06/07/0506/07/0506/07/05

Project Title: Comparison of Cytochrome P450 1A Induction in Blood and Liver Cells of Sea Otters

Project Number:	
Restoration Category:	Research and Monitoring
Proposer:	Brenda E. Ballachey and Paul W. Snyder
Lead Trustee Agency:	DOI: U.S. Geological Survey
Cooperating Agencies:	
Alaska SeaLife Center:	No
Project Duration:	1st year, 1-year project
Cost FY 01:	$19,900.
Geographic Area:	WPWS
Injured Resource/Service:	Sea otter

ABSTRACT

Sea otters in oiled areas of western PWS had elevated levels of cytochrome P450 1A (CYP1A), a biomarker of hydrocarbon exposure, measured in blood samples collected from otters in 1996-98. In summer 2001, as part of project 01423, we have proposed to resample CYP1A in blood from sea otters in oiled and unoiled areas of PWS. Herein we describe a complementary effort to project 01423. We propose also to sample liver from the captured sea otters, for assays of CYP1A, and for examination of histopathological changes. Liver CYP1A levels will be compared to those measured in blood from the same individuals. We will also assay for CYP1A in archived frozen liver samples from sea otters that were oiled and died in 1989, to enable comparison of current levels of CYP1A induction with levels in sea otters that had a known high degree of oil exposure. The results of this study will provide a basis for comparison of cytochrome P4501A induction in sea otters in 1989, in 1996-98, and in 2001, and will help determine if there is a decline in CYP1A levels over time.

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INTRODUCTION

In the NVP project (/025), sea otters were evaluated for increased levels of cytochrome P450 1A (CYP1A) in blood (peripheral blood mononuclear cells), as a biomarker of exposure to environmental hydrocarbons. Data from 1996-98 show elevations of CYP1A in otters from oiled areas, compared to those from unoiled areas.

We do not have archived blood samples from previous years that are suitable for assays of CYP1A, and so cannot compare CYP1A levels currently observed to levels that would have been present in sea otters exposed to oil in the months immediately after the 1989 oil spill. We do, however, have archived liver samples from 1989, which are suitable for the assay of CYP1A; most of those samples also have data on tissue hydrocarbon concentrations, collected as part of NRDA studies. During 1999, we have verified that RNA can be isolated from the archived liver samples (see below).

Further monitoring of CYP1A in sea otters in WPWS, in the summer of 2001, is currently proposed as part of Project 01423. Our goal herein is to supplement measurement of CYP1A in blood from those otters with assays on liver biopsies from the same individuals, to establish the relation between CYP1A in the two tissue types. We further propose to assay archived liver samples collected from sea otters that died in the summer of 1989. The comparison of liver levels from 1989 and 2001 would give an indication of the relative levels of exposure, 12 years after the spill.

NEED FOR THE PROJECT

A. Statement of Problem

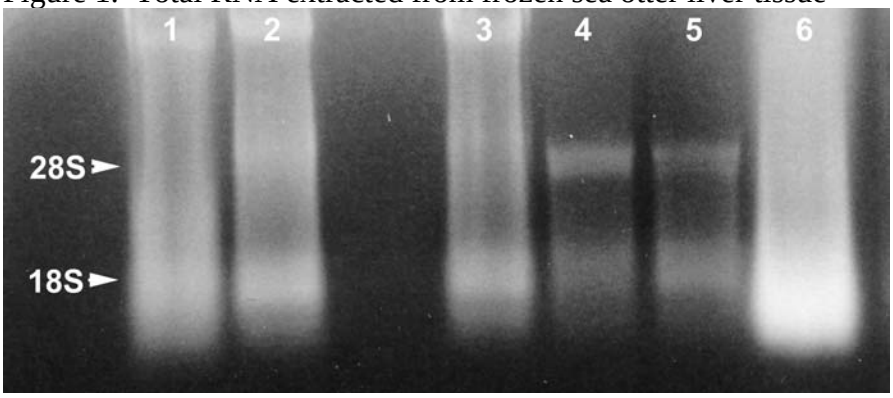
Sea otters in the most heavily oiled areas of western Prince William Sound (WPWS) have not yet recovered from the *Exxon Valdez* oil spill, based on several lines of evidence from studies conducted as part of the NVP project (/025) and the continuing sea otter work as part of project /423. Significant results on sea otters include lack of population growth in the oiled study area (Bodkin et al. 1999, Dean et al. 2000, USGS unpub. data), evidence of relatively poor survival rates of sea otters from the oiled area (Bodkin et al. 1999, Monson et al. 2000), and increased induction of CYP1A in the oiled area (Ballachey et al. 1999). Elevations in CYP1A do not appear to be due to background or natural hydrocarbon sources, as these were found to be negligible in intertidal areas of PWS (Short and Babcock 1996), nor to differential contamination of areas by PCBs (Trust et al. 2000; USGS unpub. data). Continued exposure to residual *Exxon Valdez* oil is the most plausible explanation of elevated CYP1A. Residual oil is still stranded in intertidal areas of PWS (Babcock et al. 1996, Harris et al. 2000, Hayes and Michel 1999), providing a continuing potential source of contamination. However, the extent to which continuing exposure to residual oil may be constraining sea otter population recovery is not known (Project 01423 contains objectives designed to address this question).

The NVP CYP1A data cover the period from 1996-98. At this time, comparable data on CYP1A induction in sea otters are not available from earlier post-spill years (1989-95). However, such

data would be valuable as they would provide a benchmark for evaluation of degree of exposure seen in samples collected presently to samples collected in the months post-spill, and thus a measure of the relative continuing exposure.

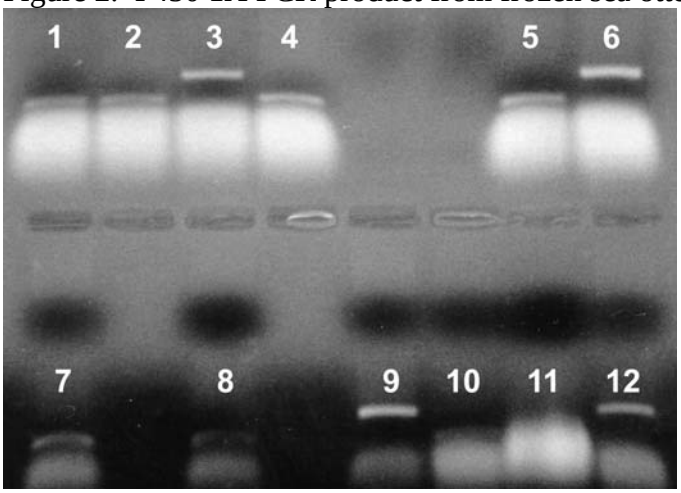
Measurement of CYP1A in sea otters in the NVP project used a quantitative RT-PCR technique on peripheral blood mononuclear cells (Vanden Heuvel et al. 1993, 1994; Snyder et al. 1999). Although there are no archived samples of blood cells that would be suitable for the RT-PCR assay, the assay can also be applied to liver or other tissue samples, and archived frozen liver samples are available. These liver samples were collected from sea otters that died in 1989 subsequent to the spill, and time of death and extent of oiling on the pelage are known. Many of these otters were exposed to large quantities of oil, and showed histopathological changes (Lipscomb et al. 1993); CYP1A levels likely were greatly elevated. Further, hydrocarbon concentrations were measured on aliquots of the same samples (Ballachey and Kloecker 1997a, b), and in many cases (where otters were heavily oiled), concentrations were well above method detection limits. Preliminary assays of archived liver samples during the last year have demonstrated that we are able to isolate RNA (Figure 1) and obtain P4501A PCR products (Figure 2) on the archived liver samples.

Figure 1. Total RNA extracted from frozen sea otter liver tissue



RNA was isolated from liver using TRI reagent protocol, Sigma. Two micrograms of each RNA sample was analyzed by electrophoresis in a 1% MOPS-EDTA-formaldehyde agarose gel and visualized by staining for 5 minutes with 100 μg/ml ethidium bromide in deionized water.
Lane 1 – VZ 081
Lane 2 - VZ 135
Lane 3 – SW 050
Lane 4 – VZ 060
Lane 5 – VZ 111
Lane 6 – VD 123

Figure 2. P450 1A PCR product from frozen sea otter liver tissue



Ethidium bromide-stained agarose gel containing PCR products resulting from amplification of sea otter liver P450 1A cDNA.
Lane 1 – VZ 081
Lane 2 – VZ 135
Lane 3 – VZ 081 positive control (glyceraldehyde-3-phosphate degydrogenase)
Lane 4 – SW 050
Lane 5 – VZ 060
Lane 6 – VZ 060 positive control
Lane 7 – VZ 111
Lane 8 – VD 123
Lane 9 – VD 123 positive control
Lane 10 – VZ 109
Lane 11 – SW 149
Lane 12 – SW 149 positive control

We propose to work in conjunction with the sea otter capture and CYP1A monitoring effort being proposed for the summer of 2001 in Project 01423. As part of that project, sea otters will be captured and blood samples taken for CYP1A evaluation. In this project, we propose to supplement the blood sampling/CYP1A effort with collection of liver biopsies from the same otters, also for analysis of CYP1A using the RT-PCR assay. This will enable us to establish the relation between CYP1A induction in blood and liver cells. We further propose to analyze 30 archived liver samples, including samples from heavily oiled otters.

The results of this study will provide a basis for comparison of cytochrome P4501A induction in sea otters in 1989, in 1996-98, and in 2001, and will help determine if there is a decline over time in CYP1A levels.

B. Rationale/Link to Restoration

This research will provide a means for us to relate present levels of CYP1A induction, measured in sea otters from oiled areas of PWS and other locations, with levels of CYP1A induction in

oiled sea otters collected in 1989, after the spill, thus providing insight into the degree of exposure currently being experienced by sea otters. It also gives an opportunity for histological examination of liver tissues from sea otters in oiled areas, which may be informative in terms of understanding apparent differences in survival rates between areas. Additionally, adaptation of the assay for liver tissues will allow us to obtain samples from other sources (e.g., natural mortalities, subsistence hunters), for monitoring of CYP1A and comparison of oiled and unoiled levels.

C. Location

The samples will be collected in western PWS. Assays of CYP1A and histopathology will be done at Purdue University.

COMMUNITY INVOLVEMENT AND TRADITIONAL KNOWLEDGE

We will interact with local communities in meetings to explain and discuss ongoing restoration projects (this effort coordinated with similar activities for project 01423).

PROJECT DESIGN

A. Objectives

1. Measure and compare CYP1A in blood (PBMC) and liver samples from sea otters captured in summer 2001.

2. Measure CYP1A in archived liver samples of oiled sea otters from 1989; compare liver CYP1A values from 2001 to 1989 samples.

3. Do histopathological examination of liver biopsies from 2001, to assess relation between CYP1A levels and histological change in the liver.

4. Relate CYP1A levels in 1989 liver samples with hydrocarbon concentrations measured previously, and histopathology collected previously on those samples.

B. Methods

In the NVP study, the RT-PCR assay (quantitative reverse transcriptase PCR assay; Vanden Heuvel et al. 1993, 1994; Snyder et al. 1999) was adapted to measure CYP1A levels in sea otters. This assay quantifies the messenger RNA (m-RNA) that codes for the CYP1A protein, and results are reported as molecules of mRNA per 100 ng of RNA. For sea otters, the assay has been applied only to peripheral blood mononuclear cells; we will adapt it for measurement of CYP1A in liver cells.

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In summers of 2001, we have proposed (project 01423) to capture 30 sea otters (15 per area) in the same areas (Knight and Montague islands) that were sampled in the NVP project, so that additional data collected can be directly compared to previous (1996-98) results. Capture and handling methods will be similar to those employed previously (Bodkin et al. 1999). Sea otters will be sedated, body measurements taken, a tooth collected for age determination, and a blood sample taken by jugular venipuncture. In addition, a liver biopsy weighing approximately 0.5 gm will be surgically collected from 10 otters per area, by a qualified veterinarian. One portion will be frozen in LN2 and a second portion fixed in formalin. Following reversal, sea otters will be released in the same vicinity as captured.

Samples (liver, blood cells, and frozen archived liver) will be shipped to Purdue University for analysis in the laboratory of Dr. Paul Snyder. CYP1A will be measured by the RT-PCR assay, and liver samples in formalin will be examined for evidence of histological change.

The data will be used to determine the relation between CYP1A in blood and liver. We will compare mean CYP1A values in liver samples from 2001 and 1989. We will look for a correlation between CYP1A in liver and histopathological change in hepatic cells. We will also relate liver histopathology and CYP1A levels to serum chemistry, including serum enzymes, measured as part of work outlined in Project 01423. Finally, for the 1989 liver samples, we will correlate total hydrocarbons in liver (data from NRDA studies) and histopathology (Lipscomb et al. 1993) with CYP1A induction.

SCHEDULE

A. Measurable Project Tasks for FY 01

July:	Capture and sampling of sea otters.
August-Sept.:	CYP1A analyses on liver samples from 2001 and from 1989, data analyses.

B. Project Milestones and Endpoints

1.	July 2001:	Collection of liver samples from live otters.
2.	Aug-Sept:	Analyses of new (year 2001) and old (year 1989) liver samples for CYP1A. Data analyses.
3.	April 2001:	Report submission - April 15, 2001.

C. Completion Date

This is a one year project. Sample collections and laboratory assays will be completed in FY2001 and a final report submitted by April 15, 2002.

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PUBLICATIONS AND REPORTS

We will provide a final report to the EVOSTC office by April 15, 2002. We anticipate a manuscript on the results to be submitted to a scientific journal in the year 2002.

PROFESSIONAL CONFERENCES

None planned for FY2001.

NORMAL AGENCY MANAGEMENT

The work proposed here is not part of normal agency management and is related specifically to research addressing oil spill restoration concerns. No similar work has been conducted, is currently being conducted, or is planned using agency funds.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project is dependent on funding of sea otter capture for monitoring cytochrome P450 as part of Project 01423; otherwise we cannot complete the stated objectives.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

This is a new project proposal.

PRINCIPLE INVESTIGATORS

Dr. Brenda Ballachey, B.S., M.S. 1980 Colorado State University, Ph.D. 1985 Oregon State University, is a Research Physiologist at the Alaska Biological Science Center of USGS, Biological Resources Division. She was Project Leader for sea otter NRDA studies from 1990 through 1996, and has been involved in all aspects of post-spill research on sea otters. She has authored or coauthored over 25 peer-reviewed publications, and is currently a co-principal investigator for the Nearshore Vertebrate Predator (NVP) project, examining effects of residual oil on health and recovery of sea otters and other NVP study species.

Dr. Paul Snyder is an Assistant Professor of Pathology and Immunotoxicology and Director of the Clinical Immunology Laboratory of the Department of Veterinary Pathobiology, Purdue University. He is also a Diplomate of the American College of Veterinary Pathologists. His research interests are in the area of mechanism-based studies on the pathology and immunology of xenobiotics on biological systems. He has been a PI on the Nearshore Vertebrate Predator project since 1995.

OTHER KEY PERSONNEL

LITERATURE CITED

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Lipscomb, T.P., R.K. Harris, R.B. Moeller, J.M. Pletcher, R.J. Haebler and B.E. Ballachey. 1993. Histopathologic lesions in sea otters exposed to crude oil. Vet. Path. 30:1-11.

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impacts of the *Exxon Valdez* oil spill on sea otters, assessed through age-dependent mortality patterns. Proc. Nat'l. Acad. Sciences, USA: in press.

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2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001
Personnel		$9.0
Travel		$2.2
Contractual		$6.3
Commodities		$0.6
Equipment		$0.0
Subtotal	$0.0	$18.1
General Administration		$1.8
Project Total	$0.0	$19.9
Full-time Equivalents (FTE)		0.1
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
		Estimated FY 2002	Estimated FY 2003		
		$0.0	$0.0		

Dollar amounts are shown in thousands of dollars.

Comments:

No costs are included for NEPA compliance, technical review session attendance, restoration workshop attendance, report writing, publications, professional conferences, or community involvement.

FY01

Prepared: 14 April 2000

Project Number:
Project Title: Comparison of Cytochrome P450 1A Induction in Blood and Liver Cells of Sea Otters
Agency: DOI

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	
Name	Position Description					
B. Ballachey	Research Physiologist	GS 12 / 04	1.0	7.0		
Technical support	Biologist	GS 7	0.5	4.0		
	Subtotal		1.5	11.0	0.0	
					Personnel Total	

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	
Description					
Airfare & per diem, Indiana - Alaska RT (Snyder)	0.7	1	15	0.1	
				Travel Total	

FY01

Prepared: 14 April 2000

Project Number:
Project Title: Comparison of Cytochrome P450 1A Induction in Blood and Liver Cells of Sea Otters
Agency: DOI

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Contractual Costs:	
Description	
4A Linkage Assays of liver for cytochrome P450 1A, histopathology 50 @ $125	
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	
Commodities Costs:	
Description	
Veterinary supplies	
Commodities Total	

FY01

Prepared: 14 April 2000

Project Number:
Project Title: Comparison of Cytochrome P450 1A Induction in Blood and Liver Cells of Sea Otters
Agency: DOI

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

New Equipment Purchases:	Number of Units	Unit Price	
Description			
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	

Existing Equipment Usage:	Number of Units	
Description		

FY01

Prepared: 14 April 2000

Project Number:
Project Title: Comparison of Cytochrome P450 1A Induction in Blood and Liver Cells of Sea Otters
Agency: DOI

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001						
Personnel		$0.0						
Travel		$0.0						
Contractual		$0.0						
Commodities		$0.0						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$0.0			Estimated FY 2002	Estimated FY 2003		
Indirect								
Project Total	$0.0	$0.0						
Full-time Equivalents (FTE)		0.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

FY00

Prepared:

Project Number:
Project Title:
Name:

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:				Months	Monthly		
	Name	Position Description		Budgeted	Costs	Overtime	
		Subtotal		0.0	0.0	0.0	
							Personnel Total

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

FY00

Prepared:

Project Number:
Project Title:
Name:

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Contractual Costs:	
Description	
Contractual Total	
Commodities Costs:	
Description	
Commodities Total	

FY00

Prepared:

Project Number:
Project Title:
Name:

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

New Equipment Purchases:	Number of Units	Unit Price	
Description			
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	

Existing Equipment Usage:	Number of Units	
Description		

FY00

Prepared:

Project Number:
Project Title:
Name: