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:..... 10100839

*Project Title: ..... Evaluating injury to harlequin ducks (**Histrionicus histrionicus**) caused by sublethal hydrocarbon exposure in Prince William Sound using species-specific cell lines*

PI Name:..... Hollmen, T. and Springman, K.

Time period covered: ..... October 1, 2009 – August 31, 2010

Date of Report:..... September 1, 2010

Report prepared by: ..... Hollmen, T., Springman, K., and Riddle, A.

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

Work Performed:

Objective 1: *Develop harlequin duck and surrogate (mallard) cell lines to evaluate injury from site-specific hydrocarbons in harlequin ducks*

FY10 Tasks: Year 3 cell line isolation

Progress:

Hepatocyte cell extractions were performed from 78 mallard (MALL), 7 harlequin duck (HADU), and two Barrow's goldeneye (BAGO) eggs. BAGO cells were tested as another potential surrogate for HADU. We continued to use the extraction protocols developed during Year 1 and 2 of the study.

Fibroblasts cell extractions were performed from two BAGO eggs, also using protocols developed previously. Forty stock vials of approximately 1.5 x 10^6 cells were archived.

Objective 2: *Develop bioassays using harlequin duck and surrogate (mallard) cell lines to assess and quantify injury due to lingering oil in PWS*

FY10 Tasks: Endpoint bioassay development

Progress:

We continued development and validation of bioassays. Our current bioassay panel consists of four assays to evaluate cellular, enzymatic, and genetic toxicity: cytopathic effect (CPE), ethoxyresorufin-*o*-deethylase (EROD) assay, lactate dehydrogenase (LDH) release, and PAH/DNA adduct formation. The CPE, EROD, and LDH assays are validated for use in testing; PAH/DNA adduct assay validation is in progress. We anticipate continuing the validation process for this assay during fall 2010. We have focused our attention to the development and validation of these four bioassays, which will offer a panel of tests to evaluate potential effects of samples collected from PWS at the levels set as objectives of our study; i.e. cellular, enzymatic, and genetic. A summary of each bioassay follows.

CPE: Results from Year 2 were compiled and a scoring data sheet was developed. This sheet, which incorporated a scoring system for observed cellular changes, was implemented in Year 3 laboratory work. Currently, data entry and analysis are underway for Year 3 CPE results.

EROD: Ethoxyresorufin-*o*-deethylase assay (EROD) is the metric of choice for assessing exposure to planar hydrocarbons, such as those of toxicological concern in oil for its sensitivity and reliability. We have developed protocols and validated them in cell lines from several species to generate consistent results. The protocols are modifications of standard protocols that are currently in use in several laboratories. Our findings show that the protocols for *in vitro* assessments are reliable and easy to follow.

LDH: Lactate dehydrogenase (LDH) is an assay that we believe complements the use of EROD for the examination of other effects beyond those associated with the biotransformation of planar hydrocarbons. It is a measure of cellular damage that has shown to be reliable. We are using a standardized kit to generate reproducible results.

PAH/DNA Adducts: When biotransformed by Cytochrome P450 1A, planar hydrocarbons can become activated. This is to say that those PAH which are known or suspected human carcinogens are at this point capable of binding to genetic material, or DNA. This binding can be visually detected and scored, according to technology available at the National Cancer Institute (NCI). We have attempted to generate these adducts using the cell lines on hand, and our results have been promising. The most limiting factor in repeating this has been the number of cells required and availability of adequate HADU source materials.

Objective 3: *Evaluate injury due to site-specific lingering oil in PWS in harlequin ducks at the cellular level*

FY10 Tasks: Test PWS samples in cell lines, data analysis

Progress:

Mallard and Barrow's goldeneye hepatocyte bioassay results

Results obtained in Year 2 were a sound point from which to expand, filling in data gaps. It was hoped that in Year 3, all necessary data would be acquired. Unfortunately, we were not able to secure adequate amounts of harlequin duck source material (eggs), and were not able to conduct all planned bioassays using cells derived from this species.

A summary of work conducted in mallard and Barrow's goldeneye cell lines during the reporting year is outlined below. We continued using mallard cell lines as reference and surrogate for assay validation and testing procedures. Barrow's goldeneye cells were used and tested as another potential surrogate for harlequin duck cells.

Mallard hepatocyte responses

This year, we used site-specific extracts (SPMD), as well as repeated reference materials (bioavailable ANS crude) and controls (various doses of chrysene and ß-naphthoflavone (BNF)). The results obtained were similar to those that were produced earlier with this cell type and these controls.

Analysis of SPMD extracts has begun with MALL hepatocyte cells, and indicated that there is a correlation between EROD response and the extract used. These will be replicated for assurance of response, and final analysis will be reported with data that have been validated.

Barrow's goldeneye hepatocyte responses

Hepatocyte cells from BAGO sources produced robust cell lines that reflected the results obtained with HADU in previous tests. Their sensitivity to chrysene and BNF will be reported when all other data are assembled. These tests will be repeated for replication.

Summary

Barrow's goldeneye hepatocyte responses were similar to those of harlequin duck cells. Initial results show that they are more sensitive to controls than either rainbow trout or mallard, but these results will be replicated prior to data submittal.

Objective 4: *Link analytical chemistry results from known oil-contaminated sites to injury assessments in harlequin ducks at the cellular level*

FY10 Tasks: Data analysis

Progress:

Integration of MALL hepatocyte results with analytical chemistry

The analytical chemistry results are similar to those previously reported from these sites (Short et al., 2008), which is not surprising as all parameters, save the date of sampling, were the same. The first set of EROD results obtained in Year 3 with MALL hepatocyte cells with SPMD extracts show expected responses that correspond with the findings from testing with extracts from those sites in other

species; these have been thoroughly discussed in other EVOSTC-funded research (Short et al., 2008; Springman et al., 2008).

A detailed analysis and discussion of MALL, BAGO and HADU hepatocyte responses will be conducted when all analytical assay results are available.

Objective 5: *Develop methods to link injury due to site-specific lingering oil in PWS in harlequin duck cell lines to harlequin duck population parameters and population level impact*

FY10 Tasks: Data analysis

Progress:

Work under this objective will be supported by results obtained under objective 3, and methods development will continue in conjunction with further analysis of laboratory results.

Future Work:

We will continue data analysis as described in our work plan and proposal. We will need to continue work on validation on one of our bioassays during the upcoming year. During the current reporting year, we were unable to secure adequate source materials from harlequin ducks to conduct all planned testing and repeats of samples collected from PWS in harlequin duck hepatocyte lines, so some additional work is required to complete testing of samples in these cell lines.

Coordination/Collaboration:

We continued coordination and collaboration with NCI/NIH on validation of bioassays of genetic toxicity, and with Dr Dan Esler on testing of samples from PWS.

Information Transfer:

A poster presentation of our project was developed to describe general information about our project to the public visiting the Alaska SeaLife Center. The poster is on display outside of our laboratory. The principal investigators attended the Alaska Marine Science Symposium in Anchorage in January 2010.

Literature Cited

Kathrine R. Springman, Jeffrey W. Short, Mandy R. Lindeberg, Jacek Maselko, Colin Khan, Peter V. Hodson, Stanley D. Rice (2008). Semipermeable membrane devices link site-specific contaminants to effects: Part I - Induction of CYP1A in rainbow trout from contaminants in Prince William Sound, Alaska. *Marine Environmental Research* 66 (5): 477-486.

Jeffrey W. Short, Kathrine R. Springman, Mandy Lindeberg, Jacek Maselko, Colin Khan, Peter Hodson, Margaret Krahn, Stanley D. Rice (2008). Semipermeable membrane devices link site-specific contaminants to effects: Part II - a comparison of lingering *Exxon Valdez* oil with other potential sources of cytochrome P4501A inducers in Prince William Sound, Alaska. *Marine Environmental Research* 66 (5): 487-498.