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, 2010 – August 31, 2011

Date of Report: ..... September 1, 2011

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*

FY11 Tasks: Year 4 cell line isolation

Progress:

Cell culture methods were developed during the previous study periods. In 2011, we performed hepatocyte cell extractions from 41 mallard (MALL) and 10 harlequin duck (HADU) eggs using extraction protocols developed during Year 1 and 2 of the study.

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

FY11 Tasks: Bioassay validation

Progress:

Validation of bioassays with standard cell lines has been completed. In our assays we used hepatocytes from MALL and HADU as well as commercially-available rainbow trout hepatocytes. These cells were tested in EROD bioassays with reference material, positive controls, solvent controls, media controls, and SPMD extracts from several sites in Prince William Sound. A summary of each of the test material follows.

Reference material: we used ANS crude oil, both neat and synthetically weathered, that was dialyzed through LDPE (low-density polyethylene) membranes and concentrated to determine the fraction of organic compounds capable of membrane passage, i.e. the bioavailable fraction.

SPMD extracts: these were SPMD extracts from various locations in Prince William Sound including Zaikoff, Disk Island, Eleanor Island, Herring Bay, and Bay of Isles, as well as the field blank used during deployment and retrieval of SPMD. As with reference material, these samples are being analyzed and the findings synthesized with analytical chemistry results.

PAH/DNA Adducts: despite promising results, the limiting factor here has been the number of cells required and availability of adequate HADU source materials as discussed in previous reports. In 2011, MALL hepatocyte cells were dosed with 2 site-specific extracts (SPMD) for the PAH/DNA adduct assay. The cell pellets, which ranged from 1×10^6- 2.5×10^6 MALL hepatocyte cells, will be forwarded to National Cancer Institute (NIH/NCI)for analysis. We look forward to completing these analyses in conjunction with NIH/NCI this fall.

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

FY11 Tasks: Test PWS samples in cell lines, data analysis

Progress:

We continued laboratory testing of samples in HADU cell lines. Results obtained with HADU cell lines in FY 11 were improved with the available cells, which were more numerous and robust. Data summaries and analyses of the assays are underway at present.

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

FY11 Tasks: Data analysis

Progress:

We requested an extension for our project in 2010 due to inadequate amount of biological source materials for required harlequin duck cell lines. This year we acquired material that produced more robust, reliable cell lines. These cell lines were tested in the bioassays with site-specific SPMD extracts, as well as a suite of controls (i.e. field blanks, media, solvent, assay and positive controls). These cell lines were used in the battery of bioassays and examinations to assess potential injury from lingering oil in HADU cell lines. Previous work included a preliminary integration of analytical chemistry data test material with the results obtained in MALL and rainbow trout hepatocytes. We will be integrating the HADU data with the chemistry data in a similar manner and comparing results between the different species.

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*

FY11 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:

Data analysis is underway. The analytical chemistry of all sites will be integrated with the data sets from MALL, HADU, and rainbow trout. Species comparisons and the nature of interactions between different homologous series will be examined. Synthesis of data sets and reporting of our findings remain for FY 12.

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:

The principal investigator attended the Alaska Marine Science Symposium in Anchorage in January 2011.

The following abstracts were presented during the reporting period:

Riddle AE, Hollmén TE, Springman K. (2011). *Poster*: Developing Cell Culture Methods and Bioassays for Harlequin Duck (*Histrionicus histrionicus*) Research. *In* proceedings of the Alaska Marine Science Symposium, Anchorage, AK, January 17-21, 2011.

Riddle AE, Hollmén TE, Springman K. (2011). Developing Cell Culture Methods and Bioassays to Assess Toxicological Responses in Harlequin Ducks (*Histrionicus histrionicus*). *In* proceedings of the Alaska SeaLife Center Research Colloquium, Seward, AK, April 6-7, 2011.