EVOSTC ANNUAL PROJECT REPORT

Recipients of funds from the *Exxon Valdez* Oil Spill Trustee Council must submit an annual project report in the following format by **Sept. 1 of each fiscal year** for which project funding is received (with the exception of the final funding year in which a final report must be submitted). **Please help ensure that continued support for your project will not be delayed by submitting your report by Sept. 1. Timely receipt of your report allows more time for court notice and transfer, report review and timely release of the following year's funds.**

Satisfactory review of the annual report is necessary for continuation of multi-year projects. Failure to submit an annual report by **Sept. 1** of each year, or unsatisfactory review of an annual report, will result in withholding of additional project funds and may result in cancellation of the project or denial of funding for future projects. **PLEASE NOTE:** Significant changes in a project's objectives, methods, schedule, or budget require submittal of a new proposal that will be subject to the standard process of proposal submittal, technical review, and Trustee Council approval.

Project Number: 090841

Project Title: ..... CYP1A1 Gene Expression Verification Study

PI Name: ..... A. Keith Miles, Liz Bowen, & Brenda Ballachey

Time period covered: ..... 7/2009 – 8/2009

Date of Report: ..... 1 September 2009

Report prepared by: ..... A. Keith Miles

Project website (if applicable): ..... N/A

Work Performed: Summarize work performed during the reporting period, including any results available to date and their relationship to the original project objectives. Explain deviations from the original project objectives, procedural or statistical methods, study area or schedule. Also describe any known problems or unusual developments, and whether and how they have been or can be overcome. Include any other significant information pertinent to the project.

We have accomplished the first objective (Whether mRNA can be successfully extracted from PBMC samples) of Phase One of the study. We have processed 80 archived, matched PBMC (peripheral blood mononuclear cells) and liver samples from individual sea otters for a total of 160 samples, which were collected from 2003 to 2006. PBMCs were isolated from heparinized whole blood in the field, by density gradient centrifugation, and cryopreserved at -80C. We assume that when collected, all samples were handled properly according to established protocols. Samples were held at the Snyder laboratory at Purdue University until early in 2009, when they were shipped to Miles and Bowen at UCDavis. Procedures conducted on samples during the period of this report follow those outlined in detail in the project proposal. Preliminary findings indicate that most of the mRNA from the PBMC and liver samples will be viable. We are in the process of completing the second objective, to determine whether or not gene expression in the PBMC samples will correlate with that in the liver tissue (expected to be more reliable). Analyses are ongoing on 80 PBMC samples. There are 12 genes of interest that we are evaluating in PBMCs and 15 in liver samples (Table 1), representing 10 different physiological systems. One slight deviation from the proposed study design is that, based on preliminary assays, we believe samples can be run in duplicate rather than triplicate (as outlined in the proposal), which will increase the efficiency of processing samples while still maintaining a high standard of quality control.

Table 1. Genes successfully sequenced for expression analysis in otters. These genes play a role in immuno-modulation, inflammation, cyto-protection, tumor suppression, reproduction, cellular stress-response, metal metabolism, xenobiotic metabolizing enzymes, antioxidant enzymes, and cell-cell adhesion. The S9 is an endogenous reference gene (aka housekeeping gene) used to normalize for varying quantities of RNA characteristic of individual organisms.

Genes of interest	*Gene function*
Aryl hydrocarbon receptor	*Responds to classes of environmental toxicants including polycyclic aromatic hydrocarbons, polyhalogenated hydrocarbons, dibenzofurans, and dioxin*
Heat shock protein 70	*Produced in response to thermal or other stress*
Interleukin-2	*Increases the growth and activity of T and B lymphocytes*
Interleukin-18	*Proinflammatory cytokine*
Interleukin-10	*Anti-inflammatory cytokine*
Cox 2	*Cyclooxygenase-2 catalyzes the production of prostaglandins which are responsible for promoting inflammation*
S9	*18S ribosomal subunit (housekeeping gene)*
Metallothionein (liver samples only)	*Modulates the bioavailability of physiological cations and the toxicity of heavy metals and modulate immune functions*
Complement cyt inhibitor	*Protects against cell death*
HDCMB21P	*(Translationally controlled tumor protein) implicated in cell growth, cell cycle progression, malignant transformation and in the protection of cells against various stress conditions and apoptosis*
DRB	*Binding of pathogens/initiation of immune response*
Thyroid hormone receptor	*Hormone-activated transcription factors bind DNA in the absence of hormone, usually leading to transcriptional repression*
CIRBP (liver only)	*Cold-shock protein; responds to cold temperature stress*
Mx-1	*Responds to viral infection*
Cytochrome P450	*Responds to xenobiotics*

Future Work: Summarize work to be performed during the upcoming year, if different from the original proposal. Describe any proposed changes in objectives, procedural or statistical methods, study area or schedule. *NOTE: Significant changes in a project's objectives, methods, schedule or budget require submittal of a new proposal subject to the standard process of proposal submittal, technical review and Trustee Council approval.*

As stated above, for Objective 1 is essentially complete, including quality control, i.e. verification of RT-PCR results with triplication of runs if the target gene and endogenous control are not similar. We anticipate completion by the target date of 30 September 2009. Work on Objectives 2 (Whether gene expression in PBMC samples correlates well with that in liver tissue, the latter expected to be more dependable), 3 (Whether expression of targeted genes in samples of otters from Knight [oiled area] differs from those from Montague Island [unoiled area]) and 4 (The relationship of gene expression in 2006 and 2007 blood samples collected using Paxgene tubes to archived PBMC and liver samples) is continuing as scheduled. Products produced are not anticipated to deviate from those outlined in the project proposal. A draft report of objectives 2 – 4 for Phase One of the project will be completed by December 2009. At that point, Phase Two can begin.

Coordination/Collaboration: Describe efforts undertaken during the reporting period to achieve the coordination and collaboration provisions of the proposal, if applicable.

We are close to initiating efforts to analyze the data in relation to origin of samples, and will collaborate with personnel at the Alaska Science Center as needed. To avoid any bias on interpretation, information on location of animals sampled (Knight versus Montague) will not be provided to Drs. Bowen and Miles (at UCDavis) until laboratory analytical procedures are completed. Co-author Stott (at UCDavis) will be engaged to confirm interpretation of results.

Community Involvement/TEK & Resource Management Applications: Describe efforts undertaken during the reporting period to achieve the community involvement/TEK and resource management application provisions of the proposal, if applicable.

There were no community involvement/TEK or resource management activities during FY2009.

Information Transfer: List (a) publications produced during the reporting period, (b) conference and workshop presentations and attendance during the reporting period, and (c) data and/or information products developed during the reporting period. *NOTE: Lack of compliance with the Trustee Council's data policy and/or the project's data management plan will result in withholding of additional project funds, cancellation of the project, or denial of funding for future projects.*

No information was disseminated during the specified reporting period.

Budget: Explain any differences and/or problems between actual and budgeted expenditures, including any substantial changes in the allocation of funds among line items on the budget form. Also provide any new information regarding matching funds or funds from non-EVOS sources for the project. *NOTE: Any request for an increased or supplemental budget must be submitted as a new proposal that will be subject to the standard process of proposal submittal, technical review, and Trustee Council approval.*

Work in FY2009 was conducted within budget. However, we request expenditures of FY2009 funds into FY2010 to complete objectives 2 – 4 on Phase One before beginning Phase Two.

We can accept your annual report as a digital file (Microsoft Word or WordPerfect), with all figures and tables embedded. Acrobat Portable Document Format (PDF) files (version 4.x or later) are also acceptable; please do not lock PDF files or include digital signatures.

Please submit reports electronically in ProjectView or by email to catherine.boerner@alaska.gov. Also, please be sure to post your annual report on your own website, if you have one.

We appreciate your prompt submission of your annual report and thank you for your participation.