PROPOSAL SIGNATURE FORM

THIS FORM MUST BE SIGNED BY THE PROPOSED PRINCIPAL INVESTIGATOR AND SUBMITTED ALONG WITH THE PROPOSAL. If the proposal has more than one investigator, this form must be signed by at least one of the investigators, and that investigator will ensure that Trustee Council requirements are followed. Proposals will not be reviewed until this signed form is received by the Trustee Council Office.

By submission of this proposal, I agree to abide by the Trustee Council's data policy (Trustee Council Data Policy*, adopted March 17, 2008) and reporting requirements (Procedures for the Preparation and Distribution of Reports**, adopted June 27, 2007).

PROJECT TITLE:	**CYP1A1 Gene Expression Verification Study – Re-Evaluation of Sea Otter Samples from the *Exxon Valdez* Oil Spill**
Printed Name of PI	**A. Keith Miles**
Email:	**keith_miles@usgs.gov**
Mailing Address	**USGS Davis Field Station 1 Shields Ave, UC Davis**
City, State, Zip	**Davis, CA 95616-5224**
Phone:	**530-752-5365**
Signature of PI:	_____ Date: _____
Printed Name of PI	**Liz Bowen**
Email:	**lbowen@usgs.gov**
Mailing Address	**USGS Davis Field Station 1 Shields Ave, UC Davis**
City, State, Zip	**Davis, CA 95616-5224**
Phone:	**530-752-5365**
Signature of PI:	_____ Date: _____
Printed Name of PI	**Brenda Ballachey**
Email:	**bballachey@usgs.gov**
Mailing Address	**USGS Alaska Science Center 4210 University Dr.**
City, State, Zip	**Anchorage, AK 99508-4626**
Phone:	
Signature of PI:	_____ Date: _____

* www.evostc.state.ak.us/Policies/data.cfm
** www.evostc.state.ak.us/Policies/reporting.cfm

FY09- FY11 Proposal
PROPOSAL SUMMARY PAGE

Project Title: CYP1A1 Gene Expression Verification Study – Re-Evaluation of Sea Otter Samples from the *Exxon Valdez* Oil Spill

Project Period: 1 June 2009 – June 30, 2011

Primary Investigator(s): A. Keith Miles and Liz Bowen, USGS Western Ecological Research Center (WERC), Brenda Ballachey, USGS Alaska Science Center, Michael Johnson, University of California Davis, Robin Keister USGS WERC, James Bodkin USGS Alaska Science Center, Jeff Stott, UC Davis

Study Location: Western Prince William Sound

Abstract: Sea otter populations in western PWS were injured as a result of the *Exxon Valdez* oil spill, with evidence for both immediate acute mortality and longer term injury from chronic exposure to oil spilled in 1989. The EVOS Trustee Council funded over a decade of studies to identify progress toward recovery of the sea otter populations, particularly in the northern Knight Island Archipelago. These projects have addressed population demographics including abundance, habitat use, and survival rates, together with biological sampling to monitor body condition using blood parameters, liver pathology, and a CYP1A biomarker to determine oil exposure. Although population abundance data indicate some level of recovery in Prince William Sound overall, recovery remained incomplete as of 2006. Recently, a 2002 report (Snyder et al.) of the CYP1A biomarker assessments of sea otter exposure to oil has been questioned, making it necessary to reevaluate this method for assessing exposure. In this study, we propose to re-test the exposure of sea otters to lingering oil by applying our recent discoveries of sea otter specific genetic primers to measure gene expression on the archived samples from these projects. Our initial studies of mink experimentally exposed to oil identified genes that were significantly altered in expression (Bowen et al. 2007). 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. We have successfully sequenced 13 genes from sea otters that were expressed in mink experimentally exposed to oil, as well as 2 additional genes that aid interpretation of stress levels in animals exposed to xenobiotics that include aromatic hydrocarbons. In phase one of the project, we will analyze the gene expression of a suite of genes from archived Peripheral Blood Mononuclear Cells (PBMC) and liver samples collected from individual sea otters in 2003-2006. If these PBMC samples produce meaningful analytic results, the project will proceed with phase two, to analyze the gene expression in PBMC samples from 1996 through 2002. This study will allow us to verify our past understanding of oil exposure of sea otters in PWS, assess the current status of recovery, and provide a reliable method for assessing recovery in the future.

Estimated Budget:
EVOS Funding Requested *(must include 9% GA)*

FY09	FY10	FY11	FY12	Total
$75,539.20	$115,959.20	$13,236.10	0	$205,734.40

Non-EVOS Funds to be used:

FY10	FY11	FY12	FY13	Total

PROJECT PLAN

CYP1A1 Gene Expression Verification Study – Re-Evaluation of Sea Otter Samples from the *Exxon Valdez* Oil Spill

A. Keith Miles and Liz Bowen, USGS Western Ecological Research Center, Brenda Ballachey, USGS Alaska Science Center, Michael Johnson, University of California Davis, Robin Keister USGS Western Ecological Research Center, James Bodkin USGS Alaska Science Center, Jeff Stott, University of California Davis

I. NEED FOR THE PROJECT

A. Statement of Problem

The *Exxon Valdez* Oil Spill (EVOS) Trustee Council has funded multiple studies to determine long term injury to sea otters from the 1989 *Exxon Valdez* oil spill in Prince William Sound, Alaska. One such study was entitled 'CYP1A1 Gene Expression in Sea Otters (*Enhydra lutris*): a Quantitative Reverse Transcriptase - Polymerase Chain Reaction to Measure CYP1A mRNA in Peripheral Blood Mononuclear Cells (Snyder, 2002). The Snyder report concluded that sea otters from areas that were heavily impacted by oil in 1989 had a higher biochemical signature than those from an unoiled reference area. In 2008 when we examined the cytochrome (CYP) P450 CYP1A1 sequences identified by Snyder et al, we did not find alignment with mammalian CYP1A1 sequences found in Genbank[1]. Further, a recent published study questioned the reproducibility of the findings associated with this report (Hook et al. 2008). We propose to re-examine the archived samples used in the Snyder study using a novel suite of genes (including CYP1A1) we have identified in sea otters to verify exposure in sea otters to oil lingering from the spill. These genes were expressed in mink exposed to crude oil in an experimental laboratory study (Bowen et al. 2007). The archived samples are splits of those that were analyzed in the Snyder study, and came from sea otters that were sampled in Prince William Sound between 1996 and 2006 under a series of EVOS restoration projects by Bodkin and Ballachey (including projects 99025, 030585, 040423, 040620 and 050775).

The Snyder report also stated that analysis of the expression of CYP1A1 in peripheral blood mononuclear cells (PBMC) by RT-PCR represented a sensitive method for evaluating potential exposure to environmental contaminants. However, several studies question the effectiveness of using peripheral blood for the detection of expression of CYP genes (Furukawa et al. 2004, Finnström et al. 2001), and that analysis of CYP1A genes alone without other bioindicators can

[1] GenBank is the National Institute of Health genetic sequence database, an annotated collection of all publicly available DNA sequences. Each GenBank entry includes a concise description of the sequence, the scientific name and taxonomy of the source organism, and a table of features that identifies coding regions and other sites of biological significance, such as transcription units and sites of mutations or modifications. Bibliographic references are included with a link to the Medline unique identifier for all published sequences.

produce spurious conclusions (Wirgin et al. 1996). Apparently, the efficacy of the CYP1A1 gene can be ephemeral in blood and reliability is variable, depending on the species (S. Teh, Toxicological Pathologist, University of California, Davis, personal communication). Liver is the preferred tissue for less variable results but understandably can be problematic to obtain. Based on experimental evidence (Bowen et al. 2007), we now recommend analysis of a suite of genes that indicate exposure and injury directly related to hydrocarbons (including the aryl hydrocarbon gene or AhR).

The primary investigators of the current proposal published a study of expression of a suite of immunologic functional genes in American mink exposed to Bunker C fuel oil (Bowen et al. 2007; Schwartz et al. 2004a,b). The genetic primers that we identified for the mink aligned closely with those for sea otters as confirmed in examination of primers in GenBank. Subsequently we have discovered and identified sea otter-specific primers of the targeted genes. We have also taken advantage of advancements in technology of human medicine by application of Paxgene® blood tubes, which immediately stabilize and preserve mRNA without an urgent need for freezing. Further, the ease of extraction, amplification, replication, and measure of the targeted genes using Real-time Polymerase Chain Reaction (RT PCR) have greatly improved over the last decade.

B. Relevance to 1994 Restoration Plan Goals and Scientific Priorities

The sea otter population in western Prince William Sound has not reached the recovery goal set by the Trustees in the 1994 Restoration Plan. The most recent recovery objective for sea otters states that the otters will have recovered when the population in oiled areas returns to prespill levels and distribution, and when biochemical indicators of hydrocarbon exposure in otters in the oiled areas are similar to those in otters in unoiled areas (*Exxon Valdez* Oil Spill Trustee Council, 2006). This project proposes to use gene expression to re-evaluate the samples taken to determine exposure to oil from sea otters in Prince William Sound from 1996 through 2006, using the archived sea otter samples that had previously been tested for the CYP1A1 biochemical indicator. The results of this project, along with the results of the other aspects of the sea otter research projects, are necessary to re-assess the recovery of sea otters as defined in the 1994 Plan.

II. PROJECT DESIGN

A. Objectives

The overall goal of this project is to determine if there is biochemical evidence in sea otters for exposure to oil following the 1989 oil spill. In Phase One of the proposed study, we will analyze gene expression of a suite of genes in archived, matched samples of PBMC and liver from individual sea otters collected from 2003 through 2006. If defendable results are obtained particularly from the older PBMC samples, then Phase Two of this proposal is to investigate gene expression in PBMC samples from 1996 through 2002. The specific objectives of this project are to determine:

1. whether mRNA can be successfully extracted from PBMC samples.
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).
4. The relationship of gene expression in 2006 and 2007 blood samples collected using Paxgene tubes to archived PBMC and liver samples.

B. Procedural and Scientific Methods

There are more than 639 PBMC and 84 liver samples still available in the archives of samples collected by Bodkin and Ballachey from sea otters in Prince William Sound between 1996 and 2006. An approximate count (i.e., sample identification numbers are diverse and will require verification) indicated that these samples represent tissues from approximately 260 sea otters captured between 1996 and 2006, and that for many otters, there are multiple (2 – 4) PBMC samples collected from the same individual and for some individuals, samples were collected in multiple years.

In Phase One of the project we will extract mRNA from 84 paired liver and PBMC samples for a total of 168 samples collected from 2003 - 2006. Peripheral blood mononuclear cells were isolated in the field from heparinized whole blood by density gradient centrifugation, following procedures outlined by the Snyder testing laboratory at Purdue University, and then the isolated cells were cryopreserved. We are relatively confident that the liver samples, which were cryopreserved immediately after collection, will produce good results. However, any variation in the field handling procedures, particularly of PBMC samples, could cause variability in results or difficulty in analysis. We will experiment with different techniques until we are satisfied with the reliability of the results obtained from the PBMC samples. Because we have replicate PBMC samples from a number of sea otters, we can conduct such methodological experiments without losing samples for the contaminant analysis.

If our results of mRNA quantification from PBMC blood from Phase 1 are successful, we then recommend determining minimal sample size required to achieve statistically acceptable variance. We project that approximately 20 samples per year per oiled and reference site would suffice from 1996 to 2002 (8 years, 320 samples total).

Our initial studies on mink identified genes that were significantly altered in expression by exposure to oil. 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. We have successfully sequenced 13 genes from sea otters that were the same as those expressed in mink experimentally exposed to oil (Bowen et al. 2007), as well as 2 additional genes that aid interpretation of stress levels in animals exposed to xenobiotics that include aromatic hydrocarbons found in crude oil. These genes include: aryl hydrocarbon receptor (AhR), CYP1A1, heat shock protein-70, Interleukin 2, Interleukin 5, Interleukin 10, Interleukin 18, Cox-2, complement cytolysis inhibitor, HDCMB21P (tumor formation), thyroid hormone receptor,

DRB (bacterial), S9, metallothionein, Mx-1 (viral), and cold shock protein (CIRBP). Descriptions of the function of these genes are provided in Table 1. The S9 is an endogenous reference gene (also called a housekeeping gene) used to normalize for varying quantities of RNA characteristic of individual organisms.

Table 1. Differential expression of genes (of interest in the proposed study) between oil-fed and control mink deduced by quantitative PCR (Bowen et al. 2007). Standard t-tests were performed and statistical significance ($P \leq 0.05$) indicated by *. Arrows indicate direction of expression difference between mink groups. Additional genes of interest are indicated by +.

Genes of interest	P value Spleen	P value CPT	Oiled mink	Gene function
Aryl hydrocarbon receptor	0.17	0.19	↓	Responds to classes of environmental toxicants including polycyclic aromatic hydrocarbons, polyhalogenated hydrocarbons, dibenzofurans, and dioxin (Oesch-Bartlomowicz et al. 2005)
Heat shock protein 70	0.02*	0.10	↑	Produced in response to thermal or other stress (Tsan and Gao, 2004)
Interleukin-2	0.04*	0.16	↑	Proinflammatory cytokine (Goldsby et al. 2003)
Interleukin-18	0.002*	0.29	↓	Proinflammatory cytokine (Goldsby et al. 2003)
Interleukin-10	0.02*	0.34	↓	Anti-inflammatory cytokine (Goldsby et al. 2003)
Cox 2	0.31	0.047*	↓	Cyclooxygenase-2 catalyzes the production of prostaglandins which are responsible for promoting inflammation (Goldsby et al. 2003)
S9	housekeeping gene			18S ribosomal subunit
Metallothionein		0.21	↑	modulate the bioavailability of physiological cations and the toxicity of heavy metals and modulate immune functions (Andrews 2000)
Complement cyt inhibitor	0.05*	0.25	↓	Protects against cell death (Jenne and Tschopp 1989)
HDCMB21P	0.07	0.13	↓	(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 (Bommer and Thiele, 2004)
DRB	0.45	0.27	=	Binding of pathogens/initiation of immune response (Goldsby et al. 2003, Bowen et al. 2006)
Thyroid hormone receptor	0.16	0.01*	↓	hormone-activated transcription factors bind DNA in the absence of hormone, usually leading to transcriptional repression (Tsai and O'Malley, 1994)
CIRBP	0.33	0.44	↑	Cold-shock protein (Nishiyama et al. 1997); responds to cold temperature stress
Interleukin-5	+	+	+	Up-regulated in response to parasites (Maizels and Balic 2004)
Mx-1	+	+	+	Responds to viral infection. (Tumpey et al. 2007)

C. Data Analysis and Statistical Methods

RNA from PBMC and liver will be isolated according to manufacturer's standard protocols (silica-based microspin technology). The extracted RNA will be treated with 10 U/µl of RNase

free DNase I (DNase, Amersham Pharmacia Biotech Inc, Piscataway, NJ) to remove contaminating gDNA at 37°C for 20 min followed by heat inactivation at 95°C for 5 min and chilling on ice. Extracted RNA will be stored at -80°C until processing and analysis.

A standard cDNA synthesis will be performed on 2 µg of RNA template from each sample. Reaction conditions will include 4 units reverse transcriptase (Omniscript®, Qiagen, Valencia, CA), 1 µM random hexamers, 0.5 mM each dNTP, and 10 units RNase inhibitor, in RT buffer (Qiagen, Valencia, CA). Reactions will be incubated for 60 minutes at 37°C, followed by an enzyme inactivation step of 5 minutes at 93°C and stored at –20°C until further analysis.

Quantitative RT-PCR: Real-time PCR systems for sea otter S9 and target genes will be run in separate wells. cDNA will be examined using an intercalating fluorescent dye PCR (Bowen et al. 2006). Each reaction will contain 500ng DNA in 25µl volumes with 20pmol SSP, Tris-Cl, KCl, $(NH_4)_2SO_4$, 2.5mM $MgCl_2$ (pH 8.7), dNTPs, HotStar Taq DNA Polymerase (Quantitect SYBR Green PCR Master Mix, Qiagen, Valencia, CA), and 0.5 units uracil-N-glycosylase (Roche, Indianapolis, IN). Amplifications will be performed in an ABI 7300 RT PCR System (Applied Biosystems, California) under the following conditions: two minutes at 50°C, followed by 15 minutes at 95°C, and 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, with a final extension step of 72°C for 10 minutes. Reaction specificity will be monitored by melting curve analysis using a final data acquisition phase of 60 cycles of 65°C for 30 seconds and verified by direct sequencing of randomly selected amplicons (Bowen et al. 2007). Final quantitation will be done using the comparative C_T method and is reported as relative transcription. All samples are performed in duplicate on the ABI 7300 RT PCR System.

Gene expression will be analyzed by relative quantitation, using the comparative C_T (cycle threshold) method, due to its ease and speed for set-up and analysis; values are expressed relative to a calibrator (weakest signal of the normalized values). First, the C_T for the target amplicon and the C_T for the endogenous control (S9) will be determined for each sample. Differences in the C_T for the target and the C_T for the endogenous control, called ΔC_T, will be calculated to normalize for the differences in the RNA extractions and the efficiency of the RT step. The ΔC_T for each experimental sample will be subtracted from the ΔC_T of the calibrator resulting in a $\Delta\Delta C_T$ value. Finally, the amount of target, normalized to the endogenous control and relative to the calibrator, will be calculated by $2^{-\Delta\Delta CT}$. Thus, all experimental samples will be expressed as a n-fold difference relative to the calibrator.

For the comparative C_T method ($\Delta\Delta C_T$ method) to be valid, the amplification efficiencies of the target and the endogenous control must be approximately equal. To determine the amplification efficiencies of S9 and the other gene of interest (GOI), six dilutions of cDNA preparations (run in triplicate) will be amplified to establish standard curves. Differences of the slopes between standard curves obtained from S9 and the GOI will be plotted against the dilution of input total RNA and the regression line calculated.

Statistical analysis: Statistical analysis of the data will be performed in NCSS (Number Cruncher Statistical System, Kaysville, UT), and Primer E (Primer v6, Plymouth UK). Population differences in gene expression will be examined using Discriminant Function Analysis whereas behavior of genes within specific individuals will be examined using Cluster

Analysis fit to Multi-Dimensional Scaling. Differences between GOI transcriptions will be analyzed with the GLM ANOVA test at the $p < 0.05$ level of significance.

D. Description of Study Area

This project will evaluate archived samples collected from sea otters in western Prince William Sound**.**

E. Coordination and Collaboration with Other Efforts

The proposed work builds directly on previous EVOS projects investigating the impact of the oil spill on sea otters in western Prince William Sound. Previous projects include numbers 99025, 030585, 040423, 040620, and 050775**.**

III. SCHEDULE

A. Project Milestones

Phase 1. Analyze archived PBMC and liver samples collected from sea otters in western Prince William Sound, 2003 – 2006. Sample analyses to be completed by Sept. 30, 2009 and interpretation by Dec. 31, 2009.

Phase 2. Analyze archived PBMC samples collected from sea otters in western Prince William Sound, 1996 through 2002. Sample analyses to be completed by Sept. 30, 2010 and interpretation by Dec. 31, 2010.

B. Measurable Project Tasks

FY09, 3rd quarter (April 1, 2009 – June 30, 2009)
Obtain project funding. Obtain archived samples from Purdue laboratory. Begin sample analyses for Phase 1 of the project.

FY09, 4th quarter (July 1, 2009 – Sept. 30, 2009)
Complete sample analyses, Phase 1, and begin interpretation of results.

FY10, 1st quarter (Oct. 1, 2009 - Dec. 31, 2009)
Complete preliminary interpretation of lab results.

FY10, 2nd quarter (Jan. 1, 2010 – March 31, 2010)
As appropriate, pending results of Phase 1, initiate sample analyses for Phase 2.

FY10, 3rd quarter (April 1, 2010 – June 30, 2010)

Continue sample analyses, Phase 2.

FY10, 4th quarter (July 1, 2010 – Sept. 30, 2010)
Complete sample analyses, Phase 2, and begin interpretation of results.

FY11, 1st quarter (Oct. 1, 2010 – Dec. 31, 2010)
Complete interpretation of results for both Phase 1 and 2.
Provide draft Final Report for submission Dec. 31, 2010.

FY11, 2nd quarter (Jan. 1, 2011 – March 31, 2011)
EVOS conducts peer review of draft Final Report

FY11, 3rd quarter (April 1, 2011 – June 30, 2011)
Respond to peer review comments and prepare Final Report.
Prepare manuscript for publication.

IV. RESPONSIVENESS TO KEY TRUSTEE COUNCIL STRATEGIES

A. Community Involvement and Traditional Ecological Knowledge (TEK)

Results of this work will be made available for communities in the oilspill area as well as the general public**.**

B. Resource Management Applications

Results of the proposed work will provide managers with additional information to make decisions regarding the progress toward recovery of sea otter populations in the oiled area of western Prince William Sound. Results will also facilitate understanding of the effects of exposure to lingering oil and any possible relationship to the delayed recovery rate of the sea otter population at northern Knight Island.

V. PUBLICATIONS AND REPORTS

An annual progress report will be submitted to the Trustee Council on 1 September 2009, 2010, and 2011. A draft final report will be submitted by Dec. 31, 2010. A manuscript reporting the findings of this project will be prepared by June 30, 2011.

VI. REFERENCES

Andrews GK. 2000. Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochem. Pharmacol. 59: 95–104.

Bommer UA, Thiele BJ. 2004. The translationally controlled tumour protein (TCTP). Int J Biochem Cell Biol. 36:379-85.

Bowen L, Aldridge B, Miles AK, Stott JL. 2006. Expressed MHC class II genes in sea otters (*Enhydra lutris*) from geographically disparate populations. Tissue Antigens 67:402-8.

Bowen L, Riva F, Mohr C, Aldridge B, Schwartz J, Miles AK, Stott JL. 2007. Differential gene expression induced by exposure of captive mink to fuel oil; a model for the sea otter. Ecohealth. 4:298-309.

Exxon Valdez Oil Spill Trustee Council, 2006. Update on Injured Resources and Services 2006. *Exxon Valdez* Oil Spill Restoration Plan. 42p.

Finnström N, Thörn M, Lööf L, Rane A. 2001. Independent patterns of cytochrome P450 gene expression in liver and blood in patients with suspected liver disease. Eur J Clin Pharm. 57:403-409.

Furukawa M, Nishimura M, Ogino D, Chiba R, Ikai I, Ueda N, Naito S, Kuribayashi S, Moustafa MA, Uchida T, Sawada H, Kamataki T, Funae Y, Fukumoto M. 2004. Cytochrome P450 gene expression levels in peripheral blood mononuclear cells in comparison with the liver. Cancer Science. 95(6):520-529.

Goldsby RA, Kindt TJ, Osborne BA, Kuby J. 2003. Immunology, fifth edition. W.H. Freeman and Company, New York.

Holland-Bartels, L. E., editor. 2002. Mechanisms of impact and potential recovery of nearshore vertebrate predators following the 1989 *Exxon Valdez* oil spill. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 99025), U.S. Geological Survey, Alaska-Biological Science Center, Anchorage, Alaska. (NTIS No. PB2004-100060)

Hook, S.E., M.E. Cobb, J.T. Oris, J.W. Anderson. 2008. Gene sequences for Cytochromes p450 1A1 and 1A2: The need for biomarker development in sea otters (*Enhydra lutris*). Comparative Biochemistry and Physiology, Part B 151 (2008) 336–348.

Jenne DE, Tschopp J. 1989. Molecular structure and functional characterization of a human complement cytolysis inhibitor found in blood and seminal plasma: Identity to sulfated glycoprotein 2, a constituent of rat testis fluid. PNAS 86: 7123-7127.

Maizels RM, Balic A. 2004. Resistance to Helminth infection: The case for Interleukin-5–dependent mechanisms. Journal of Infectious Diseases 190:427-429.

Nishiyama H, Higashitsuji H, Yokoi H, Itoh K, Danno S, Matsuda T, Fujita J. 1997. Cloning and characterization of human CIRP (cold-inducible RNA-binding protein) cDNA and chromosomal assignment of the gene. Gene 204:115-20.

Oesch-Bartlomowicz B, Oesch F. 2005. Phosphorylation of cytochromes P450: first discovery of a posttranslational modification of a drug-metabolizing enzyme. Biochem Biophys Res Commun. 338:446-9.

Schwartz JA, Aldridge BM, Lasley BL, Snyder PW, Stott JL, Mohr FC. 2004a. Chronic fuel oil toxicity in American mink (*Mustela vison*): systemic and hematological effects of ingestion of a low-concentration of bunker C fuel oil. Toxicol Appl Pharmacol. 200(2):146-58.

Schwartz JA, Aldridge BM, Stott JL, Mohr FC. 2004b. Immunophenotypic and functional effects of bunker C fuel oil on the immune system of American mink (*Mustela vison*).Vet Immunol Immunopathol. 101(3-4):179-90.

Snyder, P. W., T. Kondratyuk, B. Ballachey, and J. Vanden Heuvel. 2002. CYP1A1 gene expression in sea otters (*Enhydra lutris*): A quantitative reverse transcriptase-polymerase chain reaction to measure CYP1A mRNA in peripheral blood mononuclear cells. Appendix BIO-02.1 *in* L. E. Holland-Bartels, editor. Mechanisms of impact and potential recovery of nearshore vertebrate predators following the 1989 *Exxon Valdez* oil spill, volume 2 – appendices, *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 99025), U.S. Geological Survey, Alaska Biological Science Center, Anchorage, Alaska. (NTIS No. PB2004-1000060. *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska, USA.

Tsai M, O'Malley BW. 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451-486.

Tsan M, B Gao. 2004. Cytokine function of heat shock proteins. Am J Physiol Cell Physiol 286:739-744.

Tumpey TM, Szretter KJ, Van Hoeven N, Katz JM, Kochs G, Haller O, García-Sastre A, Staeheli P. 2007.The MX1 gene protects mice against the pandemic 1918 and highly lethal human H5N1 influenza viruses. J Virology. 81(19):10818-21.

Wirgin I, Konkle B, Pedersen M, Grunwald C, Williams J, Courtenay SC. 1996. A Comparison of cytochrome P4501A (CYP1A) mRNA inducibility in four species of Atlantic Coast anadromous fishes. Estuaries 19(4): 913-922.

VII. RESUME OF PROPOSED PRINCIPAL INVESTIGATOR

A. Keith Miles
USGS Western Ecological Research Center
Davis Field Station, 1 Shields Avenue
University of California, Davis 95616
Phone 530-752-5365; Fax 530-752-9680
Keith_miles@usgs.gov

Current Assignment: Supervisory Research Wildlife Biologist - USGS; Faculty, Graduate Group in Ecology; Associate in the California Agricultural Experiment Station, Wildlife, Fisheries, and Conservation Biology, University of California, Davis (UCD).

Education: Ph.D., Oregon State University, June 1987, Wildlife Ecology; M.S., Oregon State University, August 1976, Wildlife Biology; B.S., Howard University, June 1972, Zoology.

Current Research: Team lead of 5 biologists, 4 graduate researchers, and 14 technicians that specialize in field-oriented investigative approaches to contaminants problems and general problems of conservation of wildlife species. The goals of my research are to determine consequences of accumulation of contaminants in species of concern and their prey, and discriminate the effects caused by contaminants from those induced by other causes. I conduct studies on the effects of contaminants on community structure and the potential for accumulation of these contaminants among specific guilds of migratory aquatic birds, marine mammals, and their prey. **Collaborative UCD Studies:** Dr. Jeff Stott, Dr. Liz Bowen: Environmental signatures and gene expression patterns in sea otters; Dr. Barry Wilson: Cytochrome P-4501A induction and hydrocarbon exposure.

Current Awarded Studies:

Aquatic Bird Use of Recreated Wetlands, Salton Sea, California: Benefits and Risks. State of California, Bureau of Reclamation 2007 – 2010; Effects of mercury (Hg) on waterbirds and habitat at San Francisco Bay. State of California, CALFED 2005 – 2009; Understanding the dynamics of mercury in eared grebes, Great Salt Lake, Utah. U.S. Fish & Wildlife Service, 2006 – 2009; Snowy Plovers at Point Reyes National Seashore: Unraveling the Mystery of Mercury, U.S. Park Service. 2006 - 2009.

Relevant (recent) Journal Articles:

Miles AK, Ricca MA, Anthony RG, Estes JA. *in press*. Organochlorine Contaminants in Fishes from Coastal Waters West of Amukta Pass, Aleutian Islands, Alaska. Environmental Toxicology and Chemistry.

Miles AK, Flint PL, Trust KA, Ricca MA, Spring SE, Arrieta DE, Hollmen T, Wilson BA. 2007. Polycyclic Aromatic Hydrocarbon Exposure in Steller's Eiders and Harlequin Ducks, Eastern Aleutian Islands, Alaska. Environmental Toxicology and Chemistry. 26(12):2694-2703.

Bowen L, Riva F, Mohr C, Aldridge B, Schwartz J, Miles AK, Stott JL. 2007. Differential gene expression induced by exposure of captive mink to fuel oil; a model for the sea otter. Ecohealth. 4:298-309.

VIII. BUDGET JUSTIFICATION - $205,734

Justification for each item in the attached budget is as follows:

FY09, FY10, and FY11 (close-out)

Personnel

FY09--$44,962; funding for 3 PI's and a lab biologist to obtain, inventory, prepare lab samples for Phase I, as well as to refine techniques and examine results.
FY10--$61,492; funding for 3 PI's and a lab biologist to obtain, inventory, prepare lab samples for Phase II, as well as to refine techniques and examine results.
FY11--$8743; Close-out: funding for two PI's to prepare and complete Final Report, make presentation at the Alaska Marine Science Symposium, and write a Journal article

Travel

FY09--$1500; funding for project meeting to review results of Phase I
FY10--$2710; funding for project meeting to review results of Phase II
FY11--$2400; funding for Miles and Bowen to attend and present at the Alaska Marine Science Symposium in January 2011

Contractual

FY09-- $0
FY10--$0
FY11--$0

Commodities

FY09—168 samples @ $130/sample = $21,840; RT PCR calibration estimated at $500, miscellaneous lab supplies @ $500
FY10—320 samples @ $130/sample = $41,600; RT PCR calibration estimated at $1000, miscellaneous lab supplies @ $500
FY11—manuscript preparation and page costs, $1000

Equipment

FY09---$0
FY10---$0
FY11---$0

The equipment for this project is being contributed by the USGS laboratory at UC Davis.

IX. DATA MANAGEMENT

A. Study Design

This study will produce data on archived sea otter samples (blood cells and liver) collected in oiled and unoiled areas of western Prince William Sound from 1996 - 2006. Samples will be analyzed using Real Time Polymerase Chain Reaction (PCR) techniques to obtain data on expression of a panel of genes that indicate (1) exposure of the individual otters to xenobiotics that include aromatic hydrocarbons found in crude oil and (2) stress or injury that may be related to that exposure. Because the study is conducted on archived tissue samples, there is no field work component. The primary objective is to compare gene expression in sea otters from the oiled and unoiled areas, and to assess changes over time (1996 - 2006), as an evaluation of the status of recovery of sea otters in oiled areas. As of 2006, sea otters in heavily oiled areas of western Prince William Sound had not recovered from losses incurred in 1989, during the oil spill. The data generated will be directly related to data previously collected on sea otters in western Prince William Sound, as part of EVOSTC studies (including Projects 99025, 030585, 04023, 040620 and 050775). The variables that will allow linkage of the data to be generated in this study with data from the other studies are (1) sea otter number, which is a unique ID assigned to each otter at the time of capture, and used in subsequent years if an otter is captured more than once, and (2) the capture date. Data from previous studies includes information on capture location, otter age and sex, morphometric data, hematology and serum chemistry data, and, for a subset of the otters, data on movements, dive patterns, and survival; these data sets were described in previous proposals, and reside at the USGS-Alaska Science, Anchorage, Alaska.

In the present study, we will use Real Time PCR methods to generate a Threshold Cycle (C_T), for each of 15 genes of interest in the sea otters (see Table 1) that includes one endogenous reference gene (also called a housekeeping gene), which is used to normalize for varying quantities of RNA characteristic of individual organisms. Real Time PCR is widely used for measurement of gene expression (see Bowen et al. 2007), and advances in methodology over the last decade should allow more efficient and accurate measurement of the genes of interest, provide information on the health status of individual sea otters, and provide a greater understanding of factors constraining recovery of the population.

B. Criteria/Acceptable Data Quality

Sample preparation and Real Time PCR methodology are described in detail in the study design. To assure quality control, all samples are run in duplicate on the ABI 7300 Real Time PCR System. Samples of any duplicate runs greater than 1 C_T value difference are prepared again for Real Time PCR analysis; if the anomaly persists, the sample is eliminated from further consideration or data analyses. The ABI 7300 Real Time PCR System has an internal standard, calibration efficiency test that is executed prior to each run of subject samples to assure accuracy of results.

C. Metadata

a. Metalite Metadata information:
Identification_Information:
Citation:
Citation_Information:
Originator: USGS, A. Keith Miles, Liz Bowen, and Brenda Ballachey
Publication_Date: 20111212
Title: CYP1A1 Gene Expression Verification Study – Re-Evaluation of Sea Otter Samples from the *Exxon Valdez* Oil Spill
Geospatial_Data_Presentation_Form: N/A
Publication_Information:
Publication_Place: Anchorage, Alaska, United States
Publisher: USGS
Description:
Abstract: Sea otter populations in western PWS were injured as a result of the *Exxon Valdez* oil spill, with evidence for both immediate acute mortality and longer term injury from chronic exposure to oil spilled in 1989. The EVOS Trustee Council funded over a decade of studies to identify progress toward recovery of the sea otter populations, particularly in the northern Knight Island Archipelago. These projects have addressed population demographics including abundance, habitat use, and survival rates, together with biological sampling to monitor body condition using blood parameters, liver pathology, and a CYP1A biomarker to determine oil exposure. Although population abundance data indicate some level of recovery in Prince William Sound overall, recovery remained incomplete as of 2006. Recently, the viability of the CYP1A biomarker assessments of sea otter exposure to oil have been questioned, making it necessary to reevaluate this method for assessing exposure. In this study, we propose to re-test the exposure of sea otters to lingering oil by applying our recent discoveries of sea otter specific genetic primers to measure gene expression on the archived samples from these projects. Our initial studies of mink experimentally exposed to oil identified genes that were significantly altered in expression (Bowen et al 2007). 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. We have successfully sequenced 13 genes from sea otters that were expressed in mink experimentally exposed to oil, as well as 2 additional genes that aid interpretation of stress levels in animals exposed to xenobiotics that include aromatic hydrocarbons. In phase one of the project, we will analyze the gene expression of a suite of genes from archived Peripheral Blood Mononuclear Cells (PBMC) and liver samples collected from individual sea otters in 2003-2006. If these PBMC samples produce meaningful analytic results, the project will proceed with phase two, to analyze the gene expression in PBMC samples from 1996 through 2002. This study will allow us to verify our past understanding of oil exposure of sea otters in PWS, assess the current status of recovery, and provide a reliable method for assessing recovery in the future.

Time_Period_of_Content:
Time_Period_Information:
Range_of_Dates/Times:
Beginning_Date: 19960601
Ending_Date: 20060515
Currentness_Reference: ground condition

```
  Status:
    Progress: Planned
    Maintenance_and_Update_Frequency: As needed
  Spatial_Domain:
    Bounding_Coordinates:
      West_Bounding_Coordinate: -147.200
      East_Bounding_Coordinate: -147.983
      North_Bounding_Coordinate: 60.750
      South_Bounding_Coordinate: 60.150
  Keywords:
    Theme:
      Theme_Keyword_Thesaurus:
      Theme_Keyword: sea otters, recovery, oil exposure
    Place:
      Place_Keyword_Thesaurus:
      Place_Keyword: Prince William Sound
     Temporal:
      Temporal_Keyword_Thesaurus:
      Temporal_Keyword: not required
  Access_Constraints: None
  Use_Constraints: None
Spatial_Data_Organization_Information:
  Direct_Spatial_Reference_Method: Point
Distribution_Information:
  Distributor:
    Contact_Information:
      Contact_Person_Primary:
        Contact_Person: Brenda Ballachey
        Contact_Organization: USGS Alaska Science Center
      Contact_Address:
        Address_Type: Mailing and Physical Address
        Address:
          Alaska Science Center
          4210 University Drive
        City: Anchorage
        State_or_Province: Alaska
        Postal_Code: 99508
        Country: United States
      Contact_Voice_Telephone: 907.786.7000
      Contact_Facsimile_Telephone: 907.786.7150
      Contact_Electronic_Mail_Address: bballachey@usgs.gov
  Distribution_Liability:
Metadata_Reference_Information:
  Metadata_Date: 20090430
  Metadata_Contact:
    Contact_Information:
```

Contact_Person_Primary:
Contact_Person: Brenda Ballachey
Contact_Organization: USGS Alaska Science Center
Contact_Address:
Address_Type: Mailing and Physical Address
Address:
Alaska Science Center
4210 University Drive
City: Anchorage
State_or_Province: Alaska
Postal_Code: 99508
Country: United States
Contact_Voice_Telephone: 907.786.7000
Contact_Facsimile_Telephone: 907.786.7150
Contact_Electronic_Mail_Address: bballachey@usgs.gov
Metadata_Standard_Name: FGDC Content Standards for Digital Geospatial Metadata
Metadata_Standard_Version: FGDC-STD-001-1998

b. Dataset category:
Laboratory results: RT PCR data, cycle of threshold (C_T) for each of 15 genes of interest, plus for an endogenous reference gene.

D. Algorithms
None of our data require conversion algorithms.

E. Sample Collection, Handling, Custody, Storage
Samples were collected from sea otters captured from 1996-2006, processed and frozen in LN_2 the field, and shipped in LN_2 to Purdue University in Indiana (lab of Dr. P. Snyder) where they have been maintained in frozen storage at -80°C since their arrival. Samples will be shipped (in LN_2 transfer dewars) to the laboratory of Dr. Keith Miles at University of California, Davis, California, where they will be maintained in frozen storage at -80°C until used in analyses.

F. Analytical Instrumentation
Amplifications of cDNA (from mRNA isolated from PBMC and liver samples) will be performed in an ABI 7300 Real Time PCR System (Applied Biosystems, California).

G. Data Reduction and Reporting
Off the shelf statistical software (e.g. SAS, SYSTAT, NCSS, Primer E) will be used for descriptive statistics and between areas (oil exposed vs. non-exposed) comparisons.

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

Budget Category:	Proposed FY 09	Proposed FY 10	Proposed FY 11	Proposed FY 12	TOTAL PROPOSED
Personnel	$44,962.0	$61,492.0	$8,743.2	$0.0	$115,197.2
Travel	$1,500.0	$2,710.0	$2,400.0	$0.0	$6,610.0
Contractual	$0.0	$0.0	$0.0	$0.0	$0.0
Commodities	$22,840.0	$43,100.0	$1,000.0	$0.0	$66,940.0
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0
SUBTOTAL	$69,302.0	$107,302.0	$12,143.2	$0.0	$188,747.2
General Administration (9% of subtotal)	$6,237.2	$9,657.2	$1,092.9	$0.0	$16,987.2
PROJECT TOTAL	$75,539.2	$116,959.2	$13,236.1	$0.0	$205,734.4
Other Resources (Cost Share Funds)	$0.0	$0.0	$0.0	$0.0	$0.0

COMMENTS: Salaries are being contributed for participation in the project by: Keith Miles, Jim Bodkin, M. Johnson, and J.Stott. The equipment for this project is being contributed by the USGS laboratory at UC Davis.

Prepared April 27, 2009

FY09 - 11

Project Title: Gene Expression Verification Study
Lead PI: Keith Miles
Agency: USGS

FORM 3A
TRUSTEE AGENCY SUMMARY

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

Personnel Costs:		GS/Range/	Months	Monthly		Personnel
Name	Project Title	Step	Budgeted	Costs	Overtime	Sum
K. Miles	PI		0.5	9744.0		4,872.0
L. Bowen	co-PI		1.0	7100.0		7,100.0
Keister	Lab biologist		4.0	5580.0		22,320.0
Ballachey	co-PI		1.1	9700.0		10,670.0
Bodkin, collaborator, in-kind						0.0
Johnson, collaborator, in-kind						0.0
Stott, collaborator, in-kind						0.0
						0.0
Miles, additional salary contributed						0.0
						0.0
						0.0
						0.0
	Subtotal		6.6	32124.0	0.0	
					Personnel Total	########

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
project meeting of PI's	600.0	2	5	60.0	1,500.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$1,500.0

FY09

Project Title: Gene Expression Verification Study
Lead PI: Keith Miles

FORM 3B
PERSONNEL & TRAVEL DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

Contractual Costs: Description		Contract Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required.	**Contractual Total**	$0.0

Commodities Costs: Description	Commodities Sum
PCT & RT primers + RNA isolation + RT & Q PCR PCR reactions + DNA sequencing	
168 samples @ $130.00/sample = $21,840 All runs are duplicated on RT PCR System	21,840.0
Equipment maintenance (RT PCR calibrating)	500.0
Miscellaneous lab supplies	500.0
Commodities Total	########

FY09

Project Title: Gene Expression Verification Study
Lead PI: Keith Miles

FORM 3B
CONTRACTUAL & COMMODITIES DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY09

Project Title: Gene Expression Verification Study
Lead PI: Keith Miles

FORM 3B
EQUIPMENT DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

Personnel Costs:		GS/Range/	Months	Monthly		Personnel
Name	Project Title	Step	Budgeted	Costs	Overtime	Sum
						0.0
K. Miles	PI		0.5	9744.0		4,872.0
L. Bowen	co-PI		1.5	7100.0		10,650.0
Keister	Lab biologist		6.5	5580.0		36,270.0
Ballachey	co-PI		1.0	9700.0		9,700.0
Johnson, collaborator, in-kind						0.0
Stott, collaborator, in-kind						0.0
Bodkin, collaborator, in-kind						0.0
						0.0
Miles, additional salary contributed						0.0
						0.0
						0.0
	Subtotal		9.5	32124.0	0.0	
					Personnel Total	#########

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
project meeting of PI's	800.0	2	5	222.0	2,710.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$2,710.0

FY10

Project Title: Gene Expression Verification Study
Lead PI: Keith Miles

FORM 3B
PERSONNEL & TRAVEL DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

Contractual Costs: Description	Contract Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual Total**	$0.0

Commodities Costs: Description	Commodities Sum
PCT & RT primers + RNA isolation + RT & Q PCR PCR reactions + DNA sequencing	
320 samples @ $130.00/sample = $41,600 All runs are duplicated on RT PCR System	41,600.0
Equipment maintenance (RT PCR calibrating)	1,000.0
lab supplies	500.0
Commodities Total	########

FY10

Project Title: Gene Expression Verification Study
Lead PI: Keith Miles

FORM 3B
CONTRACTUAL & COMMODITIES DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY10

Project Title: Gene Expression Verification Study
Lead PI: Keith Miles

FORM 3B
EQUIPMENT DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

Personnel Costs:		GS/Range/	Months	Monthly		Personnel
Name	Project Title	Step	Budgeted	Costs	Overtime	Sum
						0.0
K. Miles	PI		0.3	9744.0		2,923.2
L. Bowen	co-PI					0.0
Keister	Lab biologist					0.0
Ballachey	co-PI		0.6	9700.0		5,820.0
						0.0
Miles, additional salary contributed						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.9	19444.0	0.0	
					Personnel Total	$8,743.2

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
presentation at Alaska Marine Sciences Symposium					0.0
travel for Miles and Bowen	700.0	2	4	250.0	2,400.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$2,400.0

FY11

Project Title: Gene Expression Verification Study
Lead PI: Keith Miles

FORM 3B
PERSONNEL & TRAVEL DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

Contractual Costs: Description		Contract Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required.	**Contractual Total**	$0.0

Commodities Costs: Description	Commodities Sum
manuscript preparation	1,000.0
Commodities Total	$1,000.0

FY11

Project Title: Gene Expression Verification Study
Lead PI: Keith Miles

FORM 3B
CONTRACTUAL & COMMODITIES DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY11

Project Title: Gene Expression Verification Study
Lead PI: Keith Miles

FORM 3B
EQUIPMENT DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

Personnel Costs:		GS/Range/	Months	Monthly		Personnel
Name	Project Title	Step	Budgeted	Costs	Overtime	Sum
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
Subtotal			0.0	0.0	0.0	
					Personnel Total	$0.0

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY12

FORM 3B
PERSONNEL &
TRAVEL DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

Contractual Costs: Description		Contract Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required.	**Contractual Total**	$0.0

Commodities Costs: Description	Commodities Sum
Commodities Total	$0.0

FY12

FORM 3B
CONTRACTUAL & COMMODITIES DETAIL

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 09- FY 11

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY12

FORM 3B EQUIPMENT DETAIL