United States
Environmental Protection
Agency

EPA-600/9-83-005

EPA

Research and Development

EFFECTS OF CARCINOGENS, MUTAGENS, AND TERATOGENS ON NONHUMAN SPECIES (AQUATIC ANIMALS)

FOURTH ANNUAL REPORT
NCI/EPA COLLABORATIVE PROGRAM

Prepared for

National Cancer Institute

Office of Pesticides
and Toxic Substances

Prepared by

Environmental Research
Laboratory
Gulf Breeze FL 32561

DISCLAIMER

This report has been reviewed by the U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Fourth Annual Report

NCI/EPA Collaborative Program

Fiscal Year 1982 (October 1981 - September 30, 1982)

Project 3

EFFECTS OF CARCINOGENS, MUTAGENS, AND TERATOGENS ON NONHUMAN SPECIES (AQUATIC ANIMALS).

by

John A. Couch, Ph.D., Coordinator, Pathobiologist

W. Peter Schoor, Ph.D. Biochemist

Will Davis, Ph.D., Biologist

and

Lee Courtney, Research Toxicologist

U.S. EPA, ERL, GULF BREEZE, FLORIDA

TABLE OF CONTENTS

II. Abstract 2

III. Introduction 4

IV. Objectives 5

V. Methodological Approaches 5

VI. Major Findings and Results 7

VII. Significance to Biomedical Research and Program needs of NCI and EPA 13

VIII. Future Plans 14

IX. Date Contract Initiated and Period of Contract Planned 14

X. Contractors Project Director and Project Officers 14

XI. Progress Reports on Specific Extramural Cooperative Agreements 14

XII. Publications Resulting from Program 39

EFFECTS OF CARCINOGENS, MUTAGENS, AND TERATOGENS ON NON-HUMAN SPECIES-AQUATIC ANIMALS

John A. Couch, Coordinator; W. Peter Schoor, Biochemist;
Will Davis, Biologist; Lee Courtney, Research Toxicologist

United States Environmental Protection Agency

Gulf Breeze, Florida 32561

Aquatic systems and organisms are under both laboratory and field study in order to develop indicator, screening, and modeling capabilities for detection and evaluation of risks of carcinogens, mutagens, and teratogens. Studies include both Gulf Breeze laboratory projects and complementary, extramural projects. In the fourth year of the program, several advances were made in the development of laboratory and field carcinogen assays, utilizing fishes such as the sheepshead minnow (liver lesions via benzidine and aflatoxin exposures), Menidia peninsulae (liver lesions via aflatoxin exposures), and freshwater cat fish (papillomatous-like lesions via chlorinated effluent exposures). Emphasis is still placed on the development and utilization of critical life stage exposures (e.g., embryo and newly hatched fry exposures) in order to expedite carcinogen tests and minimize time required for tumorogenic responses. Preneoplastic hepatic lesion development in Menidia at 12 weeks suggests promise for this species and exposure method. A novel approach has shown that tiger salamanders may be good biochemical and histologic indicators of the presence of certain carcinogens (polycyclic aromatic hydrocarbons - PAH's). Skin and liver tissues of the salamanders revealed induced enzyme activity (MFO system) following exposure to the PAH, perylene. Considerable field monitoring work on mollusks and carcinogenic PAH's along the coast of Oregon has revealed a positive correlation between prevalence of cellular proliferation disorders in shellfish and higher concentrations of certain PAH's in natural water.

Emphasis in biochemistry for the last year has been directed mostly toward the elucidation of the metabolism of the mixed-function oxidases in marine organisms. From continuing work on the mullet (Mugil cephalus), we have found that benzo(a)pyrene (B(a)P) is converted mainly to 3-hydroxybenzo(a)pyrene, 9-hydroxybenzo(a)pyrene and 4,5-, 7,8-, 9,10-dihydrodiols of BaP, in in vitro systems containing liver microsomes from Aroclor 1254-treated mullet. Other metabolites such as diones of B(a)P and probably tri-and tetraols have also been produced. We have also developed a procedure for the epoxide hydrolase enzyme system in order to determine the role of this enzyme on the metabolism of the polyaromatic hydrocarbons. Our studies show that UDP-glucuronosyl transferase and sulfotransferase were significantly increased in livers of rats given phenobarbital (PB), phenolphthalein, phenanthrene, BaP or 3-MC orally for 10 to 14 days. The injection of PB or 3-MC into rats, sea catfish or mullet on long-term schedule resulted in increase in liver size, and an increase in UDP-glucuronosyl transferase in livers of all three animal species. We have observed that the liver microsomal fraction of the killifish, Fundulus grandis, has a high in vitro activity when injected with a single dose of 3-MC, converting B(a)P to 1-OH, 3-OH, 5-OH, 6-OH, 7-OH, (t) 9,10-diol, (t)

7,8-diol, 1,6-, 3,6-, and 6, 12 diones, and 4,5-oxide. There may be an optimal concentration of NADPH, and this concentration may have a significant effect on the products of the *in vitro* reaction, quantitatively as well as qualitatively. These studies continue to reveal that fish have metabolic pathways similar to mammals for disposition of certain carcinogens. A brief study was conducted to determine possible interactions between the 9-OH BaP and salmon sperm DNA. The interaction between salmon DNA and 9-OH BaP was shown by relative fluroescence studies. The binding was evidenced by an eight-fold reduction in relative fluorescence yield. We are developing methods to determine the possible binding of proximal carcinogenic metabolites to DNA's of selected aquatic animals.

III. Introduction

A major problem faced by the National Cancer Institute and the Environmental Protection Agency is that of aiding in determining the fate and effects of carcinogenic pollutants in the larger environment. One way is to study wildlife populations that are widespread, but which live in environments where exposure to ambient pollutants is certain. The use of wildlife populations as surrogates for human populations may be considered to be a novel expansion or logical extension of the use of laboratory animals and animal models of human diseases as alternatives to the use of human subjects. The sharing of biologic characteristics by phylogenetically diverse species makes certain comparative approaches possible.

Problems arise, however, when such factors as selection of sensitive, indicative species, geographically adequate populations, and representative segments of the air, land, water biosphere are considered. In this regard, Office of Research and Development laboratories, such as Gulf Breeze Environmental Research Laboratory, have exemplary, pilot research programs that are investigating the use of aquatic animal species as indicators of carcinogens in the environment. The Gulf Breeze pilot research program has been under way since August, 1978 and is supported jointly by the Office of Research and Development (EPA) and the National Cancer Institute through an interagency agreement. The Gulf Breeze studies are based on the premise that the aquatic portion of the biosphere (water, biota, and sediment) is the ultimate "sink" for the runoff, fallout, and discharge of most toxic pollutants. In addition, animals living in the relatively efficient solvent, water, are more intimately exposed (total exposure through body surfaces, gills, alimentary tracts) than are species living in terrestrial or air environments. Aquatic species are also less likely to escape a dissolved or carried pollutant.

At Gulf Breeze, researchers have been studying species of fish and shellfish along the Northern Gulf of Mexico (Florida, Alabama, Mississippi) in order to determine which are good indicators of the role of carcinogenic agents in the environment. Selected species of fish have been used in both long-term and short-term laboratory exposures to determine their specific tissue, cellular, and biochemical responses to known chemical carcinogens which may occur in the environment. In addition, a significant number of cooperative agreements with principle investigators from around the nation support an extramural complemental and supplemental effort in the identification of aquatic species and systems that may serve as early warning mechanisms.

IV. Summarized Objectives

Objective 1:

Determine fate and effects of carcinogens, mutagens, and teratogens in aquatic species (individuals; populations).

Objective 2:

Determine role of aquatic species and systems in actual or potential exposure of man to carcinogens, mutagens, or teratogens (metabolism, accumulators).

Objective 3:

Develop aquatic species as bioindicator, sentinel, and model systems for use in study of risks of carcinogens, mutagens, and teratogens in the general environment (sensitive species and systems).

V. Methodological Approaches

We have underway both in-house and complementary extramural research projects. The overall project is divided into major disciplinary approaches: 1) Pathobiology and 2) Biochemistry. Therefore, methods outlined below and results in the next section will be reported under these two complementary, disciplinary headings. Detailed results of each cooperative agreement in progress during FY 82 will be included at the end of this report. The disciplinary area of each cooperative agreement will be identified by project officer (Couch - pathobiology; Schoor - biochemistry).

1. Pathobiological Methods

Fish carcinogen assay system, toxicology, and histopathology of induced lesions.

At Gulf Breeze, major emphasis was concentrated on short-term critical life-stage carcinogen exposures of several estuarine fish species. *Cyprinodon variegatus* (Sheepshead minnow), *Menidia peninsulae* (Silverside), and *Rivulus marmoratus* were exposed to the overt carcinogen aflatoxin B_1. This compound, a natural product of the metabolic activity of fungi (genus *Aspergillus*), has been shown to be of high toxicity and carcinogenicity in mammalian studies (including humans) and work with freshwater fish species. Selection of aflatoxin B_1 as a test compound was partly based on this high hazard background, to test marine species with a proven and potent carcinogen. As such, special precautions and methods were needed to insure safety.

Exposures were conducted under static conditions within a glove box. Test organisms were exposed in 3.8ℓ chambers containing 1.5ℓ seawater, toxicant, and carrier, then rinsed and transfered to clean holding facilities for development and observation. A detailed description of the test systems utilized is now in press (Courtney and Couch, 1982). Carrier controls were run under identical conditions except for toxicant. Species, test concentrations, exposure duration, carrier, and life-stage tested varied. 96 hour embryos and 120 hour newly hatched larvae of *C. variegatus* were exposed for 1 hour to

200 μg/ℓ aflatoxin B_1 using dimethylsulfoxide (DMSO) as a carrier. C. variegatus, R. marmoratus, and M. peninsulae were exposed to 1000 μg/ℓ aflatoxin B_1 (using DMSO and triethylene glycol as carriers) for exposure durations ranging from 2 to 43.5 hours. Holding and observation continues on surviving test organisms.

In extramural efforts, Hendrix at Oregon is continuing histopathologic evaluation of tissues from rainbow trout (Salmo gairdneri) exposed for 18 months to dietary benzo(a)pyrene (BAP: 1000 ppm), Aroclor 1260 (PCB: 500 ppm), and toxaphene (50 ppm). Trout embryo exposures to methyl azoxymethanol acetate (MAMA) and benzidine dihydoro chloride (BDHC) were conducted and an experiment to produce a neuroblastoma neoplasm in coho salmon and Shasta strain rainbow trout was initiated. Martin, at the University of Southern Mississippi, is examining specimens from over thirty adult and embryo C. variegatus benzidine exposures. Additional work has been done with C. variegatus involving aseptic embryo techniques. Cell culture techniques have been investigated using a Fundulus species and C. variegatus.

Grizzle, at Auburn University, is continuing laboratory and field experiments to examine causation and development of oral papillomas found on feral black bullheads (Ictalurus melas) from the final oxidation pond of a Tuskegee, Alabama sewage treatment plant.

Rose and Anderson are involved in a two part research project involving tiger salamanders (Ambystoma tigrinum) which have shown an unusually high prevalence of dermal neoplasia. Rose is studying the histopathological effects of perylene on the species and Anderson is studying metabolic activation and/or detoxification of perylene and B(a)P by tissue enzymes of A. tigrinum.

Mix, at Oregon State University, is continuing a long-term field study of mulluscs in Oregon coastal waters and expanding laboratory research. Major emphasis this year include 1) baseline measurements of arsenic, cadmium, and nickel levels; 2) methods development for trace levels of polynuclear aromatic hydrocarbons (PNAH) in seawater; 3) developing a crustacean (barnacle) bioassay system; 4) studying possible viral associations with Mytilus edulis neoplastic disorders; and 5) evaluating data previously collected on neoplastic disorders in M. edulis.

2. Biochemical Methods

Induction Studies:

Aquatic species such as mullet, killifish, flounder, sea catfish and others have been and will continue to be exposed to inducers of microsomal mixed-function oxygenase (MFO) activity either by intraperitoneal injection or direct exposure in seawater. Since periods up to one year might be necessary to induce MFO activity by water exposure, the direct injection of inducer is used at the start of the investigations in order to optimize other parameters such as metabolite and conjugation reactions. The long-term, low exposure in seawater will follow. [Schoor, Melius (CR809493), Strength (CR809673)].

Metabolite Identification:

Metabolites from the MFO reactions will be identified using high pressure liquid chromatography coupled with fluorescence detection and confirmed by stopped-flow fluorescence scanning. Metabolite standards for benzo(a)pyrene [(BaP)] have been obtained from the Illinois Institute of Technology through the courtesy of NCI. All the phenolic compounds have been chromatographed and their excitation and emission spectra have been obtained in appropriate solvents. All spectra will be stored on discs for later data manipulation.

Conjugation and Excretion Studies:

Rats are being used to make a series of conjugation products *in vivo* by injection of ^{14}C-labelled BaP. They will include glucuronides, glutathiones, and sulfates. Their occurrence in fish will then be ascertained by comparison to the standards produced in the rat. This will be helpful in determining the final dispositon of a carcinogen like BaP within the animal and in what forms the parent compound is finally passed back into the seawater.

VI. Major Findings and Progress

As noted earlier under Methodological Approaches, results and findings of studies to date are reported under the two disciplinary areas of Pathobiology and Biochemistry with extramural cooperative agreement reports at the end of this report.

1. Pathobiology

Table I summarizes the carcinogen assay/induction studies undertaken by the Gulf Breeze staff and investigators working under cooperative agreements. This work represents an investigative effort encompassing a wide range of organisms and several selected carcinogens and suspect-carcinogens. In addition to the data generated, and possibly of even greater value, is the methodology developed (both in exposure systems and support technology).

The following represents scientific results and accomplishments of the Gulf Breeze staff and associated investigators during FY 82.

At Gulf Breeze, the static exposure system utilized in the aflatoxin studies has proven to be an efficient method for short-term exposures using high hazard compounds. Each species tested performed well and the use of various life-stages presented no problems. We feel this system provides a safe and inexpensive method of high hazard single short-term or pulse exposures for a wide range of life-stages and species.

Data generated to date include some basic toxicity data with regard to aflatoxin and the three species tested,and two lesions related to the exposures. Gross necropsy and histological examination of *C. variegatus* exposed to 200 μg/ℓ aflatoxin B_1 for 1 hr. have revealed a benign hepatic polyp on several specimens. Exact nature of the lesions is still under investigation and a large number

SPECIES	AGENT	EXPOSURE	RESULTS
OYSTER	3-Methyl-Cholanthrene	Flowing seawater, 1-5 μg/ℓ for one year.	*Incipient, perivascular blood cell proliferation.
OYSTER	Benzo(a)pyrene	Flowing seawater, 1-5 μg/ℓ for one year.	No tissue response.
SHEEPSHEAD MINNOW	Trifluralin-(Herbicide)	Flowing seawater, 5 μg/ℓ, 19 months.	Focal, benign, vertebral growths-non-neoplastic.
SHEEPSHEAD MINNOW	Aflatoxin B_1	Embyro-fry static 200 μg/ℓ for one hour held for 18 months.	*Hepatic polyps (on surface of livers). Benign neoplasm.
SHEEPSHEAD MINNOW	Benzidine	Recirculated seawater, 1 ppm, for 166 days.	*Proliferataive lesions in liver; ductile proliferation; cholangioma; adenofibrosis; oval cell hyperplasia.
RIVULUS	Aflatoxin B_1	Fry xposure static, 1 ppm, 2 hours, 8 hours.	No tumor response yet.
SILVERSIDE	Aflatoxin B_1	Fry exposure, static 1 ppm, 2 hours, 8 hours. Held 12 weeks.	*Early altered foci in livers; enlarged cells; preneoplastic.
SILVER PERCH CELL LINE	Asbestos-(Crocidolite)	Asbestos in culture medium.	Nodule formation in culture around asbestos fibers.
RAINBOW TROUT	Benzo(a)pyrene	1000 ppm, diet, 12 month feeding.	Hepatocellular Carcinoma.
TIGER SALAMANDER	Perylene	Injection i.p.	*Hepatic tumor.

*Tumors or suspect neoplasms actually induced.

of specimens still remain in holding. In addition, a suspect pre-neoplastic lesion has been noted in histologic sections of M. peninsulae exposed as fry for 2 hrs. to 1000 µg/ℓ aflatoxin (using both DMSO and TEG as carriers). The lesions, noted in specimens examined 12 weeks post-exposure, consist of foci of enlarged hepatic cells. Additional specimens from the exposure are being held for continued observation and future histologic examination. No lesions have been noted in those Rivulus examined to date.

During FY 82 a final report summarizing a two-year field biomonitoring study (Aug. 1978 to Aug. 1980) was completed. This is now available in manuscript form entitled "A Prospective Study of Infectious and Noninfectious Diseases in Fishes and Shellfishes in Relationship to Pollutant Activity in Three Gulf Coast Estuaries". The main issue of the study was that certain diseases of fishes and shellfishes (including neoplasia) from coastal populations have been suggested to be related to, caused by, or enhanced by pollutant activity. Considerable data have been published from which inferences have been made that fishes and shellfishes inhabiting contaminated waters are at higher disease risk than those in cleaner environments. Most of these studies from which data derived were retrospective, epizootiological efforts, initiated after the fact because certain fish diseases were increasingly observed or because fish kills were noted or had recurred frequently in specific limited bodies of water. Prospective studies of estuaries without prior knowledge of disease prevalence to determine possibly previously undetected frequencies and relationships of diseases and pollutant residues in fishes and shellfishes have been rare. Therefore, a need existed to determine prospectively if populations of fishes and shellfishes at higher disease risks related to pollutant activity could be identified with standard sampling, pathological, and analytical methods.

The study of three northern Gulf Coast estuaries, Pensacola and Escambia Bays in Northwest Florida, Mobile Bay, Alabama, and Pascagoula Harbor in Mississippi Sound, Mississippi, was undertaken in August, 1978. The specific goals of this prospective study were to: 1) determine and compare relative contamination by select pollutants on specific sites in and among the three estuarine areas, 2) determine frequencies of known or new diseases, including neoplasms, in shellfish (oysters) and fishes at these sites among the estuaries, and 3) to examine critically any relationships between disease frequency and pollutant contamination among the selected estuarine sites in order to assess the role of pollutant activity in influencing disease prevalences in fish and shellfish populations in coastal regions characterized by varying degrees of human pollutant activity. For each of the three estuaries, three different kinds of samples (oysters, fishes and sediments) were collected quarterly for chemical analysis of pollutant residues from August, 1979 through August, 1980. One offshore control station was sampled for prevalence comparison to the estuarine stations. Monthly samples of fishes, and oysters were collected for disease analyses and diagnoses from one or more specific sites in the three estuarine systems from August, 1978 through August, 1980 (25 Month Period).

Results of this study suggest that though far from being pristine, the estuaries studied were not severely unhealthy environments at the time that the biota were sampled. However, because predictions have been made that the Gulf Coastal Plain of the southeastern U.S. will probably be the fastest growing region in the nation in the next decade, this study and others provide base lines and points of departure and comparison for studies that should be made, periodically, on the health of coastal biota in relation to increasing impact of population and industrial growth.

In extramural projects, rainbow trout exposed to dietary benzo(a)pyrene (100 ppm) have developed hepatocellular carcinoma at 24% incidence level (see Hendricks attached cooperative agreement report no. CR809344-010). Aroclor 1260 and toxaphene exposures have yet to reveal neoplasms; however, tissue examination is not complete. Methylazoxymethanol acetate (MAMA) and benzidine dihydrochloride exposures have been completed, however, all necropsy samples have not yet been processed. Preliminary indications are that MAMA is carcinogenic to trout. Mechanical problems have hindered progress on attempted neuroblastoma induction.

Martin (see attached report no. CR809347) has produced liver lesions, probably adenofibrotic or neoplastic, in C. variegatus exposed to 1, 5, and 10 ppm benzidine dihydrochloride (BDHC). Several experiments are still in progress and characterization of the BDHC produced lesion is still underway. In BDHC exposed C. variegatus embryos (50 to 500 ppm exposure concentrations), 85% exhibited developmental anomalies compared to a 0.02% rate of anomalies in controls. Aseptic C. variegatus techniques have developed to the point of successful maintenance of embryos for 60 days or more. Four C. variegatus cell lines have been developed including three fibroblastic cell lines, and a striated muscle cell line, to our knowledge, the first such line developed from fish tissue. In immunological studies, banding pattern differences have been noted between BDHC-exposed and non-exposed C. variegatus and the characterization of these differences is being pursued. Additional studies using immunoelectrophoresis, competitive enzyme immunoassay and bacteriophage neutralization show much promise in exploring the processes occurring in BDHC exposed C. variegatus.

Grizzle (see attached report no. CR809336-010) has produced lesions in caged black bullheads (Ictalurus melas) confined to a chlorinated sewage effluent pond. The lesions consisted of oral mucosa hyperplasia, developing after 2 months, and papilloma-like lesions in the mouth fornix, developing during the second year. These lesions were similar to those found on black bullheads naturally occurring in the pond. Transmission experiments and ultrastructural studies failed to indicate a viral etiology for the papillomas. Induction of glucuronosyl transferase in caged channel catfish within the pond and Ames tests with the pond water indicate the presence of a chemical possibly related to the Ictalurus melas papillomas.

The work of Rose and Anderson (see attached report no. CR807740) includes the development of a radioisotopic method to quantify B(a)P and perylene (P) metabolism in Ambystoma tigrinum. Hepatic metabolism of ^{14}C-BaP and ^{3}H-P was studied in normal and induced A. tigrinum. Uninduced animals produced about 0.2 total ^{14}C-BaP metabolites/mg protein/30 min. Methylcholanthrene was indicated

as a AAH inducer. Metabolism of ^{3}H-P was found greater than that of ^{14}C-BaP, inducers had little effect on metabolism compared to noninduced Ambystoma. Carbon 14-BaP metabolite profiles of induced and non-induced animals were compared and it was found that some inducers, including P, produced a metabolite shift away from the more potentially carcinogenic compounds. This indicates a possible protective action by inducers, such as P, for subsequent BaP exposure, similar action is frequently reported in higher animals. The results of mutagenicity testing indicate that Ambystoma is quite resistant to PAH carcinogenesis and suggests that aromatic amines may be more appropriate model carcinogens. Histopathological lesions were found in several salamanders injected with perylene, one, a liver cell neoplasm. Final evaluation of this phase of the study is not yet complete.

The research project of Mix, at Oregon State University, (See attached report no. CR808000-01-0) has produced much data related to shellfish populations along the Oregon coast and valuable investigative methodology. Baseline measurements of arsenic, cadmium, and nickel were determined for two species of mussels, and it was determined that the mussels would be excellent monitors of these metals in freshwater (Margaritifera margaritifera) and marine (Mytilus edulis) environments. The evaluation of a long term study (1976-1981) of proliferative disorders in M. edulis has continued and produced much data relating to occurrence, geographical distribution, prevalence, seasonality, and histopathology. Extensive investigation, employing a variety of techniques and methods, failed to reveal the presence of any RNA tumor virus in M. edulis exhibiting neoplastic disorders. Additionally, methods for PNAH determination in seawater and a crustacean bioassay system utilizing Pollicipis polymerus were investigated. The bioassay system exhibits great potential for testing environmental carcinogens and mutagens.

Studies of teratogenic responses have arisen from a broader research program formerly supported by EPA Energy Effects funding (R805469). The objective of the investigation was to develop an experimental test with marine animals suitable for assessing the by-products of chlorination arising from biofouling control and disinfection of coastal waters. An additional task was requested by EPA to test specific compounds. Phthalate tests revealed significant increase in endematous cardiac malformations in embryos, as well as abnormal development of vertebral and other skeletal elements among surviving fishes.

A colony of the fish Rivulus marmoratus was transferred from Bears Bluff Field Station, S.C. and the College of Charleston to Gulf Breeze EPA laboratories in 1981. With the previous studies as a foundation, we have been concentrating on refinement of screening tests for teratogenic effects. Since Rivulus marmoratus is a self-fertilizing hermaphrodite, it provides natural clones which allow data tracking on individuals (temporal variations), as well as lineages and clones and representatives of wild populations. Our research designs incorporate all stages of gametogenesis as well as post-fertilization

developmental stages.

This strategy is noteworthy inasmuch as during initial screening one does not know the breadth of potential "windows of vulnerability" to teratogenic effects. Thalidomide, for example, is teratogenically dangerous to the embryo for only very brief periods. Rodent tests were run for several years before they "modelled" established human effects.

The number of potential responses of Rivulus are broad and apparently clearly distinguishable. Classical teratogenic studies used fishes, and focused mainly on expressed gametes just prior to, or soon after fertilization. The ability to "scan" broader periods of gametogenesis, and have the relative economy of fish are among the obvious advantages of this species. Studies presently underway will refine testing procedures, and compare results from n-dibutyl phthalate, thalidomide, and hexazinone (a herbicide). Other reports presented at the NCI (Koenig, et al.) Bethesda meeting report general biology and tumorgenic responses.

2. Biochemistry

Induction of transferases (UDPGA and sulfate) was observed in mullet and in channel catfish. The rate of conjugation was confirmed by enzyme assay with conjugation using p-nitrophenol as substrate. This was determined spectrophotometrically and by TLC separation of the respective conjugates of p-nitrophenol. Metabolites formed by MFO in microsomes derived from both control and induced animals were separated by HPLC and identified. Microsomes incubated with both BaP and UDGPA enabled us to ascertain the extent of conjugation in the presence of the oxidation system.

In addition to finding BaP metabolites already reported, we believe we have found some "cis" diols as well as "trans" diols. The evidence is based on agreement of retention times of standards and unknown metabolites. Nanogram amounts of the "cis" diols have not yet allowed more positive identification. In order to establish a kinetic sequence for the metabolites, we have slowed the MFO reaction at 0°C; however considerable activity remains at that temperature.

MFO activity has been studied in the sea catfish, Aries felis, collected from coastal waters in N-W Florida. The oxidase activity was induced with 3-MC injections and tissue/body mass ratios, hepatic microsomal protein components and in vitro BaP metablism were all studied. The effect of 3-MC was measured over a 5-40 mg 3-MC/kg body mass range for 7 days and at a 20 mg 3-MC/kg body mass dose level for 1, 3, 5, and 7 day periods. Liver/body and kidney/body mass ratios increased with increasing 3-MC dose levels and treatment period. Cytochrome P-450, epoxide hydratase and cytochrome b5 reductase all increased significantly with increasing doses of 3-MC up to 40 mg 3-MC/kg body mass and with increasing time periods. A linear response was obtained in the Ames test with S. typhimurium TA 98 up to 3-MC dose level for 20 mg. In the Ames test at a 20 mg 3-MC dose level, we obtained a linear response over a period of 7 days. The hepatic

microsomal fraction from the 3-MC induced sea catfish metabolized BaP to the usual metabolites including the 3-and 9- phenols, 4,5-, 7,8- and 9,10-dihydrodiols and quinones. Metabolite production was enhanced by butylated hydroxyanisole and inhibited by 6-aminochrysene, adenosine-2'-phosphate and 1,2-epoxy-3,3,3-trichloropropane.

VII. Significance to Biomedical Research and Program Needs of NCI and EPA

The following contributions to the needs of NCI and EPA were made during FY 82:

1. The responsiveness of a number of species to environmental carcinogens has been demonstrated. The sheepshead minnows have developed proliferative liver lesions (oval cell hyperplasia, possibly adenofibrosis) resulting from benzidine exposures and benign hepatic polyps related to aflatoxin exposure. A pre-neoplastic condition, also related to aflatoxin exposure, has been produced in Menidia livers. Finally, rainbow trout developed overt hepatocellular carcinoma following treatment with benzo(a)pyrene. All three species have high potential to be used as routine carcinogen assay organisms. Additionally, the black bullhead has been successfully used to demonstrate a cause and effect relationship to an existing pollution situation. Laboratory and field responsiveness have shown it to be a good indicator organism. Finally, the tiger salamander (Ambystoma sp.) is particularly interesting as an assay or indicator organism because of its cutaneous and liver enzyme responses to the PAH, perylene. The fact that this species has shown cutaneous tumor development in contaminated environments heightens its significance as a possible indicator species.

2. Results from the Field biomonitoring study (Report entitled: "A Prospective Study of Infectious and Non-infectious Diseases in Fishes and Shellfishes in Relationship to Pollutant Activity in Three Gulf Coasts Estuaries") indicate the following: First, a low tumor epizootic in fishes suggests low risks to environmental carcinogens at locations and times sampled. Therefore, future studies should be retrospective in regard to neoplasia as an indicator of carcinogen contaminants; i.e. studies should be based on an a priori indication of tumors in certain species at certain sites. Secondly, findings indicate that oysters may be more sensitive indicators of environmental contamination and especially may provide a more representative response to focal contamination in coastal regions. Future studies should include careful monitoring for tissue and physiologic changes indicative of chemical induced stress in oysters. Furthermore, several diseases of oysters and fish were detected during the study. At least two of these, a rickettsia found in oyster digestive gland cells and ichthyosporidosis from several fish species, are important in terms of possible impact related to effects on biota and human health. These diseases and their role in the Gulf coast environment should be investigated further. Finally, the overall study suggests that, while being far from pristine, the study area was not a severely unhealthy environment at the time of investigation. This study and others now provide a base line for

future comparison. Studies should be periodically made to monitor the impact of population and industrial growth along this valuable coastal region, a region predicted to be one of the fastest growing areas of the country during the next decade.

3. Biochemical and correlated structural responses of fish liver systems seem to be relatively similar to responses of mammals to certain carcinogens. Recent results show that the mixed function oxidases are induced in fish as well as the conjugating enzymes that aid in the detoxification and excretion of compounds such as B(a)P. This permits future comparative studies to determine if biochemical methods may be incorporated in early warning or sentinel monitoring projects with fish. The biochemical studies suggest that fish may serve as animal models in carcinogen (preneoplasia) studies to complement mammalian studies.

VIII. Proposed Course - Future Plans

1. The NCI/EPA collaborative project officially concludes October 1982 and no new projects will be undertaken. During FY83, efforts will be made toward concluding research projects already underway at Gulf Breeze and by investigators under cooperative agreements. Major emphasis will be directed to preparing final reports (due October 1983) and collating reprints produced as a result of the research period.

2. An in-house study, at Gulf Breeze, will be conducted on the effects of nitrosamines on fish species using test systems developed during the NCI/EPA research program. The study will focus on endpoints of tumor induction and possible other effects (histopathological, physiological, etc.).

IX. Date Contract Initiated and Period of Contract Planned

Initiated	Expiration Date
October 1978	September 30, 1982

X. Contractors Project Director

Dr. Henry F. Enos, Laboratory Director
Gulf Breeze, ERL, EPA

Project Officers for NCI or EPA

NCI:	Dr. Herman Kraybill
EPA/ORD:	Dr. Wayne Galbraith
Prinicipal Investigator:	Dr. John A. Couch, Gulf Breeze, ERL, EPA

XI. Cooperative Agreements Funded - Progress Reports FY 82

Title: Rainbow Trout: A Model for Environmental Carcinogenesis
Prinicipal Investigator: Jerry D. Hendricks
Cooperative Agreement Number: CR-809344-010
Project Officer: John Couch

Introduction

Work is continuing on the histopathological evaluation of tissues from fish exposed for 18 months to dietary benzo(a)pyrene (BAP), Aroclor 1260 and toxaphene. The data from the BAP dietary study and the BAP intraperitoneal injection study is complete and a manuscript is in the final stages of preparation.

Trout embryo exposures to methyl azoxymethanol acetate (MAMA) and benzidine dihydrochloride (BDHC) were conducted in December, 1981 and the fingerlings are now 8 months of age. An attempt to produce a rare neuroblastoma neoplasm in coho salmon fry was unsuccessful but will be conducted again during the next year.

Current Progress

Dietary exposure of trout fingerlings to 1000 ppm BAP for 18 months resulted in a 24% incidence of liver neoplasms at 18 months (table 2). Average body weight for these fish at 18 months was 364 g (table 1). Average conversion of dry diet to fish weight for rainbow trout is 1:1 indicating that the average fish would have consumed 364 g of dry diet and consumed 364 mg of BAP. In another experiment, we injected, IP, a group of 50 trout with 1 mg BAP in .4 ml of propylene glycol monthly for 12 months and held them an additional 6 months before killing and examination. The total dose in this case was only 12 mg/fish but the incidence of hepatic tumors was 50% (table 3). This may indicate that BAP is a more potent carcinogen to trout than dietary exposure demonstrates. It is possible that much of the highly water insoluble BAP is not absorbed from the trout gut, making the effective dose much lower than what was put in the diet.

Gross examination did not reveal any neoplasms in fish fed either Aroclor 1260 (500 ppm) or toxaphene (50 ppm) for 18 months, however, a histological examination of all tissues is being conducted.

The first objective of the new project was to determine the effects of two potential carcinogens on rainbow trout embryos as a continuing effort to assess the suitability of rainbow trout as indicators of environmental carcinogenesis. We are interested in determining the carcinogenicity of two rodent carcinogens, methylazoxymethanol acetate (MAMA) and benzidine dihydrochloride (BDHC), in rainbow trout embryos as well as the effects of prior exposure of trout embryos to the environmental contaminant, Aroclor 1254 (PCB), on the carcinogenicity of MAMA and BDHC.

The 3 year-old female brood fish were removed from the usual OMP brood ration and placed on our semipurified test diet containing 200 ppm Aroclor 1254 two months prior to spawning. Two months later these fish were each given two injections of commercially prepared salmon pituitaries and induced to spawn. Eggs from seven fish were available on the same spawning date and were pooled to provide the source of PCB-exposed embryos for subsequent embryo exposure. Eggs from the same number of OMP-control brood females hormonally induced to spawn on the same day were used for comparative control embryo exposures. Egg samples from both sources taken on the day of spawning, day of carcinogen exposure (day 21 of incubation) and periodically thereafter were prepared for PCB analysis to follow PCB depuration. On day 21 of incubation, 12 groups of 200 viable embryos from both the PCB and control groups were exposed for 24 hours to MAMA and BDHC at the 0.1, 1, 10, 100 and 1,000 ppm levels. Both MAMA and BDHC were lethal to trout embryos at the 1,000 ppm level for 24 hours.

Table 1

Body weight, liver weight/body weight ratios and mortalities, within groups of trout sampled at 6, 12, and 18-months of diet exposure to benzo(α)pyrene.

Sample time	Diet	Body weight (g)[a]	Mort	$\frac{\text{Liver weight}}{\text{Body weight}} \times 100$
	CD	50 ± 10 (23)	17	1.13 ± 0.13 (23)
6 months	CD + 1000 ppm BP	41 ± 10 (29)	11	1.20 ± 0.12 (29)
	CD	195 ± 49 (32)	8	0.73 ± 0.11 (32)
12 months	CD + 1000 ppm BP	158 ± 43 (33)	7	0.83 ± 0.15 (29)[b]
	CD	425 ± 153 (109)	11	0.66 ± 0.16 (109)
18 months	CD + 1000 ppm BP	364 ± 125 (111)	9	0.69 ± 0.17 (89)

[a]Data presented as mean ± SD (no. of fish used in determining mean represents pooling of duplicate tanks).

[b]n used in determining mean does not correspond to n used for computing mean body weight, i.e. data is not biased by including weights of livers with large tumors.

Table 2

Histological Incidences of liver tumors in trout sampled following 12 and 18 months of diet exposure to benzo(α)pyrene.

Sample time	Diet	Foci of basophilic cells[a]	Carcinomas[b]
12 months	CD	0/32 - 0%	0/32 - 0%
	CD + 1000 ppm BP	4/33 - 12%	1/33 - 3%
18 months	CD	0/109 - 0%	0/109 - 0%
	CD + 1000 ppm BP	5/111 - 4.5%	22/111 - 20%

[a]Includes fish having only basophilic foci.

[b]Data presented as no. of fish with at least one tumor/total no. of fish sampled.

Table 3

Histological incidences of tumors in trout sampled 6 months after 12 monthly intraperitoneal inoculations of benzo(α)pyrene (BP) in propylene glycol (PG).

Exp. group	Tumor type	Organ	Incidence[a]
PG controls	Carcinoma	Liver	1/27 - 4%
PG + BP	Carcinoma	Liver	13/28 - 46%
	Basophilic foci[b]	Liver	1/28 - 4%
	Fibrosarcoma	Liver	1/28 - 4%
	Papilloma	Swimbladder	1/28 - 4%

[a]Data presented as no. of fish with at least one tumor/total no. of fish.

[b]Includes fish having only basophilic foci.

The 100 ppm MAMA dose was also highly toxic, resulting in high mortalities. After hatching and swimup, 100 fry from the control and three highest tolerated dose levels, (0.1, 1, 10 ppm for MAMA and 1, 10, 100 ppm for BDHC) were kept and are being fed our semipurified control diet for the 12-18 month tumor development period. A 20 fish sample will be taken 9 months after treatment (September, 1982) to assess tumor development. If tumors are present the experiment will be terminated at 12 months. If not, another 20 fish will be killed at 12 months and a decision made at this time on whether to terminate the experiment or continue for another 6 months. A few fish survived the 100 ppm MAMA treatment and were kept to determine tumor development. These fish grew very poorly, average weight was only 3.3 g after 8 months, and mortalities were frequent so the remaining 15 fish (PCB and control groups combined) were killed. All fish, but one from the control group, had grossly observable liver tumors. This information gives a preliminary indication that MAMA is carcinogenic to trout. Dose response and the effects of PCB will be determined at later samples.

The second objective in our proposal was to reenact the conditions which apparently led to an inadvertent epizootic of neuroblastoma in juvenile coho salmon reared in chlorinated-dechlorinated river water, the intent being to reproduce this tumor with subsequent investigation of the causative factors. The experiment was begun as proposed and involved exposure of coho salmon and Shasta strain rainbow trout embryos and sac fry to chlorinated-dechlorinated (sodium thiosulfate) McKenzie River water brought into our laboratory. However, an unfortunate mechanical failure of our water recirculating pump resulted in complete loss of the coho salmon fry. The trout, having reached the swimup stage, had been moved elsewhere and were not in the closed system when the failure occurred. Consequently, the trout are being monitored for tumor development as proposed. The experiment using both coho salmon and rainbow trout will be repeated during the second year of this cooperative project.

Title: Teleost Carcinogen Assay Systems
Principal Investigator: B. J. Martin, University of Southern Mississippi
Cooperative Agreement Number: CR809347
Project Officer: John A. Couch

During the year we have conducted or have in progress over thirty (30) whole organism experiments exposing *C. variegatus* to benzidine dihydrochloride (BEN). These experiments represent our efforts to reproduce and more accurately characterize the proliferative liver lesions previously induced by long-term exposure of *C. variegatus* to BEN.

1) Benzidine Exposures, *C. variegatus*. To date, we have histologically examined the livers from 44 fish that have been exposed to 1 ppm BEN for at least 200 days and the lesion was evident in 5 individuals. In an experiment in which *C. variegatus* are being exposed to 5 ppm BEN, 4 fish have been examined histologically and the liver lesion was evident in 2 individuals. One of these was sacrificed after 62 days of exposure, and the other after 132 days. Seven fish have been examined from an experiment involving exposure to BEN at 10 ppm and one individual had the lesion. Also, some *C. variegatus* that survived 96-hour exposure at high concentrations to BEN during LD-50 experiments were maintained in the

laboratory and the liver lesions were evident in one of the 2 individuals examined histologically. Since a number of the experiments from which these data are derived are still in progress, we feel that we will be able to accomplish our goal of characterizing the lesion and more accurately determining its incidence and latency period by the end of the project period.

2) Benzidine Exposures, C. variegatus Embryos. Embryos were exposed to BEN at concentrations ranging from 5 to 500 ppm, and effects were observed at concentrations of 50 ppm or higher. To date, ca. 600 embryos have been exposed to BEN at 50 ppm or higher and only 15% developed normally beyond the hatching stage. In our 300 control embryos, 77% developed normally beyond the hatching stage and only 0.02% exhibited abnormalities; therefore, the detrimental effects of BEN at these concentrations are rather obvious.

Approximately 85% of the embryos exposed to BEN at 50 ppm or higher exhibited anomalies. These anomalies, in order of frequency of occurrence, are: 1) Tubed heart syndrome with distended pericardia, 2) Poor circulation, 3) Sparse distribution of melanophores around yolk, 4) Inability to hatch, 5) Abnormal head morphology, 6) Scoliosis, 7) Faint RBC pigmentation.

Efforts are in progress to examine these anomalies histologically, and experiments are planned to study the effects of BEN on the early post-hatching stage of development.

3) Aseptic Embryo Technique. Our main efforts have been to find a suitable food regime and environmental conditions to maintain healthy C. variegatus fry for an amount of time sufficient to study the effects of exposure to carcinogens in a sterile environment. The foods we have tried were ethylene oxide-treated Mono Lake Flakes, autoclaved Tetramin, autoclaved Artemia eggs, and chlorox-treated Artemia eggs. We also conducted experiments with reduced quantities of antibiotics and no antibiotics in an effort to determine the effects of antibiotics on mortality.

Chlorox-treated Artemia eggs seem to have provided the best food regime since more animals remained healthy when fed this diet. Also, fry consistently lived longer when maintined in an antibiotic-free sterile environment; however, as one might expect, the preparations without antibiotics are much more likely to become contaminated. Thus, it was possible to consistently maintain healthy embryos on chlorox-treated Artemia eggs in an antibiotic-free sterile environment for 60+ days.

Obvious problems associated with the use of Artemia are that they are another living organism in the system and, since as yet we have no method of excluding the non-viable Artemia from the system, the unhatched eggs and uneaten Artemia accumulate in the preparation. This problem can be somewhat ameliorated by underfeeding and more frequent water changes.

4) C. variegatus Cell Line Development. We have developed four new cell lines from C. variegatus: A large cell fibroblastic line (through passage 8), a

small cell fibroblastic line that forms cellular aggregates in culture (through passage 10), a small cell fibroblastic line that does not aggregate in culture (through passage 11), and a striated muscle cell line (through passage 12). The striated muscle cell line is particularly interesting since, to our knowledge, it is the first striated muscle cell line to be developed from fish tissue.

We plan to use all four of these cell lines in toxicity and carcinogenicity studies during the coming project year.

5) Primary Hepatocyte Cell Culture Technique. Because of our initial success in developing primary hepatocyte cell cultures from *Fundulus*, we felt the development of such cultures from *C. variegatus* would not be difficult. However, after a year of effort in this regard with very little success, we have decided to work again with *Fundulus*. Our recent emphasis has been the determination of media and environment conditions that will provide healthy and morphologically differentiated cells. Although the cells do not appear completely differentiated with respect to morphology, we are conducting tests to determine their state of biochemical differentiation. If these cells prove to be sufficiently differentiated to be considered "functional hepatocytes," we will begin to use them in carcinogen assays.

6) Immunological Studies. Agarose and polyacrylamide serum electrophoresis has disclosed differences in the banding patterns of BEN-exposed and non-exposed *Cyprinodon variegatus*. Densitometer scans of the agarose electrophoresis preparations indicate that both the number and size of the peaks from BEN-exposed fish differ from non-exposed individuals. PAGE (polyacrylamide gel electrophoresis) provides a much better resolution of the serum differences, and they are clearly visable with the naked eye. When the Bio-Rad silver stain is used, we can detect significant differences by using a 1:50 dilution of only 1μg of serum; thus, allowing the conservation of serum from each individual fish for additional tests.

By immunoelectrophoresis (Graber-Williams), we are able to demonstrate differences in precipitation arcs generated from exposed and non-exposed fish. This technique is not quantitative, but it demonstrates that our antiserum is sufficiently potent for use in crossed and tandem crossed rocket procedures.

A competitive enzyme immunoassay has been developed and employed successfully with non-fish antibody. We are presently miniaturizing the procedure so that it can be used with the μl quantities of blood available from *C. variegatus*.

A bacteriophage neutralization procedure has been adapted for use in the study of the ability of *C. variegatus* to generate neutralizing factors. Results show a consistently higher level of neutralization in BEN-exposed fish (fish are immunized with MS-2 phage).

Title: Causes of Papillomas on Fish Exposed to Chlorinated Sewage Effluent
Principal Investigator: John M. Grizzle
Cooperative Agreement Number: CR809336010
Project Officer: William Davis

Laboratory and field experiments are in progress to determine the cause of oral papillomas on black bullheads in the final oxidation pond of the Tuskegee, Alabama, sewage treatment plant. Wild, adult black bullheads in the final oxidation pond had a 73% prevalence of oral papillomas between December 1979 and July 1980. A gill-net sample of black bullheads from this pond on 28 May 1982 had an oral papilloma prevalence of 57%. The occurrence of tumors in this recent sample indicated a continuation of the original, tumor inducing conditions.

Exposure of fish to water in the final oxidation pond. Black bullheads (*Ictalurus melas*), brown bullheads (*Ictalurus nebulosus*), yellow bullheads (*Ictalurus natalis*), and channel catfish (*Ictalurus punctatus*) were placed into cages in the final oxidation pond of the Tuskegee, Alabama, sewage treatment plant. Control fish were kept in cages in an earthen pond at the Auburn University Agricultural Experiment Station. The approximately 1-m^3 cages were allowed to rest on the pond bottom (sinking cages) or suspended off of the bottom by floats (floating cages). Cages were placed at three locations in the final oxidation pond: near the inlet (inlet A), approximately 60 meters from the inlet (inlet B), or near the pond outlet (outlet). Most cages were stocked with 50 fish. Table 1 summarized all cage exposures including the percentage of fish with grossly visible, oral mucosa hyperplasis or papilloma-like lesions.

The oral lesions on fish confined to cages in the final oxidation pond were in the same location occupied by oral papillomas in wild black bullheads from this pond. The hyperplasic lesions were most prevalent during the spring and regressed during the summer. Black bullheads had a higher prevalence of oral musoca hyperplasia than the other species. Lesion prevalence was higher in black bullheads stocked during FY 81 than in those stocked the following year after similar lengths of exposure. Incidence of hyperplastic lesions was also higher in black bullheads near the outlet than in those near the inlet, but low fish survival near the inlet could have influenced this result. No difference was found between fish in floating and sinking cages.

During their second year of exposure to the final oxidation pond, some of the black bullheads developed oral lesions that grossly resembled papillomas. These lesions were in the same locations in the mouth fornix as the hyperplastic lesions developing during the first year of exposure. The appearance of the lesions that developed during the second year is described in a later section of this report.

Lesions on caged fish. Lesions in both fornices of the mouth were examined from a 175-mm total length black bullhead confined for 537 days to a sinking cage near the outlet of the final oxidation pond. Grossly, these lesions appeared similar to papillomas on wild black bullheads in this pond and to the lesions on other caged black bullheads with a similar length of exposure. The surface dimensions of the lesions were approximately 8 mm by 5 mm, and each

Table 1. Summary of exposures of caged fish to the Tuskegee, Alabama, final oxidation pond.

Species	Stocking Dates	Days of exposure (as of 14 Jun 82)	Maximum percentage with oral lesions	Maximum percentage with papilloma-like lesions	Maximum percent of control fish with oral lesions
Black bullheads	21 Oct 80	603	95	80	4
	23 Feb 81	476	50	50	4
	29 Oct 81	226	18	0	0
	9 Apr 82	66	0	0	0
Yellow bullheads	12 May 81	398	0	0	14
Brown bullheads	29 Oct 81	228	0	0	0
Channel catfish	16 Jan 81	514	25	0	8.3
	29 Oct 81	231	0	0	0

lesion was raised approximately 1.5 mm above the surrounding oral mucosa. In paraffin sections of the lesion, the maximum thickness of the connective tissue between the epidermis and mucosa was 1.5 mm compared to a normal thickness of approximately 0.2 mm. The maximum thickness of the mucosal portion of the sectioned lesion was 1.2 mm compared to a normal thickness of approximately 0.2 mm. The connective tissue component of the lesion extended from the hyperemic submucosa into the epithelial portion of the lesion, but the connective and epithelial tissues was less intermingled than in larger oral papillomas of wild bullheads. These lesions were different than the hyperplasic lesions appearing soon after exposure because these papilloma-like lesions were larger, there was more folding of the lesion surface, and the connective tissue was more extensive and intermingled with the epithelial tissue than in the hyperplastic lesions.

Ultrastructure of papillomas in black bullheads. The oral papilloma of wild black bullheads from the final oxidation pond has epithelial cells resembling normal oral mucosa cells and a central connective tissue column that was continuous with the submucosa. Near the tumor surface there were mucous cells, and plasma membranes of the epithelial cells adhered closely with those of adjacent cells. Desmosomes occurred less frequently between the cells than in normal mucosa. Mitochondria, endoplasmic reticulum, and ribosomes were concentrated in the peripheral cytoplasm of the epithlial cells. These surface cells often contained glycogen particles among the tonofilaments. The mid to near basal portion of the papilloma consisted of stellate epithelial cells having more tonofilaments, fewer organelles in the cytoplasm, and more intercellular space than epithelial cells near the surface. Desmosomes occurred more frequently between the cells in the basal area than near the surface. Alarm substances cells occurred in the mid portion of the epithelium and were surrounded by stellate cells. Numerous lymphocytes were present in the basal region of the tumor. The basal epithelial cells contained numerous mitochondria, endoplasmic reticulum, and ribosomes in the cytoplasm. Plasma membrances adhered to the adjacent cells by desmosomes. Intercellular space occurred less frequently in the basal region than in the middle portion of the tumor.

Mutagenicity of the final oxidation pond water. Extracts of the pond water were tested for mutagenicity with the Ames test (Ames et al., 1975, Mutat. Res. 31:347). An acid fraction was prepared by acidifying the water to pH 1.0, extracting three times with 200 ml of 25% ether and 75% hexane per liter of water, and then distilling to remove the bulk of the solvents. The last 20 ml were evaporated under a stream of nitrogen. A basic fraction was prepared in the same manner except that the water was adjusted to pH 12-13 before extraction. Both fractions were tested with and without S-9 (Aroclor-induced rat liver enzymes). Benzo(a)pyrene was used as a positive control mutagen and was tested with and without S-9.

The acid fraction was mutagenic to *Salmonella typhimurium* tester strains TA98 and TA100 if S-9 was present, and there was a positive dose response (Fig. 1 and 2). The mutagenicity of the acid fraction was higher on some dates than on others (Fig.3) and may be seasonal. The mutagenicity of the water at the inlet and outlet was compared on two dates: there was no difference in February 1982, but mutagenicity was higher at the inlet in March 1982 (Fig.

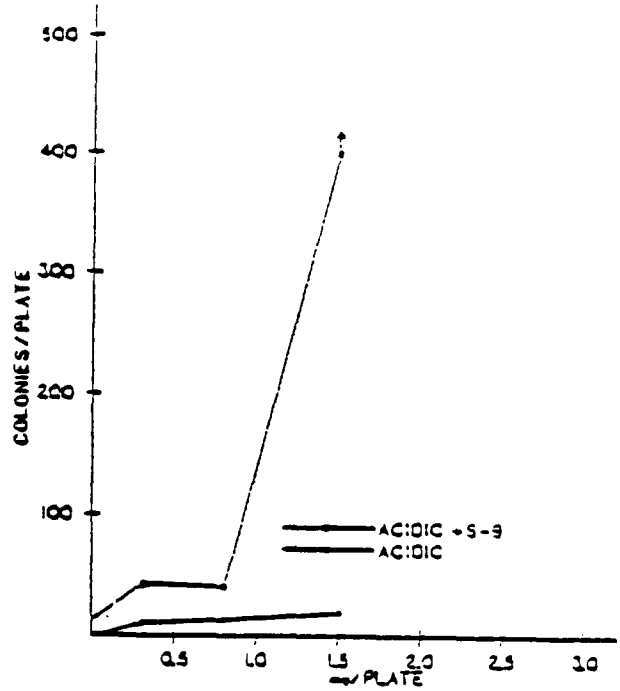


Figure 1. Response of Salmonella typhimurium strain TA 98 in the Ames test to an acid organic solvent extract of the final oxidation pond water. The water was collected at the pond outlet on 15 July 1981.

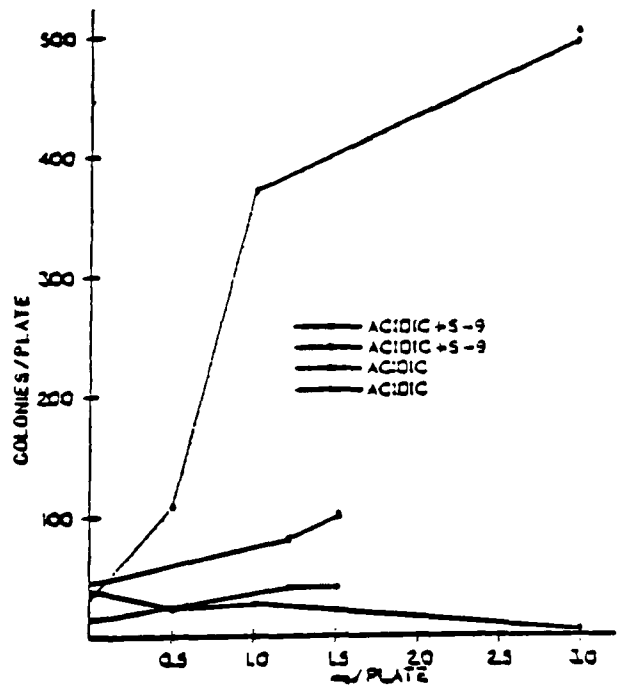
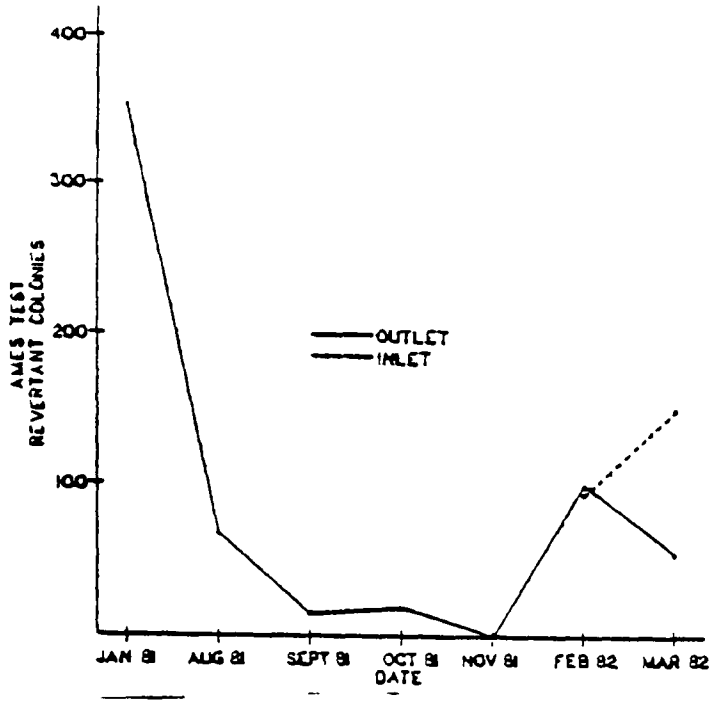
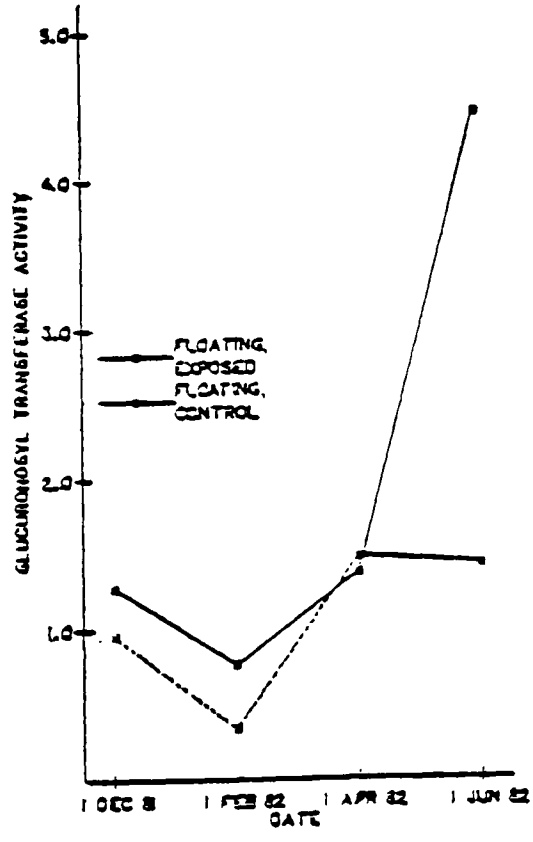


Figure 2. Response of Salmonella typhimurium strain TA 100 in the Ames test to an acid organic solvent extract of the final oxidation pond water. The water was collected at the pond outlet on 29 June 1981. The results for two trials are given on this graph.



Response of Salmonella typhimurium strain TA 100 in the Ames test to an acid organic solvent extract of the final oxidation pond water. The number of revertant colonies is expressed as the number of colonies on medium with the extract and S-9 minus the number on medium with extract only.



4. Glucuronosyl transferase activity in liver microsomes of channel catfish confined to cages in the final oxidation pond or a control pond.

3). The number of revertant colonies on plates with acid fraction but without S-9 was similar to the number of colonies on negative control plates (DMSO or S-9 only); therefore, the mutagenicity of the samples in Fig. 3 was expressed as the difference in the number of revertant colonies on plates with and without S-9. The basic fraction was not mutagenic when tested with Salmonella typhimurium strains TA98 and TA100.

Hepatic enzyme induction. UDP-glucuronosyl transferase and sulfotransferase activities in livers of black bullheads, brown bullheads, and channel catfish from cages in the final oxidation pond were measured and compared to control fish. Dr. D. R. Strength and colleagues determined the enzyme activity of these fish as part of a separate EPA cooperative agreement. Glucuronosyl transferase activity in exposed fish was generally higher than in control fish with channel catfish having the greatest induction response (Figs. 4-6). The difference between sulfotransferase activity in exposed and control fish was not consistent. The induction of glucuronosyl transferase in fish confined to the final oxidation pond indicated exposure to a toxicant. Similar induction occurs in rats and fish given oral or intraperitoneal doses of carcinogens.

Analysis of water from the sewage pond. Between September 1981 and May 1982, water leaving the final oxidation pond had 0.1 to 1.0 mg/l ammonia-nitrogen (average 0.55 mg/l) and a pH between 5.1 and 7.0. During this same period, total residual chlorine of water leaving the chlorine contact chamber before entering the final oxidation pond was 0.2 to 1.0 mg/l (average 0.53 mg/l). The residual chlorine concentration in the effluent has been lower during the experimental studies in this pond than during 1979 when the papillomas were first found in the wild black bullheads.

Water samples from the inlet to the final oxidation pond were collected on 22 January 1982 and 3 March 1982. The Environmental Health Administration Laboratory, Montgomery, Alabama, examined the samples with gas chromatography. Total volatiles, herbicides, pesticides, and PCB's were not detectable.

Transmission of papillomas by cell-free tumor homogenate. Two attempts were made to transmit papillomas by injecting homogenized, filtered papillomas into the mouth fornix of healthy black bullheads. The first group of fish was adults and was observed for 14 months after injection. The second attempt was with one-year-old fish that were observed for 11 months after injection. No signs of papillomas or hyperplastic lesions were found in any of the injected fish.

Embryo Exposures. Brown bullhead embryos were exposed to aflatoxin B_1 (0.5 or 1.0 mg/ℓ) or a concentrate of the final oxidation pond water. Controls were exposed to uncontaminated pond water or to the solvents used in the chemical exposures (dimethylsulfoxide and ethanol). Groups of 200 embryos were exposed to each treatment for one hour on the fourth day after fertilization. Embryos were kept in a closed, recirculating system and after hatching, were transferred to 3-meter diameter plastic pools. Fish were sampled after 3 and 6 months, and all remaining fish were fixed after 8 months. These fish are currently being examined. No evidence of tumor induction has been found.

3). The number of revertant colonies on plates with acid fraction but without S-9 was similar to the number of colonies on negative control plates (DMSO or S-9 only); therefore, the mutagenicity of the samples in Fig. 3 was expressed as the difference in the number of revertant colonies on plates with and without S-9. The basic fraction was not mutagenic when tested with Salmonella typhimurium strains TA98 and TA100.

Hepatic enzyme induction. UDP-glucuronsyl transferase and sulfotransferase activities in livers of black bullheads, brown bullheads, and channel catfish from cages in the final oxidation pond were measured and compared to control fish. Dr. D. R. Strength and colleagues determined the enzyme activity of these fish as part of a separate EPA cooperative agreement. Glucuronosyl transferase activity in exposed fish was generally higher than in control fish with channel catfish having the greatest induction response (Fig. 4-6). The difference between sulfotransferase activity in exposed and control fish was not consistent. The induction of glucuronosyl transferase in fish confined to the final oxidation pond indicated exposure to a toxicant. Similar induction occurs in rats and fish given oral or intraperitoneal doses of carcinogens.

Analysis of water from the sewage pond. Between September 1981 and May 1982, water leaving the final oxidation pond had 0.1 to 1.0 mg/l ammonia-nitrogen (average 0.55 mg/l) and a pH between 5.1 and 7.0. During this same period, total residual chlorine of water leaving the chlorine contact chamber before entering the final oxidation pond was 0.2 to 1.0 mg/l (average 0.53 mg/l). The residual chlorine concentration in the effluent has been lower during the experimental studies in this pond than during 1979 when the papillomas were first found in the wild black bullheads.

Water samples from the inlet to the final oxidation pond were collected on 22 January 1982 and 3 March 1982. The Environmental Health Administration Laboratory, Montgomery, Alabama, examined the samples with gas chromatography. Total volatiles, herbicides, pesticides, and PCB's were not detectable.

Transmission of papilloms by cell-free tumor homogenate. Two attempts were made to transmit papillomas by injecting homogenized, filtered papillomas into the mouth fornix of healthy black bullheads. The first group of fish was adults and was observed for 14 months after injection. The second attempt was with one-year-old fish that were observed for 11 months after injection. No signs of papillomas or hyperplastic lesions were found in any of the injected fish.

Embryo Exposures. Brown bullhead embryos were exposed to aflatoxin B_1 (0.5 or 1.0 mg/l) or a concentrate of the final oxidation pond water. Controls were exposed to uncontaminated pond water or to the solvents used in the chemical exposures (dimethylsulfoxide and ethanol). Groups of 200 embryos were exposed to each treatment for one hour on the fourth day after fertilization. Embryos were kept in a closed, recirculating system and after hatching, were transferred to 3-meter diameter, plastic pools. Fish were sampled after 3 and 6 months and all remaining fish were fixed after 8 months. These fish are currently being examined. No evidence of tumor induction has been found.

Laboratory exposures of black bullheads to sediment extracts. One-year-old black bullheads were acclimated to static-water, 40-liter aquaria, one fish per aquarium. Sediment from the final oxidation pond was collected near the inlet on 14 June 1982. Samples were extracted for organic compounds with methylene chloride and acetone according to the EPA protocol for extraction of priority pollutants from sediments.

Two types of skin exposure to the sediment extract will be used: subcutaneous injection and topical application. The sediment extract was injected on 30 July 1982, and the fish will be observed for at least one year. The topical applications will begin during August 1982.

Title: Perylene as a Possible Environmental Carcinogen: Hydrocarbon Metabolism and Mutagenesis in *Ambystoma tigrinum*
Principal Investigator: Robert S. Anderson and Francis L. Rose
Coopertive Agreement Number: CR807740
Project Officer: John A. Couch

The unusually high prevalence of various forms of dermal neoplasia in a population of tiger salamanders (*A. tigrinum*) inhabiting a sewage lagoon whose water and sediment are unusually rich in perylene has prompted this study of the possible role that this polycyclic aromatic hydrocarbon (PAH) might have in the etiology of these lesions. The work is divided into two portions: 1) the histopathological effects of perylene (P) as a carcinogen or cocarcinogen on *A. tigrinum*, under the direction of Dr. F. Rose and 2) the metabolic activation and/or detoxification of P, and a related environmental carcinogen benzo(a)pyrene (BaP), by tissue enzymes of *A. tigrinum*, under the direction of Dr. R. Anderson. The progress of this effort through the first two years is summarized in this report.

Methodological developments. A radioisotopic method used to measure BaP metabolism in mammals was adapted for use with *A. tigrinum* to quantify BaP and P metabolism. Basically, the parent compounds are converted to metabolites and conjugates which can be extracted based on their solubility in water and/or NaOH. This technique is useful to obtain an indication of the total rate of metabolism of these substrates. Once the *in vitro* conditions of reaction had been optimized this reaction procedure was used to obtain metabolite extracts in ethyl acetate which were used for HPLC separation of metabolites on a Perkin-Elmer HC ODS/SilX reverse-phase column. Metabolites of ^{3}H-P or ^{14}C-BaP were eluted in a 60-80% MeOH-H_2O gradient, the eluted samples were collected for radioactivity determinations and UV-absorbance was monitored continuously. A complete set of BaP authentic standards were used to identify by coelution all of the BaP metabolites; unfortunately a set of P metabolite references is not currently available.

Hepatic metabolism of ^{14}C-BaP and ^{3}H-P in normal and induced *A. tigrinum*. Liver homogenates from tiger salamanders mediate the *in vitro* metabolism of both BaP and P. We studied the effect of two known aryl hydrocarbon hydroxylase (AHH) inducers: aroclor 1254 (PCB) and methylcholanthrene (MC), and P on the metabolism of BaP and P. Both the metabolites in the aqueous phase (conjugates and macromolecule-bound species) and the metabolites in the NaOH phase (certain dihydrodiols and the phenolic derivatives) were quantified; their sum allowed us to estimate the total nmoles/ml liver protein/30 min produced under the various conditions.

Induction procedures used were those found effective for standard laboratory rodents: 25 mg/kg MC ip, 48 hr; 500 mg/kg PCB ip, 96 hr; 60 mg/kg P ip, 48 hr (this dosage was based on that commonly used for its isomer BaP); olive oil was the vehicle in all cases.

Table 1 summarizes the data on ^{14}C-BaP metabolism. Uninduced animals produced about o.2 total ^{14}C-BaP metabolites/mg protein/30 min; the alkali-soluble metabolites were produced in quantities more than twice that of aqueous phase metabolites. MC was the only chemical tried that produced induction, this induction would have been highly statistically significant if the variation in the induced data had been somewhat reduced by having a larger sample size. Interestingly in the MC-induced animals aqueous phase > NaOH phase metabolites, an effect also seen in the PCB-treated salamanders, although PCB injection at this level did not produce an over all AHH induction. Perylene injection had no significant effect on metabolite partitioning and was not an effective AHH inducer. ^{14}C-BaP metabolism by Mutazyme™ (a commercial preparation of aroclor induced rat hepatic microsomes designed for the Ames bacterial mutagenesis assay) was measured for reasons of comparison. Its activity per mg protein was 100-fold greater than the uninduced salamander with aqueous phase = NaOH phase metabolites.

Table 1. HEPATIC ^{14}C-BaP METABOLISM*

Ambystoma Inducer	Metabolites NaOH	Aqueous phase	Total
None	0.131 ± 0.148	0.059 ± 0.045	0.190 ± 0.130 (6)
Aroclor	0.055 ± 0.052	0.089 ± 0.038	0.144 ± 0.072 (6)
MC	0.228 ± 0.230	0.318 ± 0.339	0.546 ± 0.562 (4) ($p<0.2$)
Perylene	0.118 ± 0.117	0.056 ± 0.016	0.174 ± 0.116 (3)
Rat			
Aroclor	10.104 ± 1.098	8.044 ± 1.925	18.148 ± 3.021 (3)

*Nmoles/mg protein/30 min: X ± SD (n).

Table 2. HEPATIC ^{3}H-PERYLENE METABOLISM*

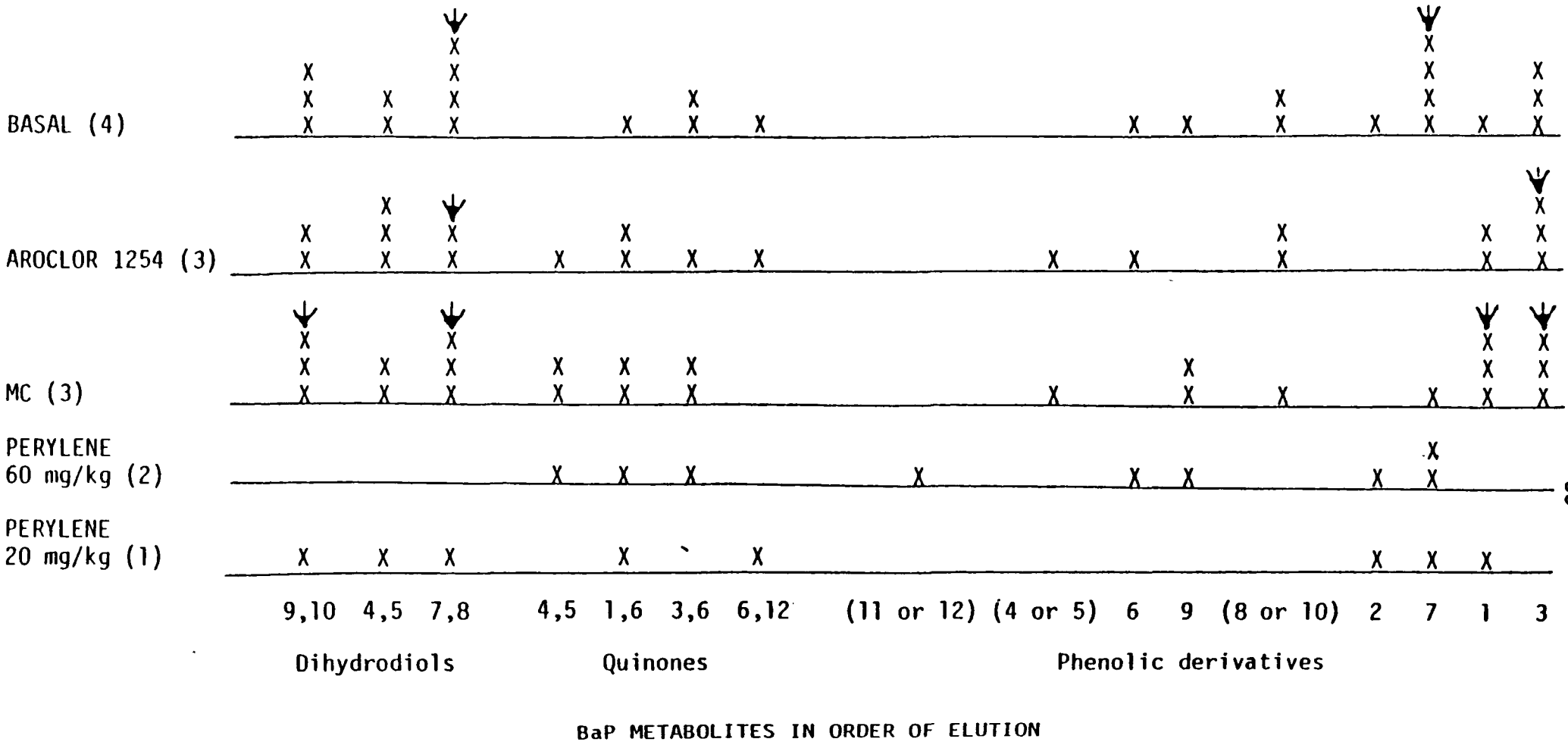
Ambystoma Inducer	NaOH	Metabolites Aqueous phase	Total
None	0.005 ± 0.010	1.302 ± 0.528	1.307 ± 0.529 (6)
Aroclor	0.192 ± 0.289	1.305 ± 0.873	1.498 ± 0.733 (5)
MC	0.144 ± 0.184	1.365 ± 0.527	1.509 ± 0.641 (6)
Perylene	0.000 ± 0.000	2.477 ± 0.288	2.477 ± 0.288 (3) ($p<0.01$)
Rat			
Aroclor	16.425 ± 1.280	56.169 ± 19.014	72.593 ± 18.483 (3)

*Nmoles/mg protein/30 min; X ± SD (n).

When assays using the same protocol as those for ^{14}C-BaP metabolism were run using ^{3}H-P as substrate an estimate of perylene metabolism was obtained (Table 2). In both induced and uninduced salamanders the majority of P metabolites were extracted in the aqueous phase. Perylene was more extensively metabolized than BaP, ~9-fold more for uninduced A. tigrinum and ~4-fold more for PCB-induced rat. Although neither PCB nor MC treatment caused significant changes in overall 3H-P metabolism, both treatments produced a significant increase in the production of NaOH-soluble metabolites. Perylene, while not a BAP-OHase inducer (Table 1), did significantly increase the rate of metabolims of ^{3}H-P in hepatic homogenates. This inductive event was accomplished by a marked increase in the production of aqueous-phase metabolites. As was the case for BaP-OHase, the specific activity of A. tigrinum P-metabolizing enzymes was much less (~70-fold) than those of the rat.

Effects of inducer on ^{14}C-BaP metabolite profiles by HPLC. In Table 3 the specific BaP metabolites produced by uninduced and induced salamanders are listed. Metabolites marked with an "X" were produced during the 30-min in vitro incubation, the number of "Xs" for a given metabolite indicates the numbers of animals in a given group that produced that metabolite. Metabolites that were not only produced by the majority of animals within a group, but were also quantitatively the major metabolite(s) of their class (diols, quinones, phenols) for that group of animls, are marked with an arrow. Basal animals produced many readily identified BaP dihydrodiols, quinones, and phenolic derivatives. The major diol is the 7,8-diol, which can serve as a precursor of the ultimate carcinogenic BaP metabolite, the 9,10-epoxide of the 7,8-dihydrodiol. In uninduced A. tigrinum the major monohydroxylated metabolite appears to be 7-OH-BaP, in contrast to mammals which produce mainly 3-OH and 9-OH BaP. In PCB- and MC-induced salamanders 7,8-diol is also a major

^{14}C-BaP METABOLITES PRODUCED BY AMBYSTOMA LIVER: RESPONSE TO AHH INDUCERS



diol, as is 9,10-diol in the case of MC induction. The pattern of BaP metabolism is shifted toward 3-OH-BaP production by PCB and MC, and 1-OH-BaP in MC-induced animals. Attempts to induce with 20 mg P/kg produced no significant alteration from the normal metabolite profile, but treatment with 60 mg P/kg caused an apparent shift away from dihydrodiol production.

Table 4. EFFECTS OF AHH INDUCERS ON AMBYSTOMA ^{14}C-BaP METABOLITE PROFILES (HPLC)

	Basal (4)	Aroclor 1254 (3)	MC (3)	Perylene 60 mg/kg (2)	Perylene 20 mg/k
Dihydrodiols	54.8 ± 13.5	52.3 ± 6.0	40.3 ± 4.0 ($p<0.2$)	0	31.0
Quinones	14.8 ± 14.2	11.3 ± 6.4	11.7 ± 5.0	20.5 ± 10.6	12.0
HO-BaPs	30.4 ± 10.9	36.4 ± 4.7	48.0 ± 3.6 ($p<0.1$)	79.5 ± 10.6 ($p<0.025$)	57.0

*X% total ^{14}C-BaP metabolites in each category ± SD (n), peak identity confirmed by coelution with authentic metabolites standards.

Another way to look at the effect of putative AHH inducers on ^{14}C-BaP metabolite profiles is presented in Table 4. In this table the actual amount of metabolites produced from each major class is presented as the % of total metabolites in each category. For example, in basal animals approximately 55% of the total BaP metabolites produced are dihydrodiols, 15% are quinones, and 30% are phenolic derivatives. Of the three inducers tested, only MC produced significant overall BP-Phase induction (Table 1); however, both MC and P cause alterations in BaP metabolite profiles (Table 4). Aroclor 1254 had no effect on relative composition of the metabolite categories. MC-treatment tended to cause a shift from diol production to phenol production. This effect was much more pronounced in the case of 60 mg P/kg, where virtually no dihydrodiols were generated and ~80% of the metabolites were phenols. The distribution of metabolites started to revert to normalcy when the dose was reduced to 20 mg P/kg. It would appear that P treatment might have a protective effect in the case of subsequent BaP exposure because it shifts A. tigrinum metabolism away from the production of potentially carcinogenic epoxides and diol-epoxides toward generation of comparatively inactive phenol derivatives. AHH induction in higher animals also is frequently reported to be protective.

Mutagenicity of BaP, P, and aromatic amines in the Ames bacterial system. We have been able to identify and quantify numerous BaP metabolites produced by A. tigrinum enzymes, and quantify P metabolism in the same species (although the exact identity of the P metabolites is unknown). However, the biological activity of the metabolites produced by salamanders has not been determined using conventional testing systems. Therefore, we tested the ability of A. tigrinum hepatic enzymes to activate BaP, P, and some carcinogenic aromatic

amines using the Ames Salmonella typhimurium tester strains. The results are given in Table 5.

Microsomal enzyme preparations from the livers of Aroclor-induced salamanders mediated the production of mutagenic aromatic amine metabolites. These preparations were generally less active, on the basis of protein concentration, than comparable rat S9 preparations, with the exception of 2-aminofluorene and 4-aminostilbene. Therefore, higher substrate concentrations were usually required to get significant mutagenesis with Ambystoma S9; in no case were the concentrations used highly toxic or directly mutagenic to the bacterial tester strains. All data presented in Table 5 have been corrected for this background, as well as for spontaneous revertants. After the appropriate corrections, it was seen that salamander mixed-function oxygenase (MFO) activated 2-aminoanthracene, 2-aminofluorene, 2-acetylaminofluorene, 4-amino-trans-stilbene, and mono- and diacetylbenzidine; however, benzidine and 4-aminobiphenyl were poorly mutagenic in this system. As was expected, the frameshift mutagen detector strains TA 1538 and TA 98 (TA 1538/pK M101) were most sensitive to the activated aromatic amines. Strain TA 100 (TA 1535/pK M101) was also useful because it detects frameshift, as well as base-pair substitution mutagens.

The generation of mutagenic aromatic amine metabolites by Ambystoma microsomes was totally inhibited by prior heating of the S9 to 56° C for 30 min. The reaction did not proceed in the absence of the NADPH-generating system usually included in the S9 mix.

Rat microsomal enzyme induction with phenobarbital produced a marked increase in the mutagenesis by benzidine, N-acetylbenzidine, and N,N-diacetylbenzidine. However, phenobarbital was not a more effective inducer than Aroclor in the activation of these substrates, or other aromatic amines, by Ambystoma enzymes.

In this study there was no evidence that either BaP or perylene were mutagenic after reaction with Ambystoma liver microsomes. Strains TA 98 and TA 100 were mutated by BaP with Mutazyme(R), as had already been reported. Another frameshift detector, TA 1537, was most useful in perylene activation studies using rat S9. Although data from only 1 concentration of BaP or perylene + Ambystoma S9 are given in Table 1, concentrations ranging from 5-250 µg/plate were tried, without any sign of mutagenesis. BaP was neither toxic nor directly mutagenic for TA 98 or TA 1537 over this range; it was slightly toxic over 100 µg/plate for TA 100. Ambystoma enzymes produce no BaP activation as measured by TA 98, TA 100 or TA 1537. Likewise perylene concentrations of 5-250 µg/plate were tested against TA 1537, the only tester strain to give a good response to rat S9-activated perylene. No perylene concentration tested was directly toxic or mutagenic; reaction with Ambystoma microsomes had no effect.

In summary, a number of carcinogenic aromatic amines when activated by liver microsomes from a salamander, Ambystoma tigrinum, are mutagenic for Salmonella tester strains sensitive to frameshift mutagens. However, 2 polycyclic aromatic hydrocarbons (PAH) (BaP and perylene) that are rendered mutagenic by mammalian microsomes are not activated by Ambystoma mixed-function

TABLE 5

COMPARATIVE ACTIVATION OF CARCINOGENS

Compounds[a]	μg	S9[b]	*his*⁻ revertants/plate[c]		
Aromatic Amines			TA 1538	TA 98	TA 100
2-Aminoanthracene (PC)	25	A	333	614	271
	5	M	686	TNTC[d]	TNTC
2-Aminofluorene (PC)	10	A	781	519	45
	10	M	600	764	139
2-Acetylaminofluorene (PC)	100	A	600	332	33
	5	M	298	343	6
4-Aminobiphenyl (PC)	500	A	10	33	31
	100	M	445	661	918
4-Amino-*trans*-stilbene (PC)	50	A	112	164	176
	50	M	300	396	456
Benzidine (PC)	500	A	10	23	39
	500	A (P)	18	19	46
	500	M	82	78	34
	500	M (P)	125	184	103
N-Acetylbenzidine (NT)	200	A	250	507	133
	10	A	345	359	76
	10	M (P)	TNTC	TNTC	518
N,N'-Diacetylbenzidine (NT)	200	A	58	51	11
	200	A (P)	43	49	8
	50	M	468	512	214
	50	M (P)	780	TNTC	527
Polycyclic aromatic hydrocarbons	μg	S9[b]	TA 1537	TA 98	TA 100
Benzo[*a*]pyrene (PC)	10	A	0	0	0
	5	M	96	382	310
Perylene (NT)	250	A	0	0	0
	5	M	113	28	28

[a] PC = procarcinogen, NT = not adequately tested for carcinogenicity.

[b] S9 sources: A = *Ambystoma tigrinum*, Aroclor-induced; A (P) *Ambystoma tigrinum*, phenobarbital-induced; M = Mutazyme®, Aroclor-induced rat; M (P) = rat, phenobarbital-induced (custom-made by Meloy Laboratories). 500 μl of S9 were used in all plates.

[c] Number of revertants minus background, including direct toxic or mutagenic effects and spontaneous revertants. All *Ambystoma* values represent the average of at least 6 determinations on 3 different animals; Mutazyme® data are the average of at least 6 runs.

[d] TNTC = too numerous to count (>1000 revertants/plate).

xidases. These results tend to support the observation that amphibians are uite resistant to PAH carcinogenesis and suggest that aromatic amines may be ore appropriate model carcinogens; possibly because amphibian mixed-function xidases carry out N-oxidation more efficiently than C-oxidation.

From the point of view of environmental carcinogenesis in relation to mbystoma, it is of interest that the recognized carcinogen benzidine can be ound in water supplies. In addition to benzidine and its salts, -aminobiphenyl and 2-acetylaminofluorene are included in a list of arcinogenic substances for which regulatory standards were promulgated by the ccupational Safety and Health Administration.

Histopathological Response of Ambystoma to Perylene:

Determination of maximum tolerated dose. Three groups of 10 neotenes each ere injected ip with 10 mg, 50 mg, or 100 mg P/0.5 olive oil. No lethality or ther harmful effects noted after 60 days. Autopsy showed much residual P in eritoneal cavity, especially in higher dose groups; P frequently triggered ranulomatous reactions.

Injection of perylene. A control group of 19 neotenes each received 2 ml olive oil ip; 19 experimental were injected ip with 100 mg P/2 ml olive oil. Animals in both groups were maintained for about 3 months. Only 2 of the controls died during this period; one died almost immediately (probably of causes unrelated to the study), the other died at about 2 months. None of the control animals had tumors of any kind. Thirteen of the experimental group died during the course of the study. Of 11 perylene-treated animals sent to Dr. John Harshbarger for study, two had epidermal papillomas and one had an unusual hepatocellular carcinoma (differential diagnosis: cholangio carcinoma). This is the first unequivocal liver cell neoplasm reported in salamanders. A commonly observed reactive lesion characteristic of the experimental group was diagnosed as a lipid granuloma arising in response to the olive oil vehicle, although they were uncommon in control animals which received olive oil alone. These lesions were composed of mixed populations of cells in which foamy histiocytes and collagen-producing fibrocytes predominated. These granulomas were often found on the surface of the lungs, liver, and kidneys.

Another major study of the effects of P, and P and BaP in combination, is currently underway; results are not yet available because of the length of time required in the carcinogen-testing protocols. Attempts to transplant cutaneous melanomas are also in progress.

Title: Carcinogens and Neoplasia in Indigenous Populations of Aquatic Organisms
Principal Investigator: Michael C. Mix
Cooperative Agreement Number: CR808000-01-0
Project Officer: John A. Couch

Major research activities during the past year were directed toward: (1) measuring baseline levels of arsenic (As), cadmium (Cd) and nickel (Ni) in indigenous populations of bivalve molluscs; (2) developing methods for measuring trace levels of polynuclear aromatic hydrocarbons (PNAH) in seawater; (3) developing a crustacean (barnacle) bioassay system that could be utilized for testing environmental levels of carcinogens and/or mutagens; (4) determining whether or not viruses are associated with the neoplastic disorders of *Mytilus edulis* from Yaquina Bay; and (5) evaluating data, gathered during the past five years, on the proliferative disorders in *M. edulis*. Results from studies in each of these areas is summarized below.

Baseline Levels of Inorganic Carcinogens (As, Cd, Ni) in Shellfish

Freshwater mussels (*Margaritifera margaritifera*) and bay mussels (*Mytilus edulis*) were examined monthly or bimonthly during a one-year period and tissue concentrations of As, Cd, and Ni were determined by either flame atomic absorption or neutron activation analysis. For *M. margaritifera*, the concentration ranges of metal (µg/g, dry weight) were: Cd, 0.9-6.2; and Ni, 1.6-19.4. These levels are similar to those reported in organisms from non-industrial streams and less than, or in the lower range of, those from industrialized rivers. For *M. edulis*, the concentrations of As ranged from 9-15 µg/g (dry weight), Ni, 1.0-6.8 µg/g and Cd, 7.0-12.5 µg/g. Both species were judged to be excellent monitors for evaluating levels of these important metals in freshwater (*M. margaritifera*) and marine (*M. edulis*) environments.

Measurement of PNAH in Seawater

The U.S. EPA's Method 610 (U.S. EPA 1979) Polynuclear Aromatic Hydrocarbons - Method 610. Federal Register, 44(223), (69514-69517) was modified for detecting and quantifying phenanthrene, fluoranthene, pyrene, benzo(c)phenanthrene, triphenylene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenz(a,c)anthracene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene, indeno(1,2,3-c,d)pyrene and coronene in seawater. This method is applicable to the determination of PNAH in water and was designed to be used to meet the monitoring requirements of the National Pollutant Discharge Elimination System.

Procedures described in EPA Method 610 and an alternative extraction process using C_{18} mini-columns (Sep-Paks; Waters Assoc. Inc.) were investigated for application. Further evaluations of various analytical components are currently being completed. These studies were undertaken to evaluate the use of Sep-Paks for extracting PNAH from water since they seemed to offer certain procedural advantages. Experiments were designed to determine percent recoveries of individual PNAH, reproducibility and an optimum flow rate. A system to pump water through the Sep-Paks was also developed, tests and evaluated for adsorptive loss of PNAH.

Development of a Crustacean Bioassay System

In this study, an *in vitro* system for culturing the eggs and larvae of the gooseneck barnacle, *Pollicipes polymerus*, was developed and tested in two bioassay experiments using the phthalate ester plasticizer dibutyl phthalate. The culture system employed aereated culture chambers composed of chemically inert substances and designed to be inexpensive, convenient to use, and to minimize external contamination of the cultures.

Pollicipes eggs were grown in synthetic seawater at 14° ± 1° C under lighting conditions of alternating darkness and very low light. Water was changed every 24 hours. Antibiotics were not necessary if prescribed methods were employed. An overall mean hatching success index of 75.5 was achieved with egg masses not exposed to dibutyl phthalate (DBP). Experiments indicated that allowing 86-96 hours after the first hatch before ending the experiment resulted in sufficient stage II larvae to be able to draw conclusions regarding molting success.

One of the DBP experiments showed there was a significant decrease in molting success in larvae from eggs exposed to 1000 ppb DBP from a week prior to first hatching to the end of the experiment. Several phthalate esters were found to be persistent contaminants in synthetic sea salts.

The results of these studies indicate that the *P. polymerus* assay system has great potential for testing environmental carcinogens and mutagens.

Viral Studies

Because of a recent report (Oprandy, *et al*. 1981. *J. Invertebr. Pathol*., 38:45-51) which indicated that hemic proliferative disorders in *Mya arenaria* may be caused by a B-type retrovirus, studies were undertaken to determine whether or not such a virus was associated with the neoplastic disorders in *M. edulis*. Extensive investigation, employing a variety of techniques and methods did not reveal the presence of any RNA tumor virus in either affected or control *M. edulis*.

Statistical Evaluations of Proliferative Disorders in *M. edulis*

The occurrence, prevalence, seasonality and histopathological progression of a cellular disorder, thought to be a hemic neoplasm, were studied in subpopulations of *Mytilus edulis* inhabiting different sites in Yaquina Bay Oregon, from 1976-1981. There were significant differences in the occurrence of the disorder that were related to geographical location. In the subpopulation with the highest levels of the disease, the prevalences ranged from 0.0% to 20% with a five-year mean of 9.8%. There was a statistically significant relationship between prevalence and season. During the five-year study period, there was a consistent pattern characterized by highest prevalences during January through March followed by a period of decline to lower levels during the summer and early fall, after which there was an increase. Data analyses revealed that there was no seasonal histopathological progression of the disorder. Numbers of stage 1 (early), 2, 3 and 4 (advanced) cases were not related to season but, rather, occurred in a random manner throughout the entire year.

XII. Publications, Reports and Abstracts from Program - Cumulative list (1978 - 1981)

Anderson, Robert S. 1978. Benzo(a)pyrene metabolism in the American Oyster, Crassostrea virginica. Ecol. Res. Series, EPA-600/3-78-009. pp. 18.

Anderson, R.S., Doos, J.E., Rose, S.L. 1982. Differential ability of Ambystoma tigrinum hepatic microsomes to produce mutagenic metabolites from Polycyclic Aromatic Hydrocarbons and Aromatic Amines.

Bunting, D.B. 1979. An evaluation of benzo(a)pyrene metabolism in an Oyster (Ostrea edulis)-Bacteria System. M.S. (Thesis) Oregon State.

Coker, S. 1980. Benzo(a)pyrene metabolism in the Sea Catfish (Arius felius). Ala. Acad. Sci. Meeting, March 21, 1980.

Couch, J.A. 1979. Vertebral dysplasia in young fish exposed to the herbicide Trifluralin. Journal of Fish Diseases. 2: 35-42.

Couch, J.A. 1979. Pollution ecology of Penaeid Shrimps. In: Pollution Ecology of Estuarine Invertebrates, pp. 236-258. Hart/Fuller (eds.) Academic Press, New York.

Couch, J.A. 1979. Carcinogens in the Aquatic Environment. Interagency Collaborative Group on Environment Carcinogenesis, NIH, Bethesda, Maryland January 10, 1979. (Minutes of Meeting).

Couch, J.A., Lee Courtney, James T. Winstead and Steven Foss. 1979. The American Oyster (Crassostrea virginica) as an indicator of carcinogens in the Aquatic Environment. In: Animal Models and Wildlife as Monitors, pp. 65-84. National Academy of Sciences, Washington, D.C.

Couch, J.S. and W.P. Schoor. 1979. First EPA/NCI Annual Report. Effects of Carcinogens, Mutagens, and Teratogens on Non-Human Species (Aquatic Animals). February, 1980, NCI Publication.

Couch, JA. and W.P. Schoor. 1980. Second Annual Report to NCI/EPA. Effects of Carcinogens, Mutagens and Teratogens on Non-Human Species (Aquatic Animals). April, 1981, NCI Publication.

Couch, J.A. and James T. Winstead. 1979. Concurrent Neoplastic and Protistan Disorders in the American Oyster (Crassostrea virginica). Haliotis, 8, 1977. pp. 249-253.

Couch, J.A., Frank G. Lowman and Ford A. Cross. 1980. Biomonitoring of Coastal Waters, - An Overview. In: Biological Monitoring for Environmental Effects, Douglas L. Wolf (ed.), Lexington Books, Mass. pp. 93-95.

Couch, J.A., Lee A. Courtney and Steven S. Foss. 1981. Laboratory of Symposium of Marine Fishes as Carcinogen Assay Subjects. Proceedings of Symposium of Princess Takamatsu Cancer Research Fund, Phyletic Approaches to Cancer, C.J. Dawe et al. (eds), Japan Sci. Soc. Press, Tokyo, (In Press).

Couch, J.A. 1982. Aquatic Animals as Indicators of Environmental Exposures, Journal Envion. Sci. Health, A17(4) 473-476.

Couch, J.A., Clyde Dawe. 1982. Mouse vs Minnow: The Future of Fish in Carcinogenicity Testing. A Symposium on the use of Small Fish Species in Carcinogenicity Testing. National Cancer Institute Monograph. In press.

Courtney, L.A., J.A. Couch. 1982. Usefulness of Cyprinodon variegatus and Fundulus grandis in Carcinogenicity Testing: Advantages and Special Problems. A Symposium on the use of Small Fish Species in Carcinogenicity Testing. National Cancer Institute Monograph. In Press.

Dunn, J.E. 1982. Development of a Marine Bioassay System for Priority Pollutants Using Larvae of the Gooseneck Barnacle, Pollicipes polymerus: A Feasibility Study. M.S. Thesis.

Elam, D., M.V. Kilgore, B. Tan and W.P. Schoor. 1979. Mixed Function Oxidase Inducibility in the Mullet and Killifish. 4th Intl. Symp. on PAH, Columbus, Ohio, October 4, 1979.

Elam, D., M.V. Kilgore and W.P. Schoor. 1979. Induction of Mixed Function Oxidase and Metabolism of Polyaromatic Hydrocarbons in Marine Organisms. 11th Intl. Congress of Biochemistry, Toronto, Canada, July 11, 1979.

Elnenaey, Elsayed, and W. Peter Schoor. A Simple High Performance Liquid Chromatography Method for the separation of benzo(a)pyrene metabolites. Presented at American Association for Clinical Chemistry 32nd Annual Meeting, Kansas City, Missouri, July 18-23, 1981.

Gregory, P.E., P.N. Howard-Peebles, R.D. Ellender and B.J. Martin. 1980. C-Banding of chromosomes from three established marine fish cell lines. Copeia, 3: 545-547.

Gregory, P.E., P.N. Howard-Peebles, R.D. Ellender and B.J. Martin. 1980. Analysis of a marine fish cell line from sheepshead: chromosomal alterations in Archosargus probatocephalus. J. Heredity 71: 209-211.

Grizzle, J.M., T.E. Schwedler, A.L. Scott. 1981. Papillomas of Black Bullhead (Ictalurus melas Rafinesque) Living in a Chlorinated Sewage Pond. J. Fish Diseases.

Harshbarger, John D., Elliot R. Jacobson, Charles E. Smith and John A. Couch. 1980. Hematopoietic Neoplasms in Invertebrates and Cold Blooded Vertebrates. In: Advances in Comparative Leukemia Research. 1979. David S. Yohn, Boris A. Lapin and James R. Blakeslee (eds.) Elsevier/North Holland. New York/Amsterdam/Oxford. pp. 223-225.

Hemingway, S.J. 1979. (1) Storage Sites of Benzo(a)pyrene in contaminated Bay Mussels (Mytilus edulis) inhabiting different sites in Yaquina Bay, Oregon. (2) Uptake and depuration of Benzo(a)pyrene in contaminated Bay Mussesl (Mytilus edulis) inhabiting different sites on Yaquina Bay. M.S. Thesis, Oregon State University.

Hendricks, J.D. 1980. Chemical carcinogenesis in fish. In: Aquatic Toxicology. L.Weber (ed.). Raven Press, New York. (In press).

Hendricks, J.D., J.H. Wales, R.O. Sinnhuber, J.E. Nixon, P.M. Loveland and R.A. Scanlan. 1980. Rainbow Trout (Salmo gairdneri) embryos: A sensitive animal model for experimental carcinogenesis. Fed. Proc. (In press).

Hendricks, J.D., R.O. Sinnhuber, P.M. Loveland, N.E. Pawlowski and J.E. Nixon. 1980. Hepatocarcinogenicity of glandless cottonseeds and refined cottonseed oil to rainbow trout (Salmo gairdneri). Science (In press).

Hendricks, J.D., and T.R. Meyers. Ganglioneuroblastomas in coho salmon reared in chlorinated-dechlorinated river water. J. Natl. Cancer Inst. (In review).

Hendricks, J.D., Meyers, T.R., Shelton, D.W., and Sinnhuber, R.O. (1982). Liver neoplasia and induction of hepatic mixed function oxidase enzymes in the rainbow trout following dietary exposure to benzo(a)pyrene. Proc. Amer. Assoc. Cancer Res. 23:58.

Hendricks, J.D., Meyers, T.R., and Shelton, D.W. Histologic progression of hepatic neoplasms in rainbow trout (Salmo gairdneri). Natl. Cancer Inst. Monogr. (In press).

Hendricks, J.D., Meyers, T.R., Casteel, J.L., Nixon, J.E., Loveland, P.M., and Bailey, G.S. Rainbow trout embryos: Advantages and limitations for carcinogenesis research Natl. Cancer Inst. Monogr. (In press).

Hendricks, J.D., Meyers, T.R., Shelton, D.W., and Casteel, J.L. The hepatocarcinogenicity of benzo(a)pyrene to rainbow trout by dietary exposure and intraperitoneal injection. (Final stages of preparation).

Hillebert, S.A., B.J. Martin and R.D. Ellender. 1980. Chronic exposure of a teleost cell line to suspect carcinogens. J. Miss. Acad. Sci. (In press).

Kilgore, M.V., and D. Elam. 1979. Mixed-Function Oxidase Inducibility in Marine Organisms. Ala. Acad. of Sciences Meeting, April 14, Florence, Ala.

Kilgore, M.V., and D. Elam. 1979. A comparatiave study of induction of mixed function oxidase activity in the rat, mullet and killifish. Ala. Acad. Sci. Meeting, March 30, Florence, Ala.

Koenig, Christopher C., M. Chasar 1982. Usefulness of Hermaphroditic Marine Fish Rivulus marmoratus as a Test Animal for carcinogenicity testing. A symposium on the use of small fish species in carcinogenicity testing. National Cancer Institute Monograph. In Press.

Koening, Christopher C., C. Abel, C.W. Klingensmithe, M.B. Maddock, 1982. Usefulness of the self-fertilizing Cyprinodontid fish, Rivulus marmoratus as an experimental animal in studies involving carcinogenesis, teratogenesis and mutagenesis. EPA Research Assistance Grant (R805469) 129 pp.

LaTouche, Y.D. 1981. Metal levels in a population of Mytilus edulis from Yaquina Bay, Oregon. PH.D. Thesis, Oregon State University.

LaTouche, Y.D., and M.C. Mix. 1981. Seasonal variations in trace metal levels in a Mytilus edulis population in Yaquina Bay (Newport, OR). The 21st Hanford Life Sciences Symposium.

LaTouche, Y.D., C.W. Bennett and M.C. Mix. 1981. Determination of Vanadium in a marine mollusk using a chelating ion exchange resin and neutron activation. Bull. Environ. Contam. Toxicol., 26: 224-227.

LaTouche, Y. D. and M. C. MIx. 1982. The effects of depuration, size and sex on trace metal levels in bay mussels. Marine Pollution Bulletin, 13:27-29

LaTouche, Y. D. and M. C. Mix. In Press. Seasonal variations of arsenic and other trace elements in bay mussels (Mytilus edulis). Bulletin of Environmental Contamination and Toxicology. 1982.

Long, R.L., and B.J. Martin. 1980. Morphology of peripheral blood cells of Cyprinodon variegatus. ABSTR. J. Miss. Acad. Sci., Vol. XXV, Suppl: 122.

Martin, B.J. 1980. Effects of petroleum compounds on estuarine fishes. Ecol. Res. Series. EPA-600/3/80-019. January, 1980.

Martin, B.J., and W.W. Greenwich. 1980. Exposure of two teleost species to polycyclic aromatic hydrocarbons. ABSTR. J. Miss. Acad. Sci., Vol. XXV, Suppl: 120.

Martin, B.J., and W.W. Greenwich. 1981. Benzidine Toxicity. J. Miss. Acad. Sci. 26 (Suppl.): 127.

Martin, B.J., and W.W. Greenwich. The Induction of Proliferative Lesions in the Sheepshead Minnow, Cyprinodon variegatus, with Benzidine Dihydrochloride. (In preparation).

Martin, B.J., R.D., Ellender, S.A. Hillebert, and M.M. Guess. Primary Cell Cultures from the Teleost, Cyprinodon variegatus: Culture Establishment and Application in Carcinogen Exposure Studies. National Cancer Institute Symposium on the Use of Small Fish Species in Carcinogenicity Testing, Bethesda, MD. December 8-10, 1981.

Meador, C.B., B.L. Middlebrooks, and B.J. Martin. Serologic Alterations in Carcinogen-Exposed Teleosts: Procedures for Preparation and Analysis of Samples From Small Fish. National Cancer Institute Symposium on the Use of Small Fish Species in Carcinogenicity Testing, Bethesda, MD. December 8-10, 1981.

Melius, P., B. Tan, M. Kilgore, D. Elam, W.P. Schoor. 1979. Product pattern and kinetic analysis of benzo(a)pyrene metabolism in Marine Organism by HPLC. ACS 31st Southeast Regional Meeting, Florence, Ala. April 14, 1979.

Melius, P., D. Elam, M.V. Kilgore, B. Tan and W.P. Schoor. 1979. Mixed-Function Oxidase Inducibility and Polyaromatic Hydrocarbon Metabolism in Mullet, Sea Catfish and Gulf Killifish. In: Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Bjorseth and Dennis (eds.), pp. 1059-1075. Batelle Press, Columbus, Ohio.

Melius, P., D. Elam, M.V. Kilgore and W.P. Schoor. 1979. Induction of Mixed-Function Oxidase and Metabolism of Polyaromatic Hydrocarbons in Marine Organisms. Eleventh International Congress of Biochemistry, Toronto, Canada. July 7-11.

Melius, P., B. Tan, M.V. Kilgore, and D. Elam. 1979. Metabolites of B(a)P in Aroclor-induced fish. Fourth ASTM Symposium on Aquatic Toxicology, Chicago, Illinois. October 16-17.

Meyers, T.R. and J.D. Hendricks. Case reports of spontaneous neoplasms in feral and laboratory-reared salmonids. J. Fish Dis. (In review).

Meyers, T.R. and Hendricks, J.D. Histopathological methods. In Principles of Aquatic Toxicology. (Rand, G.M. and Petrocelli, S.R., eds.). Hemisphere Publishing Co., New York (In press).

Meyers, T.R. and Hendricks, J.D. A summary of tissue lesions in aquatic animals induced by controlled exposures to environmental contaminants, chemotherapeutic agents and potential carcinogens. Marine Fish. Review (In press).

Meyers, T.R. and Hendricks, J.D. Histopathology of four spontaneous neoplasms in three species of salmonid fishes. J. Fish Dis. (In press).

Meyers, T.R. and Hendricks, J.D. A limited epizootic of multi-differentiating neuroblastoma in coho salmon reared in chlorinated-dechlorinated water. Submitted to J. Natl. Cancer Inst.

Mix, M.C., R.T. Riley, K.I. King, S.R. Trenholm and R. L. Schaffer. 1977. Chemical carcinogens in the marine environment. Benzo(a)pyrene in economically important bivalve mollusks from Oregon estuaries. In: Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, D. A. Wolfe (ed.), pp. 421-431. Pergamon Press, New York.

Mix, M.C., J.W. Hawkes and A.K. Sparks. 1978. The ultrastructure of cells associated with proliferative disorders in mussels (Mytilus edulis) from Yaquina Bay, Oregon. Proceedings of a Colloquium on Invertebrate Pathology: Neoplasms in Invertebrates.

Mix, M.C. and R.L. Schaffer. 19797. Benzo(a)pyrene concentrations in mussels (Mytilus edulis) from Yaquina Bay, Oregon during June, 1976 - June, 1978. Bull. Environ. Contam. Toxicol., 23: 677 - 684.

Mix, M.C., J.W. Hawkes, and A.K. Sparks. 1979. Observations on the ultrastructure of large cells associated with putative neoplastic disorder of mussels, Mytilus edulis, from Yaquina Bay, Oregon. J. Invert. Path., 34: 41-56.

Mix, M.C. 19797. Chemical Carcinogens in Bivalve Mollusks from Oregon Estuaries EPA Ecological Research Series, EPA600/3-79-034. pp. 33.

Mix, M.C. 1979. Seasonal variation in benzo(a)pyrene in bivalve mollusks from Oregon estuaries. Symposium on Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment. U.S. EPA., Gulf Breeze, Florida.

Mix, M.C. 1979. Leukemia-like disorders in bay mussels (Mytilus edulis) from Yaquina Bay, Oregon, U.S.A. Proceedings of a Symposium on Comparative Leukemia and Related Diseases.

Mix, M.C., D.L. Bunting and D.T. Abbott. 1979. Preliminary studies to evaluate the potential of using embryo and larval stages of the goose barnacle, Pollicipes polymerus, for marine bioassays. Proceedings of the Second Biennial Crustacean Health Workshop, Texas A M Univ., TAMU-SG-79-114:361-381. College Station, Texas.

Mix, M.C., S.R. Trenholm and K.I. King. 1979. Benzo(a)pyrene body burdens and the prevalence of cellular proliferative disorders in mussels, Mytilus edulis, from Yaquina Bay, Oregon. In: Animals as Monitors of Environmental Pollutants. 52-62. National Academy of sciences, Washington, D.C.

Mix, M.C., R.L. Schaffer and Y.D. LaTouche. 1980. Environmental contaminants and the occurrence of certain pathological conditions to bivalve mollusks. Proceedings of the Society for Invertebrate Pathology.

Mix, M.C., and W.P. Breese. 1980. A cellular proliferative disorder in Ostrea chilensis from Chilor, Chile, South America. J. Invert. Pathol. 36:123-124.

Mix, M.C. 1980. Utilization of bivalve mollusks for monitoring carcinogenic polynulclear aromatic hydrocarbons in estuarie environments. The Eleventh International Symposium of the Princess Takamatsu Cancer Research Fund. (In press).

Mix, M.C. Polynuclear Aromatic Hydrocarbons in Bivalve Molluscs from Oregon Estuaries. EPA Ecological Research Series. (In press).

Mix, M.C., R.L. Schaffer and S.J. Hemingway. Polynuclear aromatic hydrocarbons in bay mussels (Mytilus edulis) from Oregon. In: Proceedings of the Eleventh International Symposium of the Princess Takamatsu Cancer Research Fund. (In press).

Mix, M.C. Cellular proliferatiave disorders in bivalve mollusks from contaminated marine environments. To be submitted to Journal of Environmental Pathology and Toxicology.

Mix, M. C., S. J. Hemingway and R. L. Schaffer. 1982. Benzo(a)pyrene concentrations in somatic and gonad tissues of bay mussels, Mytilus edulis. Bulletin of Environmental Contamination and Toxicology, 28:46-51.

Mix, M. C. and R. L. Schaffer. In Press. Concentrations of unsubstituted polynuclear aromatic hydrocarbons in bay mussels, Mytilus edulis, from Oregon. Marine Environmental Research.

Mix, M. C. and R. L. Schaffer. In Press. Concentrations of unsubstituted polynuclear aromatic hydrocarbons in softshell clams (Mya arenaris) from Coos Bay, Oregon. Marine Pollution Bulletin.

Mix, M. C. In Press. The neoplastic disease of bay mussels, Mytilus edulis, from Oregon: occurrence, prevalence, seasonality and histopathological progression. Journal of Fish Diseases.

Moreno, M., and B.J. Martin. 1979. Embryologic development of sheepshead minnow, (Cyprinodon variegatus). ABSTR. J. Miss. Acad. Sci. Vol. XXIV, Suppl: 116.

Porter, R.D., and B.J. Martin. 1980. The histology of the postpharyngeal digestive tract of the sheepshead minnow, Cyprinodon variegatus. ABSTR. J. Miss. Acad. Sci., Vol. XXV. Suppl: 123.

Riley, R.T. 1978. The effects of chemical perturbation by naphthalene in glucose metabolism in the European flat oyster, Ostrea edulis: An in vivo kinetic analysis. Ph.D. Thesis Oregon State University.

Riley, R.T., and M.C. Mix. 1980. Thin layer separation of citric acid cycle intermediates, lactic acid, and the amino acid taurine. Journal of Chromatography, 189: 286-288.

Riley, R.T., and M.C. Mix. 1981. An ion -exchange technique for the concentrating of ammonia in small volumes of seawater. Marine Chemistry. 10: 159-160.

Riley, R.T., M.C. Mix, R.L. Schaffer and D.L. Bunting. Uptake and accumulation of naphthalene by the oyster, Ostrea edulis, in a flow-through system. Marine Biology. (In press).

Riley, R.T., and M.C. Mix. The effects of naphthalene on glucose metabolism in the European flat oyster, Ostrea edulis. Marine Biology, (In press).

Riley, R.T. Stimulatory effect of naphthalene on glucose transport in the oyster. Submitted to Nature.

Riley, R.T., and M.C. Mix. The pathways of glucose metabolism in the oyster, Ostrea edulis. To be submitted to Comparative Biochemistry and Physiology.

Schaffer, R.L., and M.C. Mix. A method for determining environmental levels of polycyclic aromatic hydrocarbons in bivalve mollusks by reverse phase liquid chromatography. To be submitted to Analytical Chemistry.

Schneider, S.R., J.D. Hendricks, G.H. Constantine and R.E. Larson. 1980. Tobramycin nephrotoxicity in coho salmon at subtherapeutic doses. Toxicol. Appl. Pharmacol. (In press).

Schoor, W.P. 1979. Fluorimetric confirmation of metabolites of benzo(a)pyrene from a marine fish. Fourth International Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, Ohio, October 2-4, Battelle Institute.

Schoor, W.P., and J.A. Couch. 1979. Correlation of mixed-function oxidase activity with ultrastructural changes in the liver of a marine fish. Cancer Biochemistry and Biophysics, 4: 95-103.

Schoor, W.P., Elsayed Elnenaey and Barrie Tan. 1981. Benzo(a)pyrene Metabolism in 3-Methylcholanthrene-treated sea catfish. Submitted to Carcinogenesis.

Schoor, W.P. Exposure of Fishes to Benzo(a)pyrene and Source Aspects of Analysis of Metabolites, J. Natl. Canc. Inst. in press.

Sigismondi, L.A. 1982. The Accumulation of Cadmium, Copper and Nickel in Freshwater Mussels (Margaritifera margaritifera), Sediment and Algae (Cladophora sp.) from Big Elk River, Oregon. M.S. Thesis.

Smith, A.C., and M.C. Mix. 1978. The effects of sodium chloride concentration on electrophoretic patterns of adductor muscle proteins from bivalve mollusks. Comparative Biochemistry and Physiology (Part B), 61: 169-171.

Strength, R., H.H. Daron, J.L. Aull, J.F. Wilson and W.P. Schoor. 1980. The induction of glucuronide and sulfate transferases by phenobarbital and polycyclic aromatic hydrocarbons. Fed. Proc. 39: 1694.

Strength, D.R., K.V. Sarambadal, S.L. Wang, H.H. Darron, and W.P. Schoor, Oxidation and Conjugation of BaP in Mullet. Fed. Proceed., 41, 1147(1982).

Strength, D.R., H.H. Darron, and W.P. Schoor. Comparison of Polycyclic Aromatic Hydrocarbon Metabolism in Rodent and Fish. J. Natl. Canc. Inst., in press.

Tan, B. 1979. Product Pattern and Kinetic analysis of benzo(a)pyrene metabolism in marine organisms by HPLC. S.E. Regional A.C.S. meeting, Roanoke, Va. October 25, 1979.

Tan, B. 1980. Indirect atomic absorption assay of epoxide hydrase. Ala. Acad. Sci., March 21.

Tan, B. 1980. Indirect atomic absorption assay of epoxide hydrase activity and determination of 1,2-diols via digested lead periodate. English Society of Analytical Chemists, 5th Intl. Symp., Lancaster, England, July 20-26.

Tan, B. 1980. Benzo(a)pyrene metabolism in PAH and Biphenyl treated Tilapia. Presented October 28-30, 5th Intl. Symp. on PAH's, Columbus, Ohio.

Tan, B., and J.M. Grizzle. 1980. The use of benzo(a)pyrene metabolism as a biochemical indicator in control and tumored bullhead catfish. October 28-30, 5th Intl. Symp. on Polynuclear Atomic Hydrocarbons, Columbus, Ohio.

Tan, B., P. Melius, M. Kilgore, D. Elam and W.P. Schoor. 1979. Metabolites of benzo(a)pyrene in Aroclor-induced fish. XI International Congress of Biochemistry, Toronto. Canada, July 8-13.

Tan, B., and P. Melius. 1980. Responses of Hepatic Enzymes of Tilapia to PCB and PAH modifiers. American Assoc. Biol. Chemists Meeting, New Orleans. June 2-5. Abstract published in Fed. Proc.

Tan, B., P. Melius and M.V. Kilgore. 1980. Determination of 1,2-diols by direct atomic absorption with digested lead periodate. Anal. Chem. 52:602-604.

Tan, B., D. Elam, E.V. Kilgore and W.P. Schoor. 1979. Metabolites of Benzo(a)pyrene in Aroclor 1254 treated mullet. 4th ASTM Symp. on Aquatic Toxicology. October 16-17, 1979. Chicago, Illinois. (In press).

Tan, B. Responses of hepatic enzymes of Tilapia to biphenyls and polyaromatic hydrocarbons. Submitted to Biochem. Pharmacology.

Tan, B. Comparative metabolism of polyaromatic hydrocarbons in marine organisms. Invited review article being prepared for Journal of Comparative Biochemistry and Physiology.

Tan, B., and P. Melius. Kinetic product patterns and mechanistic pathways of benzo(a)pyrene metabolism in Aroclor-treated mullet. Submitted to Biochem. Biophys. Acta.

Tan, B., D. Elam, M.V. Kilgore, and W.P. Schoor. Mixed function oxidase inducibility and polyaromatic hydrocarbon metabolism in mullet, sea catfish and gulf killifish. 4th Intl. Symp. on Polynuclear Aromatic Hydrocarbons Chemistry and Biological Effects. Battelle Press. (In press).

Tan, B., M.V. Kilgore, D. Elam and W.P. Schoor. Metabolites of benzo(a)pyrene in Aroclor 1254 treated Mullet. 4th Symp. on Aquatic Toxicology, ASTM (In press).

Tan, B. 1981. Indirect Atomic Absorption Spectrometric Assay for Epoxide Hydrolase. Analytical Letters, 14(B5), 311-322.

Tan, B., P. Melius, and J. Grizzle. 1981. Hepatic Enzymes and Tumor Histopathology of Black Bullheads. Polynuclear Aromatic Hydrocarbons Chemical Analysis and Biological Fate 5th International Symposium. Battelle Press.

Tan, B. and P. Melius. 1981. Responses of the Hepatic Enzymes of a Teleost Fish to trans-Stilbene Oxide Treatment. Bull. Environm. Contam. Toxicol. 26, 801-806.

Tan, B., P. Melius, and P. Zieglor. 1982. A Simple Gas Chromatographic Method for the Study of Organic Solvents: Moisture Analysis, Hygroscopicity, and Evaporation. Journal of Chromatographic Science, Vol. 20. 213-217.

Trenholm, S. and M.C. Mix. The lethal and sublethal effects of ionizing radiation of juvenile Pacific oysters (Crassostrea gigas). Radiation Research. (In press).

Trenholm, S.R. and M.C. Mix. 1978. Regeneration of radiation damaged digestive tissue in juvenile Pacific oyster (Crassostrea gigas). J. Invert. Pathol. 32: 249-257.

Trenholm, S.R. 1978. Effects of X- and Gamma irradiation on the juvenile Pacific oyster, Crassostrea gigas. M.S. Thesis Oregon State University.

ERL,GB 0267

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/9-83-005	2. Problem Oriented Report	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE EFFECTS OF CARCINOGENS, MUTAGENS, AND TERATOGENS ON NONHUMAN SPECIES--AQUATIC ANIMALS		5. REPORT DATE
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) J.A. Couch, W.P. Schoor, W. Davis, and Lee Courtney		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory Office of Research and Development Gulf Breeze, FL 32561		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This report describes the fourth year of a joint U.S. Environmental Protection Agency (EPA) and National Cancer Institute (NCI) effort to develop laboratory and field assays, using fishes as indicators of the presence of certain carcinogens (polycyclic aromatic hydrocarbons). The study has revealed that fish have metabolic pathways similar to mammals for disposition of certain carcinogens.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE