DOE Progress Report

1996- 1997

Environmental Analysis of Endocrine Disrupting Effects from Hydrocarbon Contaminants in the Ecosystem

The overall objective of the basic research grant is to characterize the potential of common hydrocarbon contaminants in ecosystems to act as endocrine disruptors. The three major lines of research include 1) a biotechnology based screening system to identify potential hormone mimics and antagonists. 2) an animal screening system to identify biomarkers of endocrine effects. and 3) a literature review to identify compounds at a variety of DOE sites that need to be examined for endocrine disrupting effects. By relating results obtained from this research project to contamination problems at various DOE sites. CBR will provide data and information on endocrine disrupting contaminants to DOE for consideration in risk analyses for determining clean-up levels and priorities needed at the sites.

One co-investigator. Dr. Steven Arnold. is no longer working on the project as he has left the Center for Bioenvironmental Research. His duties have been assumed by Dr. Ann Cheek. with the support of an additional technician contributing to the project.

I. Biotechnology Screening System

Objectives

Determine the ability of PAHs identified in Louisiana's Bayou Trepagnier to function as hormone mimics or antagonists at environmentally relevant concentrations.

Results

Yeast genetically engineered to produce human estrogen, progesterone, glucocorticoid, or thyroid hormone receptors were exposed to a pane1 of polyaromatic hydrocarbons (PAHs) known to occur in Bayou Trepagnier. The ability of the chemicals to interact with hormone receptors was determined by a colorimetric assay. From a panel of 22 PAHs, seven compounds that interact with steroid/thyroid receptors were identified. Concentrations at which these compounds interacted with receptors were below the concentrations measured in the sediments of Bayou Trepagnier, suggesting that PaH-induced endocrine disruption could occur at this site (Table 1). In general, the PaHs had inhibitory activity, antagonizing hormone-induced responses in the yeast system.

II. Preliminary animal screening

Objective.

Examine the ability of the yeast screen to predict hormonal activity of chemicals in whole animals. specifically frog tadpoles (*Xenopus laevis*).

Results.

Dibenz[a.h]anthracene (DBA) was identified as anti-estrogenic in the yeast screen (see Table 1). A tadpole assay was designed to assess the *in vivo* anti-estrogenicity of this compound. Estrogen delays tadpole metamorphosis. while thyroid hormone accelerates metamorphosis (Hayes. 1997, *Am. Zool.*, 37: 185-194). In the anti-estrogenicity assay. tadpoles were given estrogen alone (0.4 µM = 109 ppb), DBA alone (0.5 µM = 139 ppb), or DBA + estrogen in the absence of thyroid hormone and in the presence of thyroid hormone. Time to completion of metamorphosis (tail resorption) was measured. In this assay. estrogen tended to delay naturally occurring metamorphosis, but the effect was not statistically significant. Estrogen did significantly delay thyroid hormone induced metamorphosis. DBA alone did not cause mortality. nor did it alter the rate of metamorphosis. When DBA was given in combination with an inhibitory dose of estrogen. the estrogen-induced delay was reversed. suggesting that DBA interacts with the estrogen signaling pathway in animals.

III. Biomarker ana!ysis

Previous DOE supported work:

Since the cytokine IL-1ß with its associated type 1 receptor serves as an endocrine molecule linking the immune system and the nervous system. alterations in either component by exposure to environmental contaminants represents a form of endocrine disruption. In previous work supported by the DOE, we showed that 1) IL-1ß and its type 1 receptor are expressed in the developing nervous system of the frog, *Xenopus laevis* (Jelaso et al, 1998, *J. Comp. Neurol.*. in press). and 2) exposure of larval frogs to the organometal, methyl mercury chloride, depletes IL-1ß in specific neural structures (Jelaso etal., 1997, *Neurotoxicology*, 18 (3): 841-850) at very low environmcnta1 concentrations (50 ppb). Since IL-1ß regulates itself and the type 1 receptor in a feed-forward manner, depletion of IL-1 may have serious consequences for the frog's neuroimmune and endocrine systems.

Objectives of current work:

To create RNA probes to assess whether tadpoles (*Xenopus laevis*) exposed to organometals and/or hydrocarbon contaminants show altered regulation of IL-1 mRNA's.

Results:

Primers for polymerase chain reaction (PCR) were designed from the mouse IL-1ß cDNA sequence. The PCR product covered 487 base pairs of the 1339 total base pairs in the mouse cDNA (36%). The PCR product using frog cDNA as template was approximately 487 base pairs. This product was cloned into the pGl M-1 basy cloningvector and sequenced for use as a probe to screen a Xenopus cDNA library and for in situ hybridization and Northern blot analysis.

A *Xenopus* cDNA library is being probed for the full length IL-1ß cDNA which will be sequenced. Similar work will be carried out to create a probe for studying the frog IL-1 type 1 receptor. With these tools in

hand, future experiments will determine how contaminants alter IL-1ß and type 1 receptor gene expression. In addition. studies will characterize the acute and chronic consequences of alteration of IL-1ß system components on tadpole development and. ultimately. population fitness. an important issue since frog populations are in decline worldwide.

IV. Literature review and project integration

Science Applications International Corporation (SAIC) subcontracted with CBR and initiated work in February 1997.

Objectives:

Prepare a comprehensive study on contaminants, located at various DOE sites, that are the same as or have similar characteristics to the contaminants that CBR has identified as potential endocrine disruptors from Bayou Trepagnier.

To prepare a study of priority contaminants at DOE sites based on Site needs assessments.

To prepare an analysis to correlate the basic research and results to DOE/EM Focus Areas.

To coordinate with CBR to integrate the grant research results and information on CBR's Web page to show the overall significance of the research.

SAIC researched and reviewed information on Site needs from various DOE databases. reviewed various EM documents relating to clean-up goals and priorities. and initiated listing of priority contaminants at specific DOE sites. In the next year, SAIC will complete the literature review of studies on contaminants and priorities and will begin coordination work for integrating research results on the CBR's Web page. Environmental Concepts Made Easy.

V. Xavier Subgrant

Kinetic exclusion assays were developed on the KinExA™ instrument (Sapidyne Instruments, Inc., Boise, ID) to quantify the thermodynamics and kinetics of binding reactions between the human estrogen receptor and individual compounds that compete with 17-ß-estradiol for the hormone binding site on the receptor. Three different covalent conjugates of ß-estradiol with bovine serum albuman (BSA) were surveyed for their ability to capture and permit the quantification of free,unliganded estrogen receptor in the KinExA: the 17-hemisuccinate: the 6-(0-carboxymethyl)oxime; and the 3-(O-carboxymethyl)oxime derivatives. Each ß-estradiol-BSA conjugate was adsorption-coated onto polymethylmethacrylate beads and deposited in a

packed bed above the porous screen in the observation/flow ceil of the spectrofluorimeter. Solutions with different concentrations of human estrogen receptor (PanVera, Inc.. Madison. WI. or J. McLachlan. Tulane University) were then percolated through the beadpack. followed sequentially by a mouse monoclonal antibody directed against the receptor and a fluorescein-labeled goat anti-mouse antibody. The level of fluorescence observed from the beadpack was then used to quantify the number of receptor molecules captured from the solution. All three conjugates served to capture and quantify the free receptor in solution. The efficacy of capture was as follows: 6-CMO >. 17-hemisuccinate >> 3-CMO. Standard curves of instrument response as a function of the estrogen receptor concentration were linear up to 10 nM.

When reaction mixtures of the receptor and soluble ß-estradiol at equilibrium were subjected to separation and quantification on the KinExA, an equilibrium dissociation constant of 6×10^{-10} M was determined for the solution binding reaction regardless of the identity of the immobilized capture reagent on the beads. These promising preliminary data indicate that the KinExA can be exploited to quantify the interaction of any compound that binds to the estrogen receptor in a manner that is mutually exclusive with that of 17-ß-estradiol.

Similar efforts to devise a cell-free assay on the KinExA for the binding of hormone-stimulated estrogen receptor to its response element on DNA are in progress. Initial efforts to immobilize the DNA on the beads using biotinylated DNA and beads adsorption coated with avidin or strepavidin were unsuccessful. The principal problem was that each of these proteins appeared to lose significant levels of biotin-binding activity upon adsorption to the hydrophobic surface of the polymer beads. Adsorption of an anti-biotin monoclonal antibody to the beads retained significantly greater binding activity against biotinylated biopolymers. but the concentration of labeled DNA thus captured was still insufficient to drive the effective capture of hormone-stimulated receptor. Efforts to improve the immobilization procedures of DNA for assay improvement will continue. This is actually a more important assay to develop. since it measures both binding to the hormone receptor *and* the subsequent activation of the receptor to enable binding to the appropriate response element on the DNA.

VI. Publications:

Jin, L., D.Q. Tran, C.F. Ide, J.A. McLachlan, and S.F. Arnold. 1997. Several synthetic chemicals inhibit progesterone receptor-mediated transactivation in yeast. Biochem. *Biophys. Res. Commun. 233: 139-146.*

D.Q.Tran, C.F. Ide, J.A. McLachlan, and S.F. Arnold, 1997.The anti-estrogen activity of selected polynuclear aromatic hydrocarbons in yeast expressing human estrogen receptor. *Biochem. Biophys. Res. Comm., 229: 1 02- 108.*

Table 1.

Screening Results from the Biotechnology System. Lowest dose at which stimulatory (+) or inhibitory (-) activity occurred in a yeast-based assay measuring ability of the various PAHs to interact with each receptor. BTP = Bayou Trepagnier, nt = not tested, - = no activity.

PAH tested	Estrogen Receptor		Progesterone Receptor		Glucocorticoid Receptor		Thyroid Receptor		highest conc. in BTP (ppb)
	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	
acenaphthene	-	-	-	-	-	-	-	-	345
acenaphthylene	-	-	-	-	-	-	-	-	37
anthanthrene	-	-	-	-	nt	nt	-	-	not detected
anthracene	-	-	-	-	-	-	-	-	1922
2,3-benzanthracene	-	-	-	-	-	-	-	-	6267
benzo[b]fluoranthene	-	-	-	-	-	-	-	-	5243
benzo[j]fluoranthene	-	-	-	-	-	-	-	-	not detected
benzo[k]fluoranthene	-	-	-	-	-	-	-	-	1430
2,3benzofluorene	-	108 ppb	-	-	-	216 ppb	-	-	not detected
benzo[a]pyrene	-	126 ppb	-	-	-	-	-	-	9664
chrysene	-	-	-	-	-	-	-	-	23,590
6-hydroxychrysene	-	246 ppb	-	123 ppb	-	-	-	-	not detected
dibenz[a,c]anthracene	-	-	-	-	nt	nt	-	-	
dibenz[a,h]anthracene	-	139 ppb	-	-	-	-	-	-	4330
7,12-dimethylbenz(a)anthracene	-	-	-	-	nt	nt	-	-	
fluoranthene	-	-	-	-	-	-	-	-	2353
fluorene	-	-	-	-	-	-	-	-	114
1-hydroxypyrene	-	-	-	218 ppb	-	-	-	-	not detected
indeno[1,2,3,cd]pyrene	-	-	-	-	-	-	-	-	7018
naphthalene	-	-	-	-	-	-	128 ppb	-	236
phenanthrene	-	-	-	-	-	-	-	-	1916
6,8dimethylbenzanthracene	nt	nt	nt	nt	-	256 ppb			not detected