

Project Number 8

**Bioavailability and Genotoxicity of Produced Water Discharges Associated
with Offshore Drilling Operations**

Gary W. Winston
Department of Biochemistry
Louisiana State University
Baton Rouge, LA 70803

Jay C. Means
Institute for Environmental Studies
Louisiana State University
Baton Rouge, LA 70803

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ABSTRACT

As the aquatic environment is a major sink for numerous contaminants, the elucidation of biochemical responses to contaminants by aquatic animals becomes increasingly important and is the basis of this research. Unfortunately, there were administrative delays resulting from a protracted negotiation concerning indirect cost charges. This was resolved after several months, and the grant was initiated Nov. 1, 1990. A permanent postdoctoral research associate is now in training and it is anticipated that she will be well trained to perform biological evaluations from the first round of samplings and laboratory exposure studies scheduled for mid-summer. Training of personnel in trace determination of several specific positional isomers of alkylated naphthalenes, phenanthrenes and dibenzothiophenes is in progress. The mean detection limits achieved for tissue samples (<2 g wet weight) by selected ion monitoring gas chromatography/mass spectrometry are 1.0 ppb. Field validation studies on use of mixed-function oxidase (MFO) in chemically contaminated sites e.g., Devil's Swamp in Baton Rouge. Evidence of induction of aspects of this parameter in the contaminated site was observed in pooled microsomal fractions of livers of exposed channel catfish (*Ictalurus punctatus*) and those from a pristine reference site (Ben Hur research farms, Louisiana State University). *Fundulus heteroclitus* is an important test organism for the present studies. Preliminary determinations of the responsiveness of MFO of embryos, larvae and adults of this species towards cyclophosphamide (CP) and ethylmethane sulfonate (EMS), indicate that whereas adults are potentially efficacious sentinel organisms for detecting exposure to these chemicals, eggs and larvae might not. Further studies will be required under varied exposure regimens before rigorously excluding the use of embryos and larvae of this species.

PROJECT GOALS AND OBJECTIVES

The present study evaluates a suite of biochemical markers recently identified as sensitive sublethal indices of environmental impact on several test organisms through detailed studies of: 1) the bioavailability of dissolved and sediment-bound normal, alkylated and heterocyclic aromatic hydrocarbons to benthic invertebrates and demersal fish, eggs and larvae; 2) the ability of both benthic and pelagic organisms to metabolize these compounds; and 3) the genotoxicity of the compounds and metabolizes in benthic organisms and demersal fish. The data generated during these studies will allow environmental managers to assess the potential impacts and both ecological and human environmental risks associated with increased discharges of petroleum associated hydrocarbons. Moreover, MFO is still recognized as one of the most robust biochemical indicators that is not elevated by normal physiological stress e.g., heat, hyper- and hypo-salinity, hyper- and hypo-oxia. The chromosomal aberration assay has similar advantages. As the aquatic environment is a major sink for numerous contaminants, the elucidation of biochemical responses to contaminants by aquatic animals becomes increasingly important. The epidemiology of highly elevated rates of idiopathic lesions and neoplasia among some populations of aquatic animals inhabiting polluted environments is in most cases far clearer than is generally the case for human cancers suspected of being related to environmental pollution.

ACCOMPLISHMENTS TO DATE

To date we have hired a highly qualified research associate, Dr. Zuo Mei Yan, and have begun a program of training in the various aspects of the MFO and genotoxicological parameters for use in both the laboratory and field studies. Dr. Yan is now proficient in the conduct of one of the most robust MFO assays used in biomarker research namely, the evaluation of O-dealkylation reactions from substituted alkoxyphenoxazones. Dr. Yan has also been trained to perform the *umu* mutagenicity assay, a short-term bacterial assay which measures the responsiveness of *Salmonella typhimurium* to various mutagens including, promutagens i.e., those which require metabolic activation for mutagenic expression and

direct-acting mutagens i.e., those that do not require metabolic activation for mutagenic expression. Dr. Yan is now being trained in Dr. Means' laboratory on various aspects of **genotoxicity** assessment and in newly developed analytical methodologies for the extraction, identification and quantification of normal, **alkylated** and **heterocyclic** aromatic hydrocarbons associated with produced waters in sediments and biological tissues. It is anticipated that Dr. Yan **will** be well trained to perform the biological evaluations from our first round of samplings and laboratory exposure studies scheduled for the middle of this summer. Further accomplishments have been infield validation of the use of the **arylhydrocarbon** hydroxylase assay in chemically contaminated sites including **Devil's** Swamp and Capital lake in Baton Rouge. Clear evidence of induction of this parameter in the contaminated site was observed in **pooled microsomal fractions** obtained from livers of exposed channel catfish (*Ictalurus punctatus*) and channel catfish from a pristine reference site (Ben Hur research farms, Louisiana State University). As *Fundulus heteroclitus* is an important test organism for the studies proposed in this initiative, preliminary studies were undertaken to determine the responsiveness of the **MFO** system of embryos, larvae and adults of this species towards **cyclophosphamide (CP)** and **ethylmethane sulfonate (EMS)**, two benchmark mutagens that have been documented with respect to their genotoxic effectiveness on this species. The data indicate that whereas adults are potentially efficacious sentinel organisms for detecting exposure to these chemicals, eggs and larvae might not be. Further studies will be required under varied exposure regimens before rigorously excluding the use of embryos and larvae of this species.

Microcosm Development for Sedi' merit Exposures of Estuarine and Marine Organisms:

The goal of this aspect of the research was to **develop a microcosm** system which **could** be used to study the **bioaccumulation** of **alkylated PAH** from contaminated sediments under a variety of conditions. As suggested by the **scientific** advisory board, we wanted to be able to use the microcosms for studies involving **benthic** organisms. Figure 1 shows the microcosm design which was developed and which has been used to study **abiotic** processes as well as preliminary biotic accumulation of **alkylated PAH from** contaminated sediments over time courses up to 120 days.

Ultra-trace techniques for aromatic hydrocarbon analysis in biological tissues:

In order to determine effects of previous sediment contamination on sublethal responses of organisms, analytical measurements of the amounts of compounds associated with **petrogenic** contamination entering the organisms (dose) **are** necessary. The **alkylated** and **heterocyclic polynuclear aromatic hydrocarbons (PAHs)** **were** chosen as the focus of the analytical methods development effort as these are the dominant chemical species present and new methods were required to ensure adequate analytical resolution at low detection limits **from** PAHs having other sources such as combustion. In order to meet this need and to serve the broader goals of the proposed research, we embarked upon an extensive methods development effort which was directed at the trace determination of a number of specific positional isomers of **alkylated naphthalenes, phenanthrenes** and **dibenzothiophenes**. Table 1 lists the compounds for which trace detection methods have been developed. The mean detection limits achieved for tissue samples (Q g wet weight) using selected ion monitoring gas chromatography/mass **spectrometry** are 1.0 ppb.

SIGNIFICANT FINDINGS

Table 2 shows that the specific content of **cytochrome P-450** and **cytochrome b5** are markedly elevated in the liver **microsomal** fraction of fish from Devil's Swamp as compared to that of the reference Ben Hur fish. In conjunction with the elevated **cytochromes** is an increase in the activities of NADH and NADPH-dependent **cytochrome c reductase**.

Figure 1. Microcosm design for sediment resorption studies.

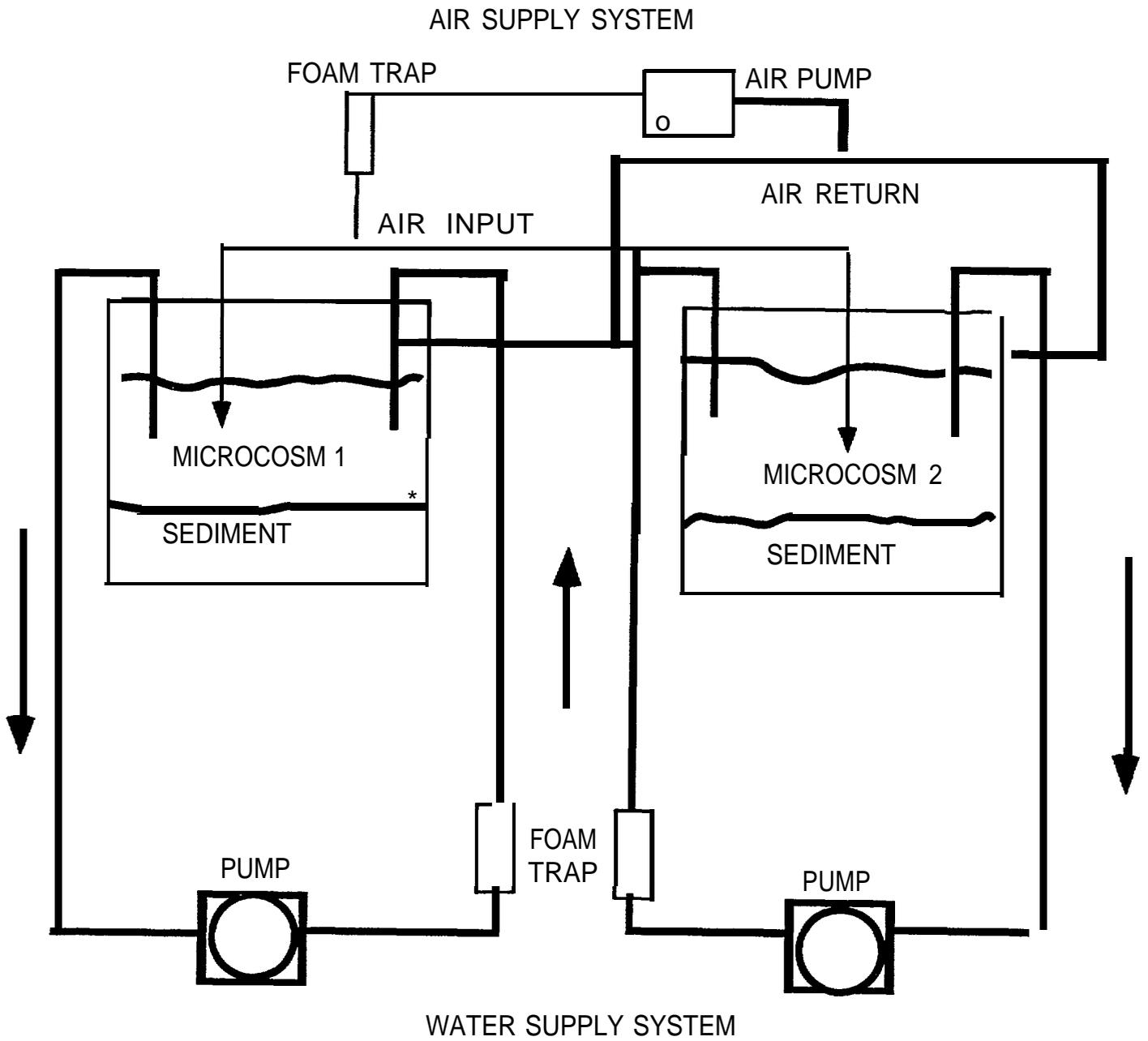


Table 1. Target Analytes

Peak No.	Analyte	Abbreviation	Primary Ion	Confirming ion
1	Naphthalene	Naphthalene	128	129
2	2-Methylnaphthalene	2-MN	142	141
3	1-Methylnaphthalene	1-MN	142	141
4	2-Ethylnaphthalene	2-EN	156	141
5	1-Ethylnaphthalene	1-EN	156	141
6	2,6/2,7-Dimethylnaphthalene	2,6/2,7-DMN	156	141
7	1,3/1,7-Dimethylnaphthalene	1,3/1,7-DMN	156	141
8	1,6-Dimethylnaphthalene	1,6-DMN	156	141
9	1,4/2,3-Dimethylnaphthalene	1,4/2,3-DMN	156	141
10	1,5-Dimethylnaphthalene	1,5-DMN	156	141
11	Acenaphthylene	Acenaphthylene	152	153
12	1,2-Dimethylnaphthalene	1,2-DMN	156	141
13	2-Isopropylnaphthalene	2-IPN	170	155
14	1,8-Dimethylnaphthalene	1,8-DMN	156	141
15	Acenaphthene	Acenaphthene	153	154
16	Fluorene	Fluorene	166	165
17	Dibenzothiophene	Dibenzothiophene	184	185
18	Phenanthrene	Phenanthrene	178	179
19	Anthracene	Anthracene	178	179
20	4-Methyldibenzothiophene	4-MDBT	198	197
21	2/3-Methyldibenzothiophene	2/3-MDBT	198	197
22	1-Methyldibenzothiophene	1-MDBT	198	197
23	3-Methylphenanthrene	3-MP	192	191
24	2-Methylphenanthrene	2-MP	192	191
25	4/9-Methylphenanthrene	4/9-MP	192	191
26	1-Methylphenanthrene	1-MP	192	191
27	4,5-Dimethylphenanthrene	4,5-DMP	206	191
28	3,6-Dimethylphenanthrene	3,6-DMP	206	191
29	3,5-Dimethylphenanthrene	3,5-DMP	206	191
29	2,6-Dimethylphenanthrene	2,6-DMP	206	191
30	2,7-Dimethylphenanthrene	2,7-DMP	206	191
31	3,9-Dimethylphenanthrene	3,9-DMP	206	191
32	1,6/2,5/2,9-Dimethylphenanthrene	1,6/2,5/2,9-DMP	206	191
33	1,7-Dimethylphenanthrene	1,7-DMP	206	191
34	1,9/4,9-Dimethylphenanthrene	1,9/4,9-DMP	206	191
35	Fluoranthene	Fluoranthene	202	101
36	1,5-Dimethylphenanthrene	1,5-DMP	206	191
37	1,8-Dimethylphenanthrene	1,8-DMP	206	191
38	1,2-Dimethylphenanthrene	1,2-DMP	206	191
39	9,10-Dimethylphenanthrene	9,10-DMP	206	191
40	Pyrene	Pyrene	202	101
41	Benzo(a)anthracene	Benanthracene	22a	226

Table 1. (cont'd.)

Peak No.	Analyte	Abbreviation	Primary Ion	Confirming ion
42	Chrysene	Chrysene	228	226
43	Benzo(b)fluoranthene	Benzo(b)fluor	252	253
44	Benzo(k)fluoranthene	Benzo(k)fluor	252	253
45	Benzo(a)pyrene	Benzo(a)pyrene	252	253
46	Indeno(1,2,3-cd)pyrene	Indenopyrene	276	278
47	Dibenz(a,h)anthracene	Dibenzanthracene	278	276
48	Benzo(g,h,i)perylene	Benzoperylene	276	278
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Deuterated Internal/Surrogate standards				
	d8-Naphthalene	d8-Naph	136	
	d10-Acenaphthene	d10-Ace	164	
	d10-Phenanthrene	d10-Phen	188	
	d12-Chrysene	d12-Chrys	240	
	d12-Perylene	d12-Peryl	264	

Table 2. Cytochrome Content and Flavoprotein Reductase Activities of Liver Microsomes from Devil's Swamp and Ben Hur Farm Channel Catfish.

Microsomal Sample	Cytochrome P-450 (nmol/mg) ^a	Cytochrome b5 (nmol/mg)	NADPH-cyt c reductase (nmol/min/mg)	NADPH-cyt C reductase (nmol/min/mg)
Ben Hur				
Male (6)	0.42 ± 0.06 ^b	0.09 ± 0.02	54 ± 6	268 ± 21 ^d
Female (4)	0.38 ± 0.05	ND ^c	46 ± 5	200 ± 8
Devil's Swamp				
Male (4)	1.0 ± 0.1	0.17 ± 0.02	146 ± 10	789 ^d
Female (3)	1.2 ± 0.0	ND	149 ± 18	366 ± 30

^amg of microsomal protein. ^bValues are the means ± SD of 3 determinations made on pooled liver microsomes prepared from the number of fish in parentheses. ^cnot determined. ^dmean of 2 determinations on pooled samples. Assays were carried out as described under methods.

Table 3 shows that **AHH** was approximately 2-fold and 4-fold higher for males and females, respectively in the Devil's Swamp fish as **compared** to Ben Hur fish. Moreover, **alpha-naphthoflavone (ANF)**, a highly selective inhibitor of AHH activity catalyzed by **cytochrome(s) P-450** that **are** induced by **polynuclear** aromatic hydrocarbons inhibited **AHH** catalyzed by **microsomes** from Devil's Swamp male and female fish by 89 and 79 %, respectively. On the other hand, ANF **inhibition** of the Ben Hur **microsomal AHH** activity was appreciably less than that of the Devil's Swamp system. The elevated activity in the Devil's Swamp fish appears therefore to be consistent with activities that are associated with PAH and/or PCB exposure.

PROBLEMS OR DELAYS ENCOUNTERED AND PROPOSED SOLUTIONS

The primary problem encountered in the initiation of this project was an administrative delay of the award from June 1 until November 1, 1990. This **delay** was due to a heretofore unprecedented grants management problem owing to the fact that the **P.I.s** were affiliated with two independent administrative units, the Center for Energy Studies and the College of Basic Sciences. This resulted in a protracted negotiation concerning indirect cost charges which was resolved after several months, with the initiation of the grant set at Nov. 1, 1990.

REVISED SCHEDULE FOR REMAINDER OF PROJECT

By arrangement with the principal investigators, the remainder of the three years of funding on this grant will proceed as if the origination date of the grant were November 1st. The delays in staffing of the grant have been overcome and the addition of students and technician support from **simultaneous** grants held by Dr. Means will more than compensate for the lost time.

PROJECT PARTICIPANTS

Dr. Gary W. Winston, Associate Professor, PI-study design, biochemical indicators
Dr. Jay C. Means, Professor, PI-study design, analytical **chemistry, cytogenetic** assays
Ms. Debra J. **McMillin**, Research Associate-analytical methods development
Dr. Miles Kirchin, Research **Associate-MFO** assays, EROD assays, **umu** assay
Dr. **Zuo-Mei Yan**, Research **Associate-MFO** assays, **cytogenetics**, microcosm studies

RELATED PUBLICATIONS AND PRESENTATIONS

The following publications which relate to the ongoing project have been published during the grant **period**:

Garcia-Martinez, P., **A.K.D. Hajos, D.R. Livingstone and G.W. Winston**. Metabolism and mutagenicity of **4-nitroquinoline N-oxide** by **microsomes** and **cytosol** of digestive gland of the mussel *Mytilus edulis* L., Marine Environmental Research. (In press). 1991.

Daniels, C.B. and J.C. Means. Assessment of the **genotoxicity** of produced water discharges associated with **oil** and gas production using a fish egg and **larval** test. Marine Environmental Research. **28:303-307**. 1990.

Daniels, C.B., C.B. Henry and J.C. Means. Coastal oil drilling produced **waters**: Chemical characterization and assessment of the genotoxicity using **chromosomal** aberrations in *Cyprinodon variegatus*. pp 356-371 in Aquatic Toxicology and Risk Assessment, eds. Landis and Van Der Schalle, ASTM, Philadelphia, PA, 1990.

Table 3. Aryl Hydrocarbon Hydroxylase (AHH) Activities of Devil's Swamp and Ben Hur Channel Catfish Liver Microsomes: Effect of alpha-Naphthoflavone (ANF).

Fish Sample	AHH (pmol min ⁻¹ mg ⁻¹)	-ANF	+ANF ^a	ANF Effect (%inhibition)
Devil's Swamp				
Male (4)		55.1 ± 7.2	6.3 ± 2.2	-89
Female (3)		41.5 ± 1.4	8.9 ± 1.9	-79
Ben Hur Farm				
Male (6)		8.5 ± 1.2	5.6 ± 1.2	-34
Female (4)		11.3 ± 1.9	5.9 ± 1.2	-48

^aANF concentration is 0.1 mM. ^bMean ±SD of four determinations made on pooled liver microsomes from the number of fish shown in parentheses. Values are for a 10 min incubation. AHH was assayed according to Nebert and Gelboin (1968).

PROPOSAL SUBMITTED AND GRANTS RESULTING

The following proposals have been submitted that relate to this ongoing grant proposal:

Design of a local **biomonitoring monitoring** program for oil **refinery** effluents **in** the San Francisco Bay-Delta **System**, June, 1991; Western States Petroleum **Association-FUNDED**.

Gulf of Mexico Offshore Operations Monitoring Experiment Phase I Sublethal Responses to Contaminant Exposure, June, 1991; US Minerals Management Service-**PENDING**.

