo and temperature was held at approximately 25°C. Larvae were fed Artemia nauplii throughout the test.

The observed success of metamorphosis for the experiment is shown in Fig. 1. Total percentage metamorphosis and survival at the completion of the test is given in Table 2.

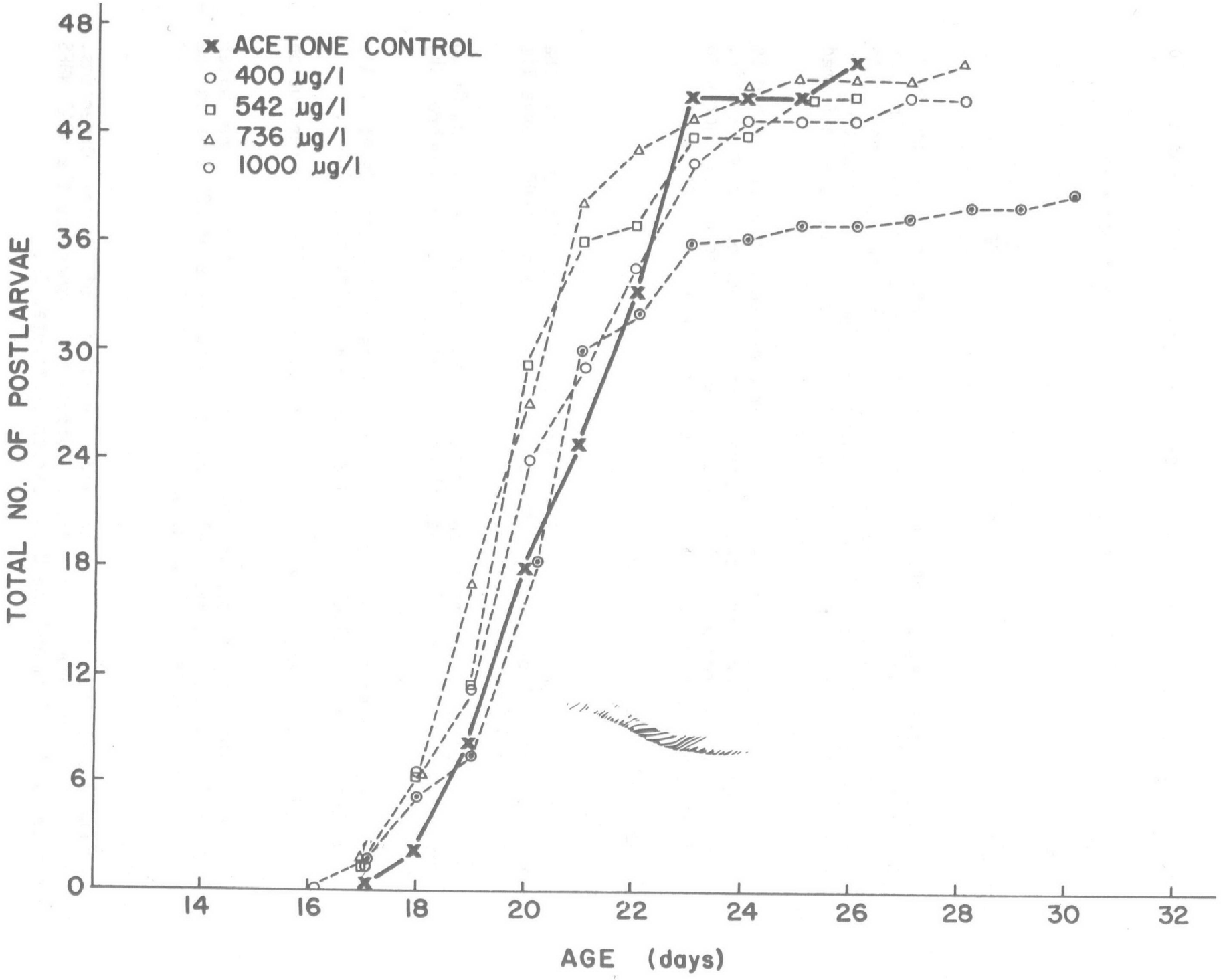

ACETONE CONTROL
400 µg/l
542 µg/l
736 µg/l
1000 µg/l
TOTAL NO. OF POSTLARVAE
AGE (days)
0
6
12
18
24
30
36
42
48
14
16
18
20
22
24
26
28
30
32

Table 2

Percentage survival and metamorphosis of grass shrimp exposed to Altosid.

Altosid, µg/ℓ	Survival.	Metamorphosis,
Acetone Control	65	77
400 µg/ℓ	48	73
542 µg/ℓ	53	73
736 µg/ℓ	56	76
1000 µg/ℓ	32	63

Research concerning effects of endrin, a chlorinated hydrocarbon insecticide, on the life-cycle of the grass shrimp was begun. The 96-hr LC50 of Endrin to 1st day P. pugio larvae was 1.0 µg/ℓ in first tests. Exposure to concentrations from 0.34 to 3.4 µg/ℓ caused a significant reduction in growth of the larvae from the controls (a=0.01). These tests were performed in the same flow-through system as were the Altosid tests.

A 500 ml. diluter with exposure aquaria has been constructed and is being readied for uptake and spawning studies on P. pugio, using Endrin. Completion of these studies should yield an application factor for Endrin to be used in establishing water quality criteria for marine invertebrates.

Meetings, Conferences, and Workshops

Hansen, D.J.

Presented a paper at the AIBS meeting at Corvallis, Oregon. The presentation was part of a summary of research conducted by NERC Corvallis research laboratories.

Participated in preparing a manuscript at a meeting of the Working Group on Principles for Developing Coastal Water Quality Criteria in Dubrovnik, Yugoslavia.

Presented a paper on the effects of PCB's on estuarine animals at EPA's National Conference on Polychlorinated Biphenyls at Chicago, Illinois.

Testified in Albany, New York on the matter of PCB's in the Hudson River.

Discussed the use of application factors for setting marine water quality criteria at the ERL, Duluth and the occurrence of PCB's in marine waters at an OTS meeting in Washington, D.C.

Bahner, L.H.

Attended Symposium on Pollution and Physiology of Marine Organisms, Millford, CT. November 3-6, 1975. Symposium sponsored by the NOAA NMFS laboratory at Millford and the Belle Baruch Institute, SC.

Nimmo, D.R.

Attended Symposium on Pollution and Physiology of Marine Organisms, Millford, CT. November 3-6, 1975. Presented paper entitled "Effects of cadmium on the shrimps, Penaeus duorarum, Palaemonetes pugio and Palaemonetes vulgaris (Nimmo, D.R., Lightner, D.W. and Bahner, L.H.).

Schimmel, S.C.

Attended meeting of American Society for Testing Materials at St. Louis, MO. October, 1975. Discussed acute bioassay and bioaccumulation methods and hazard evaluation.

ESTUARINE ECOSYSTEMS BRANCH

Objectives

The objectives of the Estuarine Ecosystems Branch are to determine the pathways, biological effects and fate of hazardous organic and inorganic pollutants in estuarine ecosystems, simulated as well as *in natura*. Specific responsibilities include the design of laboratory microcosms, and a conceptual estuarine mathematical model for assessment of routes, rates, effects, sources and sinks of pollutants as integrated into the overall model. The approach in the design of these systems is to establish a simple and basic system for the screening of pollutants. A secondary objective is to develop a system to the point where it can be used as a rigorous scientific tool in establishing the effects of stress applied to an estuary.

Status of Projects

Effect of Mirex on Predator-Prey Interaction in an Experimental Estuarine Ecosystem: Schoor, Tagatz, Ivey and Borthwick

Tests of 14- to 16-days duration were conducted to determine the distribution and sublethal effects of mirex in an experimental estuarine ecosystem. Observations of the system and water data indicated a healthy community that appeared to be of sufficient size not to be stressed by removal of replicate samples. The water remained clear, turbidity averaging 0.6 nephelometric unit (range 0.2 to 1.8). No algal growth was observed in the tanks during Experiment 1, but small amounts were visible near the end of Experiment 2. Dissolved oxygen ranged from 7.2 to 8.5 ppm, but was usually at the saturation level (8.1 ppm). Overall pH of the water averaged 7.2 (range 7.0 to 7.4). All plants survived, some showed new growth, and most of their leaves remained green. Shrimp were closely associated with the plants, eating leaf detritus or epiphytic material on the leaves or both.

Concentration of mirex in the water averaged 0.025 µg/ℓ (range 0.015-0.050 µg/ℓ) for Experiment 1, 0.046 µg/ℓ (0.017-0.11 µg/ℓ) for Experiment 2 (one day predation), and 0.044 µg/ℓ (0.011-0.13 µg/ℓ) for Experiment 2 (two days predation).

Mirex was translocated from water to sand and biota. All components sampled during 16-days of exposure in Experiment 1 contained mirex. Only trace amounts (< 0.02 mg/kg) occurred in sand. Mirex was not detected in *Thalassia* at day 1, but subsequent concentrations in leaves (trace to 0.033 mg/kg) were as great as 1,300X the average concentration in the water. Roots had less mirex (not-detected, less than 0.020 mg/kg, to 0.024 mg/kg) than did leaves (trace to 0.033 mg/kg). Mirex in shrimp ranged from trace amounts to 0.20 mg/kg. Concentrations in shrimp increased with time and were as great as 8,000X the average concentration in the water. After three days exposure, pinfish contained 0.050 to 0.063 mg/kg mirex, up to 3,800X that in the water.

In both experiments, an alteration of predator-prey interaction due to the effect of mirex was evident. There was no significant difference (α = 0.05) survival of grass shrimp in control and treated tanks after 13-days exposure in the absence of predation by pinfish. However, there was a significant difference (α = 0.01) in survival after one, two, or three days of predation by pinfish (Table 3). Survival of shrimp after three days of predation was based on two, instead of three, treated tanks due to death of a pinfish in the third tank.

That more deaths due to predation occurred in the treated tanks than in the control tanks could be interpreted as an alteration of the normal behavior of either shrimp or pinfish by mirex; It is believed, however, that the behavior of only the grass shrimp was altered. This is supported by the experiments of Lowe et al. (1971), who found that mirex was acutely toxic to grass shrimp, but on the basis of mortality, pathology, and observations for symptoms of pesticide poisoning, had no apparent affect on pinfish.

Thus, while the criterion of death may be useful in single-species bioassay, in an ecosystem bioassay an effect on the predator-prey interactions may have far greater implications regarding the total welfare of the system. In addition, concentrations at which this behavioral effect occurs are quite low and come closer to the environmental levels.

Effect of Mirex on Lugworm Ecosystem: Schoor and Borthwick

An inexpensive bioassay system has been developed to estimate pollutant effects on the benthic community. The system is composed of washed beach sand, turtlegrass and periphyton on the sand surface, artificial seawater and the lugworm (Arenicola cristata). Observations are made of surface phenomena, indicative of lugworm activity, including feeding funnels (head shafts), respiratory tubes (tail shafts), and egg masses. Preliminary experiments have demonstrated that the pesticide Mirex taken into the substrate by the burrowing and feeding activity of the lugworm seemed to affect the behavior of this species.

Environmental Chambers: Schoor and Borthwick

Two fiberglass equipment shelters have been purchased and adapted to provide self-contained facilities for static estuarine system bioassays. Artificial light within 91% of the natural daylight spectrum can be provided for any pre-selected photoperiod. A wide range of temperatures may also be selected. These facilities provide space for simultaneous experiments in up to thirty-six 180 liter aquaria under controlled conditions.

DETERMINATION OF THE SITE(S) OF ACTION OF SELECTED PESTICIDES BY AN ENZYMATICIMMUNOBIOLOGICAL APPROACH. Grant R 803458. Mississippi State University. Principal Investigator: Robert B. Kock. Project Officer: W. Peter Schoor.

The objective of this project is to develop antibodies to Kelevan, an "active" derivative of Mirex which inhibits the ATP'ase system of fire ants. These antibodies will be used to determine if the inhibited enzyme system

Table 3 - Survival of grass shrimp after 13 days exposure to mirex in the absence of predation and after an additional 1 to 3 days of exposure and predation by two pinfish per tank. (Numbers in parentheses indicate numbers of shrimp in tanks before deaths occurred)

No. tanks (Control/ Treated)	Avg. concn. mirex (µg/l)	Survival after 13 days		Chi-square[a]	No. days of predation	Survival after predation		Chi-square[a]
		Control	Treated			Control	Treated	
1/1	0.046	82% (63)	68% (63)	NS	1	44% (52)	23% (43)	4.57*
1/1	0.044	78% (63)	81% (63)	NS	2	16% (49)	0% (51)	9.05**
3/3	0.025	94% (189)	88% (189)	NS	3	24% (177)	4%[b] (115)	19.39**

[a]NS = non-significant; *significant at 5% level (χ^2, 1 d.f. = 3.84); **significant at 1% level (χ^2, 1 d.f. = 6.63).

[b]Based on two, instead of three, treated tanks due to death of a pinfish in the third tank.

can be reactivated in vitro by the removal of Kelevan through competitive binding of the highly specific antibodies.

The initial objective of this project was to reduce the ketone functional group of Kelevan by the Wolff-Kishner method to the methylene unit and then to attach the reduced product through its N-hydroxysuccinimide derivative to bovine serum albumin (hereafter called BSA). Although several attempts were made with the Wolff-Kishner method, reduction of the ketone function has not been achieved.

Fortunately, Dr. R. O. Hutchins of Drexel University has developed an alternate method for reducing ketone to methylene units. His method, which involves the formation of the ketone tosylhydrozone followed by reduction of the hydrazone with the highly selective reagent sodium cyanoborohydride, can be used with esters present and, therefore, will be used on Kelevan directly.

The whole sequence of attachment reactions on Kelevan itself (ketone group present) has been studied in order to gain some familarity with the reactions. Kelevan has been hydrolzyed under acid conditions to the free acid. Treatment of the free acid with N-hydroxysuccinimide resulted in the formation of a colorless crystalline compound with a m.p. of 245° C and an infrared spectrum compatable with the desired adduct. Other spectral data (ms and nmr) and elemental analysis data are being collected now. Most bondings to BSA seem to result in about 5 moles of nonprotein material being bonded per mole of BSA. Therefore a 25 fold excess of the succimide derivative (149 mg) was added to a 100 ml aqueous solution of 100 mg of BSA which had been buffered to about pH 7.5 with bicarbonate and HCl. The succinimide derivative was dissolved in 0.5 ml of tetrahydrofuran (THF) and added dropwise to the BSA solution. Preliminary checks indicated this amount of THF would not denature BSA. The suspension was allowed to stand overnight in the refrigerator to complete the formation of the BSA-Kelevan (BSA-K) adduct. The volume of the solvent was reduced by freeze-drying, and the residue was then subjected to gel-filtration.

Preliminary experiments have been conducted on the effect of the Kelevan- Nhydroxysuccinimide derivative (KS), prepared as described above, on the ATP'ase activities from catfish brain homogenate fraction. The data (Table 4) showed that the KS inhibited the three ATP'ase activities with pronounced effect on Na^+ -K^+ and oligomycin-sensitive Mg^{2+} ATP'ase activities. A freeze-dried sample of the clear portion (a small sediment was discarded) of the reaction mixture in which the BSA-K adduct was formed on the ATP'ase activities was also tested (see Table 4). At 1.0 and 4.0 µM concentrations in the reaction mixture, BSA-K inhibited Na^+ -K^+ and oligomycin-sensitive Mg^{2+} ATP'ase activities.

The 1.0 to 4.0 µm Kelevan concentrations (Table 4) were determined assuming that 5 moles of KS interacted with one mole of BSA. However, it is possible that additional KS was absorbed onto the BSA by charge-transfer complex formation, hydrophobic interaction, and unreacted KS not sedimented. The latter concentrations were ignored because there is no way of estimating their values at this time.

Table 4. Effects of Kelevan** and BSA Derivatives on the ATPase activities from a Catfish Brain Homogenate Fraction

Kelevan Derivatives	Concentration (μM)	$Na^+ - K^+$ ATPase	Specific Activity Mg^{2+} ATPase Oligomycin Sensitive	Insensitive
Control	none	17.5	6.1	8.7
KS**	20	4.3	1.1	6.9
BSA-K	∿1*	15.7	4.2	9.2
BSA-K	∿4*	15.3	4.3	7.6
BSA-K (Sephadex effluent)	∿1*	17.7	6.4	9.9
BSA-K (Sephadex effluent)	∿10*	13.7	5.2	12.0

* μM concentrations are estimates of Kelevan added to the reaction mixture in the various forms listed.

** KS - Kelevan N-hydroxysuccinimide

*** Kelevan was tested in earlier studies and found to be nearly as inhibitory to the ATPase activities from Catfish brain homogenates as was the reduction product of Kepone[5].

In order to remove unreacted KS from the protein solution, a portion of the freeze-dried BSA-K derivative was dissolved in buffer and passed through a Sephadex G-75 column using 0.02 M imidazole (pH 7.5) buffer as the mobile phase. Most of the 280 nm absorbing material was collected in the void volume effluent. The effluent sample was freeze-dried and tested for ATP'ase inhibition. The amount of BSA-K added to the reaction mixture was again calculated assuming 5 moles of KS was covalently bound to BSA. However, it is assumed that most, or at least a significant portion, of the non-covalently bound KS was removed by the Sephadex treatment.

The BSA-K sample from the Sephadex column showed less effect on the ATP'ase system compared to the BSA-K sample before it was passed through the Sephadex column (Table 4). Of possible interest is the stimulation observed of the oligomycin-insensitive Mg^{2+} ATP'ase (Table 4, 10 µM BSA-K, Sephadex-effluent). No explanation for this observation is available at present. The above results indicate that the Kelevan inhibition of ATP'ase activity is maintained even after forming an adduct with BSA.

The above results are very promising in indicating that Kelevan, a derivative of kepone from which a mono-hydrogen mirex derivative may be prepared, can be covalently bound to BSA. The observation that the BSA-K adduct was still somewhat inhibitory to ATP'ase activity after passing through a Sephadex G-75 column was not necessarily expected because of the size of the molecule. However, if this preliminary observation provides to be correct, it could indicate that antibodies to Kelevan used as a hapten would be able to interact with and remove Kelevan from the inhibited enzyme.

The following paper describing this research is currently in press:

Desaiah, D. and R. B. Koch. Preliminary investigation on the effects of Mirex and its derivitives on ATP'ase activities on Fire Ants. J. Agr. Food Chem.

FATE AND EFFECTS OF ATRAZINE IN SALT MARSH ECOSYSTEMS. Grant R 803835. Auburn University. Principal Investigator: Donald E. Davis. Project Officer: W. Peter Schoor.

The objective of this research is to investigate the accumulation, degradation, and effects of atrazine on a model micro-ecosystem consisting of organisms from a salt marsh representing four trophic levels. During the first 6 months investigations were initiated aimed at determining the level oof C14 atrazine and its metabolites in individual components of the ecosystem, beginning with the primary autotroph Spartina alterniflora. During the first six month study period two experiments were initiated simultaneously. The first was to determine the tolerance of S. alterniflora to atrazine and study its effects on growth. The second was to develop methods for the isolation and identification of atrazine and its metabolites in S. alterniflora. A similar experiment was conducted with a previously studied plant, Sorghum vulgare (sorghum), to compare the metabolites formed in Spartina and to aid in their identification.

Tolerance of S. alterniflora to atrazine and its effects on growth.

S. alterniflora plants were collected from the marsh at Sapelo Island, GA., and maintained at Auburn Univ. in Hoagland's nutrient solution in 2-liter beakers in a growth chamber having a 14 hour photoperiod with 60% relative humidity, a temperature of 28°C and 30 Klux of light provided by a mixture of incandescent and fluorescent lamps. The photoperiods were followed by 10 hour dark periods at 60% relative humidity and 24°C. Healthy plants were divided into five lots of 16 plants each. The plants were weighed and the number of leaves per clump of plants, and the height of the plants were recorded. They were then transferred to 1-liter plastic beakers containing 900 ml of Hoagland's solution containing: 0, 5×10^{-8}; 5×10^{-7}; 5×10^{-6} or 5×10^{-5} M atrazine. Each beaker contained four plants and each treatment was replicated four times. Results of this experiment are given in Table 5. Generally, the values of all parameters measured decreased with increasing concentrations of atrazine. The lowest concentration used in this experiment, 5×10^{-8} M, had little apparent effect on growth. However, in spite of the above effects on the weight, number, and length of plant parts, even those plants growing in the highest concentration of atrazine (5×10^{-5} M) showed no obvious symptoms of unhealthy plants such as chlorosis, necrosis, wilting, etc.

Isolation and identification of C atrazine metabolites from alterniflora.

Plants were grown as described above. Leaf sections from S. alterniflora and Sorghum vulgare were divided into 25 samples each of 20 g which were placed in 250 ml flasks containing 150 ml 0.1M phosphate buffer containing 25uCi ring-labeled atrazine plus nonlabeled atrazine to give a final concentration of 9×10^{-5} M. Flasks were incubated for 24-hr under 30 klux of light at 28°C. Tissues were then homogenized in 400 ml of 80% ethanol, filtered, and the combined extracts of each species were concentrated under N_2, diluted to 1000 ml with deionized water, and partitioned three times with 1000 ml chloroform. The chloroform and aqueous extracts were concentrated and stored at -5°C. Plant debris collected during filtration of the original homogenate was re-extracted with 200 ml portions of chloroform, and the extracts were concentrated and combined with the original chloroform phase. The plant residue was also stored at -5°C.

Atrazine and any metabolites in the chloroform, aqueous, and insoluble fractions will be isolated and identified. At this time only the chloroform fraction has been studied. The chloroform extract was taken to dryness under N_2, dissolved in hexane, and placed on top of a silica gel (60-200 mesh) column (25 x 2 cm). The column was washed successively with petroleum ether (PE), 25% diethyl ether, 50% diethyl ether in PE, 100% diethyl ether, ethyl acetate, methanol and water. Each fraction was again placed on a silica gel column and washed with the respective solvent while ca. 5 ml fractions were collected. Tubes containing radioactivity were combined and taken to dryness.

Pigments, mostly chlorophyll and its degradation products, were at this point inseparable from the radioactive materials. Attempts to separate the pigment from the radioactive substances by thin-layer chromatography were unsuccessful. First attempts using microparasil columns with a high

TABLE 5. Atrazine tolerance Expt. I

Effect of various concentrations of atrazine on growth of Spartina, A 5 days after treatment

Atrazine Conc. (M)	Fresh wt. of root (g)	Fresh wt. of shoot (g)	No. of leaves	Total height (cm)	Dry wt. of root (g)	Dry wt. of shoot (g)
Control	19.65	27.26	15.56	68.25	3.29	7.18
5×10^{-5}	7.53	7.85	5.13	45.88	0.67	2.60
5×10^{-6}	7.92	13.79	9.88	61.13	1.14	4.64
5×10^{-7}	9.44	16.64	11.19	63.06	1.43	5.54
5×10^{-8}	13.70	24.11	15.0	69.71	2.01	7.90

Each value is the average of four replications with four plants in each replication.

pressure liquid chromatograph to separate the pigments and radioactive substances were also unsuccessful. However, separation was achieved with an anion ion-exchange column. While the preparative procedure is slow, isolation of a single radioactive component from the 100% diethyl ether fraction has been accomplished. This compound has been tentatively identified as atrazine. Similar results were obtained with the 100% diethyl fraction of Sorghum vulgare. Radioactive constituents of other fractions (ethyl acetate, methanol) of the chloroform extract are being isolated in a similar manner. The identity of each radioactive component will be confirmed by mass spectrometry. Preliminary separations by thin-layer chromatography and autoradiography suggest that only one radioactive metabolite is present in each remaining fraction of the chloroform extract; these are anticipated to be the dealkylated derivatives of atrazine.

Methods that will provide for the separation of plant pigments from radioactive substances on a preparative scale and thereby speed up the analyses are being developed. Apparently, such a separation of pigments from atrazine metabolites in chloroform extracts has not been completely accomplished in other reported studies. While the study has been delayed by the difficulty encountered in separating pigments from the radioactive metabolites, the problem has been resolved for chloroform extracts. Similar problems are not anticipated with the other, non-pigmented, components of the model ecosystems.

BIOCIDE RESIDUES IN A MARSHLAND-ESTUARY ECOSYSTEM. Grant R 801185. Rice University. Principal Investigator: Frank M. Fisher, Jr. Project Officer: Thomas W. Duke.

Pesticides used for the control of agricultural pests may ultimately enter adjacent aquatic ecosystems. The ultimate fate and effects of these synthetic substances in such systems is basically unknown. Information about this potential impact is necessary in order to develop and implement environmental controls and application guidelines. This research project will determine the fate and effects in wetlands of pesticides used to control insect pests in agricultural fields. The specific objectives of this project are to describe and quantify the movement and accumulation of specific pesticides in a Texas Gulf Coast marshland ecosystem. Residues of aldrin, dieldrin, endrin, lindane, heptachlor epoxide, and DDT and its metabolites will be examined in representative species of several trophic levels, including oysters, clams, blue crabs, shrimp and carnivorous fish. Water and sediment samples will be analysed. Emphasis in the final year of research will be given to the pesticide carbofuran, a compound of special interest as it is now being used as a substitute chemical for dieldrin and DDT.

This project has been completed and the final report is in preparation.

Meetings, Conferences, and Workshops

Borthwick, P.W.

Attended ADP coordinators meeting at Research Triangle Park, N.C., August 19-20, 1975, to discuss Agency and ORD policies and procedures.

MICROBIAL AND PHYSIOLOGICAL MECHANISMS BRANCH

Objectives

The Microbial and Physiological Mechanisms Branch conducts research on the metabolism of toxic organic compounds by marine organisms. Metabolites are isolated and identified. Possible accumulations of substrates or metabolites in food chains are investigated. Research is currently conducted on the microbiological metabolism of hydrocarbons and chlorinated pesticides. The effects of hydrocarbon and pesticide metabolites, including cholinesterase inhibiting pesticides on marine organisms, is being investigated.

Status of Projects

Fate of Nitrilotriacetic Acid: Bourquin

Investigations into the fate of nitrilotriacetric acid in estuarine waters has been completed. Tests on the effects of salinity on NTA degradation by a soil Pseudomonas sp. showed decreasing degradation with increasing salinities. Several bacteria were isolated from 11-Mile Creek, a freshwater stream, which degraded NTA readily after a 2-week induction period. No microorganisms from a saline environment were capable of degrading NTA in the laboratory.

Microbiology of Surface Films: Bourquin

Studies on the microbiology of surface films indicate dense populations of bacteria and fungi in surface microlayers of most estuarine environments sampled as compared to samples from 10cm depths. Additionally, preliminary gas chromatographic - mass-spectroscopic data indicates hydrocarbons of C_{16} -C_{34} carbon chains with alkane/alkenes and some aromatics present in naturally occurring surface films. Studies are continuing to determine microbiology and chemistry of surface films which may be useful in determining water quality through the use of microbial indicies.

Metabolism of Aromatic Hydrocarbons: Bourquin

Laboratory studies on the metabolism of aromatic hydrocarbons have yielded limited promising results for the co-metabolism of chlorinated pesticides. Studies on biphenyl metabolism show the compound to be degraded through the dihydrodiol tocatechol. Thin-layer chromatography has been used to show the existence of the diol. Additional studies are planned with biochemical mutants which are blocked for hydrocarbon degradation and accumulate metabolites. Future studies include both mutant and growth studies on eucaroytic metabolism of polycyclic aromatic hydrocarbons.

Efforts are being made to determine the rate of degradation of organophosphate pesticides in artificial salt-marsh environments. Data accumulated on the stability of these compounds under various physicochemical parameters by Gulf Coast Research Laboratory (W. W. Walker) is being incorporated into these studies.

Effects of Carbamate Pesticides: Coppage

Research on short-term effects of carbamate pesticides on cholinesterase activity in the central nervous system of fishes was completed and a publication of findings is in press. Tests of effects of 140 day exposure of sheepshead minnows to malathion on brain cholinesterase was completed in cooperation with Bionomics/Marine Lab. In cooperation with the Bioassay Branch, initial tests were completed for brain cholinesterase inhibition caused by 144-168 hrexposures of sheepshead minnows to 10-560 µg diazinon/ℓ. Techniques are being developed for study of effects of methyl parathion on cholinesterase in brains of exposed estuarine fish. Findings will be useful in determining concentrations of pesticides that may be harmful to fishes by inhibition of enzyme essential to nerve impulse transmission and will aid in interpreting enzyme inhibition in fishes from the environment.

Effects of Toxicants on Ciliate Protozoa: Cooley

The objectives of this research are to: 1) determine effect of specific organic and inorganic toxicants, single and in combination, on population growth of ciliate protozoa; and 2) determine whether and to what extent these toxicants are accumulated by the test organisms.

Experiments on the effect of methoxychlor and cadmium chloride, singly and in factorial combinations, on 24-hr growth rate and population size of the ciliate protozoan, Tetrahymena pyriformis, have been completed and statistical analysis of population growth data is nearly complete. Chemical analyses of cells and media to determine extent of bioaccumulation of methoxychlor and of cadmium in 7-day cultures are nearly completed. Tests on toxicity of malathion to T. pyriformis at constant temperature and salinity are complete and tests at varying salinity and temperature are expected to be completed during the First Quarter, 1976.

EC50's for cadmium chloride, tested singly, were 11.1 ppm for 24-hr growth-rate reduction and 12.5 ppm for 96-hr population-size reduction. EC50's for methoxychlor, tested singly, were unobtainable at concentrations up to 5 ppm. The EC50's for methoxychlor in the presence of 10 ppm cadmium were 1.02 ppm for 24-hr growth rate reduction and 1.6 ppm for 96-hr population site reduction. The same concentrations in the presence of 15 ppm cadmium chloride caused more than 99% reduction of both 24-hr growth rate and 96-hr population size. The interaction of the ions in the presence of 15 ppm cadmium chloride caused more than 99% reduction two toxicants thus produced EC50's at lower concentrations than did either toxicant alone. It is unlikely that such concentrations of cadmium and methoxychlor would be encountered in estuarine waters, but in exceptional situations, they might occur in sediments.

INSECTICIDE PERSISTENCE IN NATURAL SEAWATER AS EFFECTED BY SALINITY TEMPERATURE, AND STERILITY. Grant R 803941. Gulf Coast Research Laboratory. Principal Investigator: William W. Walker. Project Officer: Al W. Bourquin.

The objective of this research effort is to determine the effect of salinity, temperature, light, and sterility on th persistence and degradation of representative organophosphorus and organochlorine insecticides in natural seawater. Surface water samples varying in salinity from zero to 30 ppt will be amended with insecticide chemicals and incubated for varying lengths of time under varying conditions of light, temperature and sterility. Insecticide residues and degradation products will be sought by gas-liquid and thin-layer chromatographic methods. Malathion, parathion, methyl parathion, diazinon and methoxychlor are currently being investigated.

MICROBIAL INTERACTIONS WITH PESTICIDES IN ESTUARINE SURFACE SLICKS. Grant R 803141. Georgia State University. Principal Investigator: Donald G. Ahearn. Project Officer: Al W. Bourquin.

Indegenous microorganisms of natural and man-medicated surface slicks are being investigated for their interactions with selected organochlorine pesticides. The effects of heptachlor and other selected pesticides on the metabolism of hydrocarbons and fatty acids are being evaluated using labelled (C14) heptachlor and hexadecane in a model system. The accumulation and/or biodegradation of pesticides by bacteria, yeasts and molds is being considered in regard to their primary role in the food chain.

Bacteria and fungi from North Sea surface slicks have been identified. Thirty-six representative isolates have been tested for growth on hydrocarbon substrates. All isolates grew well on alkanes and are being tested for growth on a mixed hydrocarbon substrate using both axenic and mixed culture systems. Predominate numbers of bacteria were in agreement with previous results. Studies have been initiated on extracellular enzymes to help determine emulsification factors which aid in formation of surface films.

New Methology Developed: A rapid qualitative screening test for determining the range of concentrations of heavy metals to use to determine EC50's in protozoan population-growth studies. The test requires only 24 hrs, exposes the protozoa to graded concentrations of the toxicant in La Motte cells held in a Petri dish moist chamber, and uses death and loss of ability to swim as criteria of effect.

Meetings, Conferences, and Workshops

Coppage, D.L.

Attended and gave paper at the 105th Annual Meeting of the American Fisheries Society, Sept. 10-13, 1975 Las Vegas, Nevada.

Attended and gave paper at Symposium on Pollution and Physiology of Marine Organisms, Nov. 3-6, 1975, Milford, Connecticut. Co-sponsored by MACFC, National Marine Fisheries Service and Belle W. Baruch Institute for Marine Research, Univ. N. Carolina.

Bourquin, A.W.

Attended and gave paper at Society for Industrial Microbiology Meeting, Aug. 1975, Kingston, R.I.

Attended and gave invited presentation at the 3rd International Biodegradation Symposium, Kingston, R.I., Aug. 1975.

Invited presentation at Gulf Coast Research Laboratory, December, 1975, Ocean Springs, MS.

Attended Estuarine Research Federation Meeting, Galveston, TX, Oct. 1975.

Technical Assistance

Coppage, D.L.

Data, review material and commentary were provided to the Office of Water and Hazardous Materials, Office of Pesticide Programs, Criteria and Evaluation Division on "Fate and Significance in the Environment" of several pesticides for their "Initial Scientific and Mini-Economic Reviews". Data and commentary on pesticides were also provided to the Office of Water and Hazardous Materials to write "Quality Criteria for Water" as required by the Federal Water Pollution Control Acts Amendments of 1972.

Bourquin, A.W.

Served on hearing panel for Adjudicatory Hearing for City of Philadelphia dumping of sewage sludge off Atlantic coast.

Completed study on fate of nitrilotriacetic acid in estuarine waters for OR&D Headquarters.

PATHOBIOLOGY, TOXIC INTERACTIONS BRANCH

Objectives

The Pathobiology, Toxic Interactions Branch plans, conducts and manages research concerned with the effects of toxicants on marine and estuarine species at the subcellular, cellular, tissue and organ levels. Use of contemporary tools, instruments, and techniques of pathology such as light and electron microscopy, histochemistry, tissue culture and microtechniques (Table 6) provide data and knowledge necessary to understand how pollutants damage aquatic organisms. This information is used to help assess the significance of numbers and figures that go into establishing water quality criteria (Table 7). Pathobiology is further concerned with understanding interactions between natural diseases and man-made disease agents such as pollutants. Research is conducted on the use of aquatic species as indicators of carcinogens, mutagens, and teratogens in the environment. Development of methods that utilize aquatic species as early-warning indicators for human health and ecosystem damage has high priority in pathobiology and GBERL.

The philosophy of the pathobiology branch is based on the tenet that an understanding of mechanism(s) of toxicity and damage in aquatic species is the best background upon which to predict an manage the impact of toxicants in aquatic ecosystems.

Status of Projects

Structural and functional changes in the gills of crustacea after exposure to heavy metals and organics: Couch

It has been found that cadmium exposure is associated with death of gill epithelial cells. Electronmicroscopy has revealed that osmoregulatory, ion regulatory, and respiratory cells die and become necrotic resulting in blackened gills. Levels of 718 ppb $CdCl_2$ in water for 30 days and less produces gill destruction and death. This information will be used by the Office of Water and Hazardous Materials ocean dumping program and the Office of Pesticide Programs in setting Standards, particularly for heavy metals.

Effects of interactions of biocontrol and chemical agents: Couch

Using the shrimp-virus system in interactive bioassays (see Figure 2) with polychlorinated biphenyals, it has been found that exposure to PCB's of groups of shrimp with only a few infected individuals results in up to 70% increase in virus infected individuals. These results will be used by the Office of Pesticides in registration of biocontrol agents (i.e. viruses) as pesticides.

The use of aquatic species as indicators of the Environmental Carcinogens: Couch

Research on this project is just beginning. Oysters will be used as test animals for select suspect carcinogen exposure. A conceptual paper on

TABLE 6

PATHOBIOLOGY

FOUR GENERAL, COMPLEMENTARY LEVELS			
EPIZOOTIOLOGY	GROSS	MICROSCOPIC	ULTRASTRUCTURAL
PATHOLOGIC EFFECTS OF POLLUTANTS ON POPULATIONS	DISSECTION	HISTOLOGY	SUBCELLULAR CHANGES,
	CASE HISTORY	CYTOLOGY	CYTOCHEMISTRY
	----------	HISTOCHEMISTRY	FUNCTIONAL LESIONS
----------	INVOLVES	----------	----------
MORBIDITY	INDIVIDUALS	INVOLVES SYSTEMS,	EFFECTS ON STRUCTURE
MORTALITY		ORGANS, CELLS	AND FUNCTIONS IN
			LIVING SYSTEMS
			MERGE

TABLE 7

APPLICATION OF RESULTS

1. USE REPRODUCIBLE PATHOLOGIC SIGNS * CHARACTERISTIC OF SPECIFIC TOXICANT CONCENTRATIONS TO DEMONSTRATE SUBLETHAL INJURY.

   * DAMAGE SIGNS OR SYNDROMES MAY BE AT ANY ONE (OR MORE) OF THE LEVELS:

   EPIZOOTIC,

   GROSS,

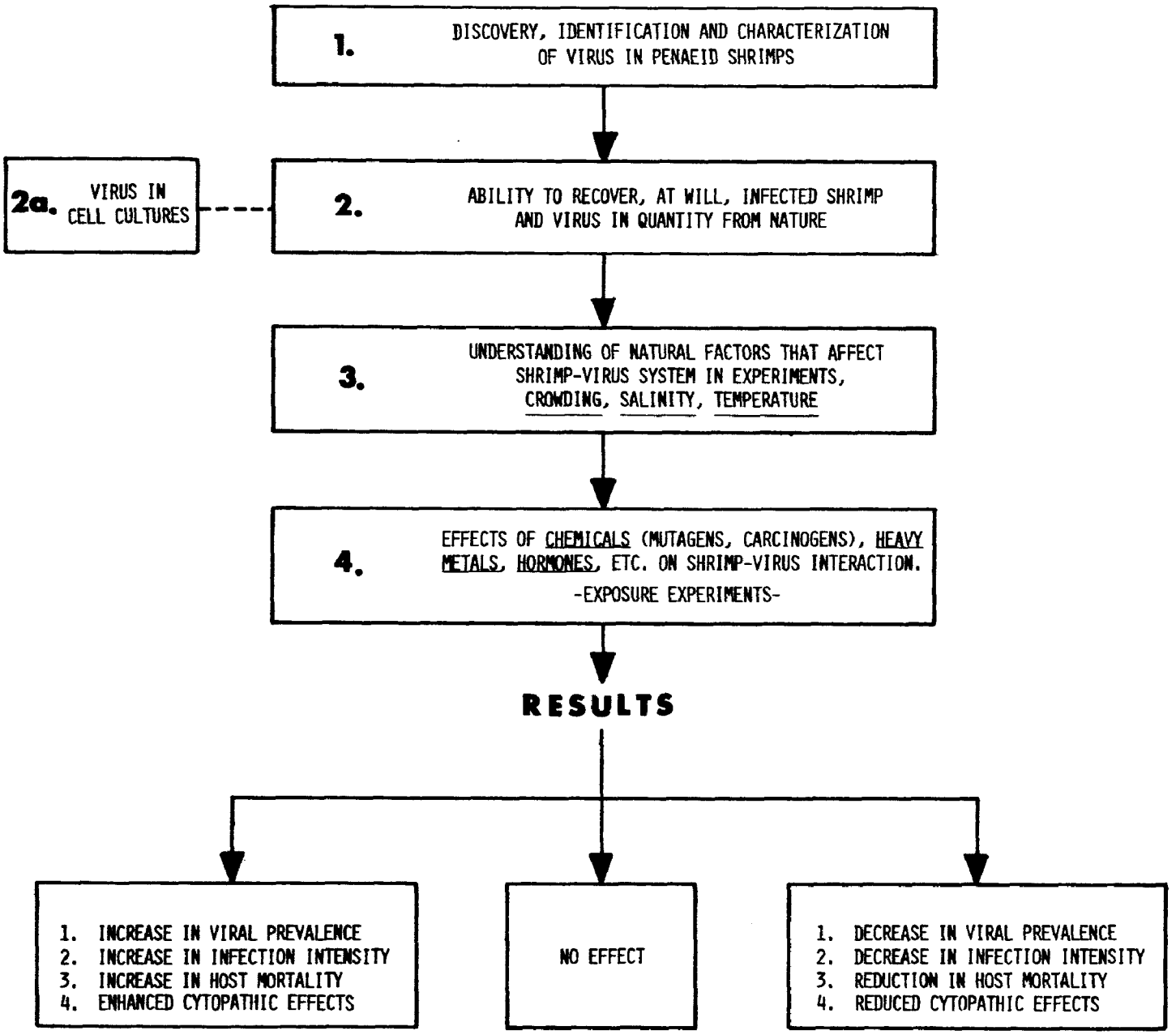
   MICROSCOPIC

   ULTRASTRUCTURAL

2. APPLY SUBLETHAL DAMAGE LEVELS IN ESTABLISHING MAXIMUM "SAFE LEVELS" FOR TOXICANTS, OR (AFTER THE FACT) TO SHOW INJURY FOR ENFORCEMENT PURPOSES.

SHRIMP-VIRUS MODEL
for
INTERACTIVE BIOASSAY

CHRONIC, SUBLETHAL EFFECTS



use of aquatic species for carcinogen indicators has been written (Joint US/Soviet Journal - in press). The Office of Health and Ecological Effects will use these results for determining threats of specific pollutants as carcinogens.

Pathology service: Couch

This is a continuous project providing diagnostic, descriptive and advisory service to projects of other branches in the interpretation of pathological responses of test organisms. Gill and pseudo-branch damage in fish exposed to chlorine at the Bears Bluff EPA laboratory has been described. Levels of chlorine sufficient to damage gills of estuarine fishes was determined.

BIOCHEMICAL CHARACTERIZATION, INSECT CELL LINE CULTURE, AND DETERMINATION OF HOST SPECIFICITY OF PINK SHRIMP NUCLEAR POLYHEDROSIS VIRUS. Grant R 803395. University of Texas. Principal Investigator: M. A. Summers. Project Officer: John A. Couch.

Shrimp baculovirus proteins and DNA have been characterized (DNA mol. wt. @ 50 x 10^6) and Kleinschmidt preparations of virus DNA molecules have been photographed.

This information and these data will permit comparisons and separations of the shrimp virus and similar insect viruses that are being developed as viral insecticides. Biochemical characterization of the shrimp virus is necessary for future correct identification of the virus in field studies and laboratory studies in which the virus is used as part of experimental models for study of effects of pollutants on host-pathogen relationships.

New Methodologies: Pathobiology has outlined the potential use of the shrimp-virus system for bioassays of interactive effects of chemicals and pollutants in estuarine animals (Figure 2). This outline is suggested as a model for understanding how low level pollutant exposure might enhance or increase the effects of natural pathogens in estuarine species, or how a pollutant might upset the host-parasite relationships in estuarine ecosystems.

Meetings, Conferences, and Workshops

John A. Couch

Attended and presented an invited paper to the III International Congress of Virology in Madrid, Spain during September. The paper and a data demonstration was about the shrimp baculovirus.

Represented Dr. Duke and GBERL at Conference on the Review of EPA Estuarine and Marine Research in Washington, D.C. in June.

BEARS BLUFF FIELD STATION

Objectives

Bears Bluff Field Station is uniquely situated among pristine salt marshes to conduct experiments on estuarine and marine organisms in the South East Atlantic coast. In this regard an overall research objective is the scientific assessment of impact of man's activities on estuarine/marine coastal ecosystems. Within this broad area the laboratory specifically coordinates EPA marine research in ecological effects of the biocides used in power generation plants to control marine growth and disinfectants applied in municipal sewage treatment to control pathogens. These efforts include development of bioassays, assessment of estuarine habitats, marine chemical analytical techniques, and specific studies in the areas of marine organism development, reproduction, toxicology, chronic effects, food webs, nutrient and pollutant assimilation, physiological and biochemical measurements, data analysis, etc. The field station facilities consist of outdoor ponds, a high capacity flowing sea water system, physiological labs, marine productivity analysis equipment, chemical analysis laboratory, a reference library, small boats and marine sampling equipment, temperature control sea water systems, and telecommunications equipment.

Status of Projects

During the period July through December, 1975, research activities at Bears Bluff Field Station were directed towards scientific evaluation of effects of chlorination processes to marine ecosystems. This effort involved both investigations conducted at the field station, and initiation of three grant-supported projects.

Marine Ecosystem Test Unit Experiments: Davis, Erikson, Crane, Yoakum and Foulk

Mixed species experiments (Marine Ecosystem Test Units - METU), consist of an array of ninety-six, 38-liter flowing water units, each receiving 40 liters/hr of unfiltered marine water from the tidal North Edisto River, containing entrained natural planktonic organisms and suspended particles. Thirty-two of these units are "harvested" each 30-days, another set (32) each 60-days and a third set (32) each 120-days. Within each harvest set three levels of continual chlorination are applied using sodium hypochlorite adjusted to calculated levels of 0.0 (control), 0.125, 0.250, and 0.500 ppm at the delivery head. The control and each of the chlorination levels is triplicated for three sets of eight test tanks. Chlorination delivery rates are maintained by the use of syringe pumps. Amperometric titration analysis can detect only traces of total residual oxidants at the highest level cited.

Harvests are followed by identification and enumeration of specimens in each unit. The enumeration requires 5-8 people to sort and identify. The principal question under consideration is: Will continuous low levels of sodium hyperchlorite affect the settling and development of species in a benthic estuarine community?

This experiment, conducted in outdoor tanks, fulfills the need for a bioassessment technique which correlates data from laboratory single-species bioassays with data from field experiments. Furthermore, it produces data representing a summation of all the potential ecological effects of chlorine introduced into marine waters, regardless of the state of the art of chemical detection.

In addition, individual trays of oysters (well known bioaccumulators), have been placed in the water collector systems downstream from the community test tanks. These oysters will provide samples for body burdens of potential halogenated organics formed during degradation of chlorine. No oyster tissue analyses have been completed at the time of this report.

During the reporting period the species enumeration effort developed a technique to increase the rate of sorting. Utilizing colloidal sillica and adjusting specific gravity, small crustaceans such as the very abundant amphipods are rapidly separated from detritus and other organisms. This has significantly accelerated the rate of data output.

Preliminary statistical analyses of the first two harvests (June/July 1975) indicate excellent uniformity of data among dosage replicates, and organisms in communities in the test units.

Single Species Bioassays: Davis and Middaugh

Single species bioassays are being conducted on such fishes as spot (Leiostomus xanthurus); mummichog (Fundulus heteroclitus), silversides (Menidia menidia), and striped bass (Morone saxitilis).

Some of the results at this time from fish bioassays include:

Short term studies with juvenile spot (10-15 mm TL) showed that they were sensitive to chlorination of flowing sea water. So-called "total residual chlorine" in these bioassays was determined by amperometric titrator. Chemically speaking, however, it is not residual chlorine that is being measured in sea water systems. The estimated incipient LC50 at 10°C was 0.12 mg/l and at 15°C, 0.06 mg/l instrument readings. Spot also showed temperature dependent avoidance responses to chlorination in experiments run simultaneously to the LC50 exposures. Concentrations avoided were in general similar to the estimated incipient LC50's at respective test temperatures of 10 and 15°C (Middaugh et al., in press).

Histological monitoring of juvenile spot demonstrate damage to gill tissue in specimens exposed to chlorine at 1.5 mg/l instrument reading. Histopathological sections of pseudobranchs from control, untreated specimens show plump epithelial cells surrounding lamellar capillaries. Histological sections of pseudobranchs from specimens exposed to chlorination levels yielding instrument readings of 1.5 mg/l for 95 minutes show separated epithelial membranes, with a scalloped border; this has been shown not to be a normal artifact.

In work with early life history stages of the striped bass, we found that the eggs were less sensitive than newly hatched prolarvae and that the sensitivity of prolarvae and larvae to chlorination decreased with increasing age.

Through an extramural grant (R 803872) to Dr. Morris Roberts at the Virginia Institute of Marine Science, bioassays are being conducted with selected invertebrate species including the oyster crab (Penopeus herbstii), hermit crab (Pagurus sp.), and blue crab (Callinectes sapidus), and coot clam (Mulinia lateralis).

In the area of halogen chemistry in sea water, Dr. James H. Carpenter has completed a state-of-the-art review paper for the EPA-ERDA sponsored "Environmental Impact of Water Chlorination" symposium, Oak Ridge, 22-24 October, 1975. Results of preliminary work (Grant R-803893) on the inorganic chemistry of chlorine added to sea water has underscored the lack of basic knowledge in the area. Experiments conducted by Dr. George Helz, University of Maryland (Grant R 803839) have demonstrated that within a few seconds of addition of chlorine to sea water, bromine complexes are activated which proceed to oxidize organic compounds present. Additionally, Dr. Carpenter has demonstrated that the currently accepted analytical technique of amperometric titration does not represent total residual chlorine (TRC) in sea water, as has been generally assumed. Chloro-bromo complexes are demonstrably present and their activity and ecological effects must be assessed. Carpenter's work is proceeding into identification of halogenated organics induced by chlorination processes. These marine chemical revelations present considerable and important insight into the extreme difficulty posed to EPA in establishing scientific criteria for regulation of all oxidation compounds (chlorine, bromochloride, and ozone) used in marine waters, especially inshore coastal zones.

Students from the College of Charleston Biology Division are conducting community studies on experimental effluent ponds at Bears Bluff. Their efforts, jointly supported by Bears Bluff Field Station and a "SOS" grant from National Science Foundation focuses on the species invasion, recruitment, and development of communities in marine habitats. One pond receives effluents from METU and laboratory chlorine bioassays. Another pond receives only "control" or non-exposed sea water. One year's effort has been completed and summarized and the students are initiating their second year of sampling.

ISOLATION AND STUDY OF CHLORO-ORGANICS RESULTING FROM CHLORINATION OF SEAWATER. Grant R 803893. University of Miami. Principal Investigator: James H. Carpenter. Project Officer: William P. Davis.

Identification and quantification of halogenated compounds formed when chlorine is added to seawater. Initial emphasis will describe the inorganic and organic reactions which lead to long lasting by-products. Followup studies will investigate the complex and physical chemical fate of identified compounds in simulated and natural marine ecosystems, including complexing with sediments, or uptake and bioaccumulation by organisms.

Analytical capability will be applied both to on-site experiments investigations and community studies by the Bears Bluff Field Station.

FIELD INVESTIGATION OF CHLORINATED AND BROMINATED ORGANIC COMPOUNDS FORMED IN SALINE POWER PLANT COOLING WATERS. Grant R 803839. University of Maryland. Principal Investigator: George R. Helz. Project Officer: William P. Davis.

(1) Identify and quantify the halogenated organic compounds produced by power plants; (2) Determine chemical factors controlling yields of produced compounds by study of power plant cooling waters under different conditions in the Chesapeake Bay Region. Included in the first year's funding is a workshop for chlorine/halogen investigators researching chemical and ecological impacts in marine ecosystems.

SUBLETHAL EFFECTS OF CHLORINE ON MARINE VASCULAR PLANTS AND DECAPOD CRUSTACENANS. Grant R 803872. Virginia Institute of Marine Science. Principal Investigator: Morris H. Roberts. Project Officer: William P. Davis.

Determine the effects of chlorine (and induced compounds) on marine organisms including impact upon decapod crustacean development and effects upon selected mollusks and other marine organisms.

Meetings, Conferences, and Workshops

Davis, W.P.

EPA Office of Toxic Substances Workshop: Use of Model Systems in Environmental Fate and Effects Testing of Toxic Materials, Annapolis, Maryland, 3-4 September, 1975.

EPA-ERDA "Workshop on Use of Microcsms in Settling Water Standard Criteria", Pensacola, Florida, 16-17 September, 1975.

Ocean Disposal Bioassays, Pensacola, Florida, 18-19 September, 1975.

Estuarine Research Federation Biannual Symposium, Galveston, Texas, 6-10 October, 1975.

EPA-ERDA "Environmental Impact of Water Chlorination", Oak Ridge, National Laboratory, 22-24 October, 1975.

Presented EPA position to the Conference/Workshop "Environmental Studies Program for the South Atlantic Outer Contental Shelf Area, 14-17 October, 1975.

Technical Assistance

Technical assistance rendered by Bears Bluff Field Station personnel include comments on a DEIS from Fish and Wildlife Service to reinterpret the Lacey Act as a regulatory authority to restrict importation and possession

of exotic organisms, including tropical fishes. Our comments became realized when Congressman Leggett later urged U.S.F.W.S. to revise their strategy in this endeavor.

Personnel at Bears Bluff Field Station provide manuscript reviews to National Marine Fisheries Service Fishery Bulletin, and grant proposals to EPA, ERDA, and National Science Foundation, on a regular basis.

Further technical assistance to BLM was provided at the "Live Bottom, Biological Sensitive Areas" Meeting, Charleston, in January 1976. Review of EPA assistance to OCS Lease sales was given to Region IV representative to other meetings and reviews.

LABORATORY ANALYTICAL SERVICES BRANCH

Objectives

This branch provides analytical support to other Research Branches of GBERL. This support consists of conducting chemical analyses of pesticides and other organics in marine water, sediment and biota as required.

1184 samples were analysed for pesticides and related organics during the reporting period.

A method for the detection of the metabolites of malathion has been developed. The technique offers, for the first time, a means of monitoring this organophosphate pesticide in the aquatic environment. Exposure of fish to malathion showed detectable levels of the monocarboxylic acid of malathion within 30 minutes whereas the parent compound, malathion, was never detected.

An analytical adaptation laboratory has been established. In this laboratory, techniques are developed for analyses of pollutants in the marine environment. Liquid chromatography is being evaluated as a means of analyzing for some of the carbamate and organaphosphate pesticides and their degradation products. A considerable amount of time has been devoted to adapting methods for the analyses of diazinon, endosulfan, methyl parathion and kepone in marine water and biota.

Meetings, Conferences, and Workshops

Wilson, A.J.

Attended a workshop on analytical methods for the analyses of kepone on January 14, 1976, at EPA Research Triangle Park, North Carolina.

Cook, G.H.

Attended 12th Annual Pesticide Review Conference, Palm Beach, Florida July 14, 1975. Presented papers entitled "Determination of malathion, malaoxon, mono-and Dicarboxylic Acid of Malathion in fish, oysters, and shrimp tissue."

Moore, J.C.

Attended 12th Annual Pesticide Review Conference, Palm Beach, Florida, July 14, 1975. Presented papers entitled "Uptake and depuration of malathion and its hydrolysis products in pinfish and the correlation of residues with acetylcholinesterase inhibition."

Technical Assistance

Analysis of whale and manatee tissues for pesticides and polychlorinated biphenyl compounds for EPA Office of Pesticides.

Analysis of sea herrings for pesticides for the Organization of Economic Cooperation and Development.

Analysis of kepone residues as requested by the State of Virginia and EPA Region III.

II. TECHNICAL ASSISTANCE

Approximately 200 man-days of technical assistance (TA) was provided to groups or agencies outside of the laboratory from June through December, 1975. The recipients of this assistance were universities, professional societies, state agencies, federal agencies (other than EPA), EPA offices (other than OR&D) and foreign countries or international organizations. This TA does not include numerous telephone conversations with private citizens on environmental questions and/or issues.

Some specific examples of Technical Assistance follow:

PCB Hearings - State of New York vs. General Electric

In December, 1975 Del Nimmo and David Hansen prepared statements and testified for the State of New York on the PCB problems in the Hudson River. GE plants on the upper Hudson were implicated in PCB-contamination of the river and aquatic life. Since that time, the courts have ruled in favor of the State of New York.

Bioassay Organisms supplied to EPA, Region IV

Juvenile marine fish and crustaceans were reared in the laboratory by the Bioassay Branch of ERL, Gulf Breeze and supplied to the Surveillance and Analysis Division of Region IV. These organisms were used for in situ, flowing water bioassays to evaluate toxicity of effluents from various industrial plants within Region IV. Mobile laboratory trailers are used by Region IV personnel for these surveillance operations.

Technical Assistance involving foreign travel

Dr. Tudor Davies traveled to Moscow in December to participate in the joint US-Soviet working group on Water Pollution. This group is developing and accomplishing a program of joint projects in the Management of Drainage Basins - Protection of Lakes and Estuaries and Water Quality Criteria. This specific activity entailed an assessment of the State of River Basin Management Modelling and Large Lake Management Modelling in both countries through a symposium and subsequent publication and the development of work plans for future cooperation.

Dave Hansen traveled to Dubrovnik, Yugoslavia to attend a meeting of the Working Group on the Principles for Developing Coastal Water Quality Criteria. A manuscript will be published by FAO.

Analyses of sea herring for the Organization of Economic Co-operation and Development (OECD)

The Laboratory Analytical Services Branch, headed by Alfred J. Wilson, is participating in the OECD program of wildlife sampling and analysis over a three year period to determine the levels of pesticides and related compounds in the environment. A shipment of sea herring, Clupea harengus, collected off the North Atlantic coast is currently being analysed.

Ocean dumping activities

In July of 1975 Dr. Bourquin and Dr. Davis served on the Adjudicatory Hearing Panel for the Philadelphia sewage disposal hearings. State and federal agencies are pursuing alternatives to the present practice of barging and dumping Philadelphia sewage in the Atlantic Ocean.

Kepone in the James River, Virginia estuary

In December of 1975 the laboratory became involved in the potential health and ecological effects of Kepone (a chlorinated hydrocarbon insecticide) contamination in the lower James River. Our involvement with the Kepone problem during the period covered by this report (July-December 1975) was limited to planning, validation of analytical methods, and initial analyses of shellfish exposed to Kepone. However, this laboratory in cooperation with the Virginia Institute of Marine Science (VIMS), has proposed an action plan for ecological research specifically related to the James River Estuary and the "Kepone incident". This research is planned to answer the following specific questions:

1. Determine the concentration of Kepone in organisms that are commercially harvested in the James Estuary and are direct routes of the carcinogen to human food. Determine the effective strategies that can be used to reduce present levels of Kepone, i.e., transplanting of shellfish, etc.

2. Determine direct toxic effects of Kepone on the individual organisms and potential ecological effects on the James River estuary.

3. Determine the fate and persistence of the insecticide as a hazardous compound in the estuary.

This research will require a considerable amount of technical assistance during the next six months. Studies are presently underway at Gulf Breeze to determine uptake, accumulation and loss of Kepone from oysters and at VIMS to determine in-situ depuration rates by removing oysters with known levels of Kepone from the James River and placing them in "clean" waters for subsequent analysis.

III. LABORATORY PUBLICATIONS

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Special EPA Publication

The Ocean Dumping Bioassay Committee, chaired by Dr. Duke, has prepared a manual entitled "Bioassay Procedures for the Ocean Disposal Permit Program." Staff members from the Environmental Research Laboratories at Gulf Breeze, Narragansett and Corvallis contributed to this manual. The manual is now in press.

The bioassay procedures given in this manual were developed to provide tests for conducting toxicity evaluations of waste materials considered for ocean disposal under EPA's Ocean Disposal Permit Program. Nine bioassay procedures are described; three of which are considered "special" and are not recommended for routine use. The procedures specify the use of various organisms representing several trophic levels. Both flow-through and static tests are included. Methods given vary in their utility and complexity of performance. The procedures are not intended to be considered "standard methods," but are to be used as reference methods or official methods depending on the judgement of the EPA Regional Administrator responsible for the management of the permit program.