Exxon Valdez Oil Spill
Restoration Project Final Report

Lingering Oil: Bioavailability and Effects of Prey and Predators

Restoration Project 030585 Part II
Final Report

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Study History: This project began in 2002 with the approval of a 1 year plan by the *Exxon Valdez* Oil Spill (EVOS) Trustee Council to further investigate persistence of shoreline oiling from the 1989 spill, and recovery of sea otters and harlequin ducks in oiled areas of western Prince William Sound. Project 030585 has two parts: Part I, conducted by NOAA Auke Bay Laboratory, is entitled **"**Bioavailability of PAH from oil patches and impacts to prey species", and Part II, "Lingering Oil: Bioavailability and Effects to Prey and Predators", which included research on the sea otters and harlequin ducks. Part II was closely integrated with other ongoing research on recovery of sea otters and harlequin ducks conducted in Project 02423, as well as with Project 01534, "Comparison of Cytochrome P450 1A Induction in Blood and Liver Cells of Sea Otters". The foundation for these projects (//423, //534, //585) was provided by the Nearshore Vertebrate Predator Project, 95025-99025, designed to assess recovery of the nearshore ecosystem affected by the *Exxon Valdez* spill. In the present study, approaches to assessing recovery of sea otters included (1) endoscopy of livers, enabling assessment of gross pathologies and collection of biopsies for histology, and (2) measurement of the biomarker cytochrome P4501A (CYP1A) in blood cells of sea otters. In this report we present gross and histological observations of livers, and CYP1A induction in blood cells, of sea otters caught in 2002. Additional blood and liver samples have been collected from sea otters captured in 2003 (Project 030620), and further analyses and interpretation of the liver CYP1A and histopathology findings as well as sea otter locations relative to residual oil, will be included with the final reports for Project //423 (scheduled for completion in late 2003) and //620.

Abstract: Sea otters (*Enhydra lutris)* in the most heavily oiled areas of western Prince William Sound have not recovered from the EVOS, based on estimates of otter abundance and survival rates, and increased induction of the biomarker for aromatic hydrocarbons, cytochrome P4501A (CYP1A). Exposure of sea otters to lingering oil appears to be a likely mechanism explaining lack of full recovery. This study was initiated to obtain further information on oil exposure, using the cytochrome P450 1A (CYP1A) biomarker and gross and histological observations of the liver, and to examine relations between CYP1A levels and residual oil along shorelines in areas occupied by the sea otters. Previous work (Projects //025, //423 and //534) found that sea otters in oiled areas of western PWS had elevated levels of CYP1A, in blood samples collected in 1996-98 and in blood and liver samples collected in 2001. In summer 2002, we resampled sea otters in oiled and unoiled areas of PWS, to monitor health and blood CYP1A values. Liver was also sampled from these otters for examination of histopathological changes. We observed significantly higher CYP1A levels in sea otters from the oiled area than in those from the unoiled area. Grossly, livers of sea otters from the oiled area generally were swollen and pale in color, whereas livers of sea otters from

the unoiled area generally appeared to be normal. However, the incidence of histopathological lesions of the liver was relatively similar in liver samples from both areas. Harlequin duck livers were also collected (captures done as part of Project 02423) for histology; however, those data will be included in the harlequin duck section of the final report for Project //423, in November 2003. Sea otters captured in 2002 were radiotagged and continue to be monitored (Project 030620). Analysis of CYP1A levels in sea otters relative to known locations of residual oil on beaches will be completed when final data from Part I of this project (02585) are available.

Key words: CYP1A, *Exxon Valdez*, oil spill, sea otters, harlequin ducks.

Project Data: Data will be kept in digital format (MS Excel) at the USGS Alaska Science Center in Anchorage.

Citation:

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INTRODUCTION

Sea otters in western Prince William Sound (PWS), Alaska, were exposed to large quantities of oil following the 1989 *Exxon Valdez* spill and mortality was high, particularly in areas with heavy shoreline oiling. In the western Sound overall, sea otter numbers have increased during the 1990's and the population is considered to be recovering. However, studies conducted in 1996-1998 as part of the NVP program (Restoration Project //025) and continued as part of Restoration Project //423, have provided evidence that sea otters in the area of northern Knight Island, where much of the shoreline was heavily oiled in 1989, have not fully recovered from oil spill injury (Bodkin et al. 2002; USGS unpublished data). Sea otter abundance at northern Knight Island was at about 50% of estimated pre-spill abundance since1993, has declined since 2001, and is currently at about 16% of the estimated pre-spill abundance (Bodkin et al. 2002, Dean et al. 2000, USGS unpublished data). Analysis of ages at death of beach-cast sea otters found before and after the spill, identify elevated mortality rates of sea otters that survived the spill, as well as those born after 1989, as a factor delaying recovery (Monson et al. 2000). Cytochrome P450 1A (CYP1A), a biomarker of aromatic hydrocarbon exposure, was elevated in blood samples collected from sea otters in oiled areas (primarily around northern Knight Island) during 1996-98 (Bodkin et al. 2002), and also in samples collected in 2001 (Ballachey et al. 2003). Further, serum enzymes, particularly gamma glutamyl transferase (GGT), are elevated in sea otters from the oiled area, suggesting liver damage in those animals. Overall, these observations implicate exposure to oil as a factor limiting recovery of sea otters (Ballachey et al. 2002, Bodkin et al. 2002). However, based on a comparison of CYP1A levels from 1996-98 and 2001, there are indications that exposure to residual oil is diminishing.

This study was conducted in summer 2002 to obtain further data on induction of CYP1A in sea otters from an oiled area, and to assess liver pathologies in sea otters and harlequin ducks from oiled areas. An additional objective was to determine if individual otters and ducks with very high P450 levels reside in areas known to be contaminated with residual oil (data on residual oil to be collected by NOAA Auke Bay Lab, Part I of this project). Primary findings of the study include (1) elevated CYP1A levels in sea otters from the oiled area compared to those in the unoiled area, and (2) similar incidence of histopathological changes in sea otters in both the oiled and unoiled areas. Harlequin duck histological findings will be included with the final report for Project //423 (November 2003). Sea otters captured in 2002 in the oiled area were implanted with radio-transmitters, and are continuing to be monitored for location and survival (Project //620), and thus the final analysis of sea otter CYP1A and liver observations relative to known areas of residual oil will be completed as part of the final report for Project //620.

OBJECTIVES

1. Assess liver function and incidence of liver abnormalities in sea otters from oiled and unoiled areas (FY02).

2. Monitor P450 induction in sea otters in oiled and unoiled areas, as an indicator of ongoing aromatic hydrocarbon exposure (FY02).

3. Assess incidence of liver abnormalities in harlequin ducks from oiled and unoiled areas (FY02).

4. Relate P450 and liver findings to residual oil concentrations in capture areas (FY02).

5. Closeout: data analyses, writing and submission of final report and publications (FY03).

METHODS

In summer of 2002, sea otters were captured in western PWS, at northern Knight Island (oiled area) and Montague (unoiled area, in the vicinity of Stockdale Harbor and Port Chalmers) Island. Capture and handling methods were similar to those employed previously (Bodkin et al. 2002). Sea otters were sedated, body measurements taken, a tooth collected for age determination, and a blood sample taken by jugular venipuncture. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood by density gradient centrifugation and isolated PBMC were cryopreserved in liquid nitrogen. In addition, three liver biopsies (weighing approximately 0.5 gm total) were surgically collected, using endoscopic procedures, from up to15 otters per area. Two biopsies were frozen immediately in liquid nitrogen and a third biopsy fixed in neutral buffered formalin. During the endoscopy procedure, gross appearance of the liver was noted. Following reversal, sea otters were released in the same vicinity as captured.

Liver samples in formalin and cryopreserved PBMC and liver samples, as well as frozen archived liver samples from 1989, were shipped to Purdue University for analysis in the laboratory of Dr. Paul Snyder.

In the NVP study, the RT-PCR assay (quantitative reverse transcriptase PCR assay; Vanden Heuvel et al. 1993, 1994; Snyder et al. 2001) was adapted to measure CYP1A levels in sea otters. This assay quantifies the messenger RNA (mRNA) that codes for the CYP1A protein, with results reported as molecules of mRNA per 100 ng of RNA. Previously, for sea otters, the assay had been applied to peripheral blood mononuclear cells as well as to liver samples. However, in 2002, a new method for quantification of CYP1A, using real-time PCR, was implemented as it is more

efficient than the method previously used. (Note: A full description of methods and references for the real-time PCR method will be included in the final revised version of this report.)

Histopathology: Liver samples in formalin were processed for histology and sections were examined microscopically.

RESULTS

CYP1A in 2002 samples: Peripheral blood mononuclear cells were collected from 10 sea otters in the unoiled area and 29 sea otters from the oiled area (Table 1 and 2). Induction of CYP1A was higher in from sea otters in the oiled area (Figure 1) than in their counterparts from the unoiled area (t-test, $t = -3.37$, $P<0.005$; Figure 1).

Liver Examinations and Histology, 2002 samples: Liver biopsies were obtained from 27 sea otters in the oiled area, and 10 in the unoiled area. Endoscopy procedures allowed visualization of the livers, as the endoscope was connected to a television monitor. Consequently, gross abnormalities, if present, could be readily observed.

Gross observation: Many animals (24 of 27 in the oiled area and 3 of 10 in the unoiled area) had mildly to moderately swollen, pale livers that may be attributable to the vacuolar and fatty change seen microscopically. This was likely the result of fat and glycogen mobilization. A few animals had evidence of capsular fibrosis and/or multifocal military white foci. The significance of the capsular fibrosis is undetermined and in many species it is an incidental finding. Microscopically, the military white foci were small areas of inflammation characterized as microgranulomas.

Microscopic observations of the liver: There was no difference between areas in the incidence of histological findings (Table 3). Inflammation, vacuolar and fatty change, necrosis, and eosinophilic foci were scored on a scale of 0 – 4 (see Table 3). Inflammation was characterized as either small accumulations of lymphocytes and rarely neutrophils or as microgranulomas. The cause of the inflammation was not readily apparent. We observed varying degrees of hepatocellular vacuolation and fatty change, non-specific changes that are likely attributed to mobilization of fat. Foci of necrosis were noted in some animals, most commonly in the center of the microgranulomas. In some animals there were single or multiple foci characterized as eosinophilic foci. Eosinophilic foci consisted of enlarged hepatocytes with abundant eosinophilic cytoplasm resulting in distinct tinctoral properties compared to adjacent hepatocytes. In some species, foci with similar microscopic appearances are attributable to a proliferation of smooth endoplasmic reticulum (SER). Electron microscopy would be required to identify SER. In the fisher rat, eosinophilic foci are often positive for γ-glutamyltransferase (GGT).

DISCUSSION

The CYP1A data collected in 2002 show a similar pattern to data collected in previous seasons (1996-98, 2001), in that greater induction of the CYP1A enzyme is consistently observed in sea otters from the oiled area in all years. Because a newer, more efficient molecular assay was implemented in 2002, it is difficult to directly compare results from previous years with 2002, and to comment on whether or not we are seeing a decline in CYP1A in oiled areas. Other studies have shown a high correlation between results obtained by the two methods (quantitative reverse transcriptase PCR and real-time PCR) (Stevens et al. 2002, Millson et al. 2003). Further testing of sea otter samples from 2002 and earlier years is planned to verify the comparability of the two methods and to address the issue of a decline in CYP1A induction over time. Nevertheless, these findings support previous conclusions (Bodkin et al. 2002) that lingering oil continues to constrain recovery of sea otters.

The higher proportion of sea otters with pale, swollen livers in the oiled area, relative to the unoiled area, is of unknown significance in consideration of no difference between areas when livers were examined histologically. In 2001, 3 of 15 sea otters from the oiled area had livers with severe abnormalities (Ballachey et al. 2003) but similar observations were not made for any of the 2002 captures. Lesions seen histologically in 2001 and 2002 were generally similar.

Additional findings and conclusions from this study, regarding liver pathologies (sea otters and harlequin ducks), CYP1A levels, and sea otter locations relative to residual oil on shorelines, will be incorporated into the final reports for Projects //423 and //620.

ACKNOWLEDGEMENTS

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Table 1. Sea otter identification and capture data from Knight Island, 2002.

Otter#	VHF Freq	Capture Status	Capture Date	Sex	Age class	Tooth age	Weight (kg)
SO-02-01	165.407	NEW	7/17/2002	F	A	4	24.1
SO-02-02	.	NEW	7/17/2002	F	P	.	9.1
SO-02-03	165.578	NEW	7/17/2002	F	J	1	19.5
SO-02-04	165.618	NEW	7/17/2002	F	J	2	18.2
SO-02-05	165.087	NEW	7/18/2002	F	A	10	26.4
SO-02-06	164.113	NEW	7/18/2002	F	A	5	23.6
SO-02-07	165.180	NEW	7/18/2002	F	A	3	24.1
SO-01-24	165.863	RECAP	7/20/2002	F	A	7	20.5
SO-01-28	165.732	RECAP	7/20/2002	M	A	6	32.3
SO-02-08	165.022	NEW	7/20/2002	F	A	6	25.0
SO-02-09	165.552	NEW	7/20/2002	F	A	7	24.5
SO-97-21	165.137	RECAP	7/20/2002	M	A	11	38.6
SO-98-38	165.353	RECAP	7/24/2002	M	A	11	36.4
SO-01-27	164.966	RECAP	7/25/2002	M	A	5	30.5
SO-02-21	.	NEW	7/25/2002	M	P	.	11.8
SO-02-22	164.461	NEW	7/25/2002	M	A	8	34.1
SO-96-08	164.886	RECAP	7/25/2002	F	A	12	21.4
SO-02-23	164.483	NEW	7/26/2002	F	A	5	23.6
SO-02-24	165.296	NEW	7/28/2002	F	A	5	25.9
SO-02-25	164.275	NEW	7/28/2002	F	J	1	18.6
SO-02-26	165.164	NEW	7/28/2002	F	J	1	17.3
SO-02-27	164.986	NEW	7/28/2002	F	A	7	28.9
SO-02-28	165.372	NEW	7/28/2002	M	A	8	34.1
SO-98-29	165.337	RECAP	7/30/2002	M	A	11	43.2
SO-02-30	.	NEW	8/1/2002	F	P	.	10.0
SO-97-25	164.552	RECAP	8/1/2002	F	A	5	22.7
SO-02-31	164.943	NEW	8/3/2002	M	A	2	27.5
SO-97-28	164.469	RECAP	8/3/2002	F	A	5	24.5
SO-02-32	164.775	NEW	8/4/2002	M	A	11	35.0
SO-02-33	164.183	NEW	8/4/2002	F	J	1	19.5

Table 2. Sea otter identification and capture data from Montague Island, 2002.

Otter#	VHF Freq	Capture Status	Capture Date	Sex	Age class	Tooth age	Weight (kg)
SO-02-10	·	NEW	7/22/2002	F	A	8	27.3
SO-02-11	·	NEW	7/22/2002	F	A	4	22.3
SO-02-12	·	NEW	7/22/2002	M	A	8	34.5
SO-02-13	·	NEW	7/22/2002	F	J	1	20.0
SO-02-14	·	NEW	7/23/2002	F	A	8	25.5
SO-02-15	·	NEW	7/23/2002	F	A	9	25.0
SO-02-16	·	NEW	7/23/2002	M	A	4	30.0
SO-02-17	·	NEW	7/23/2002	F	A	5	20.5
SO-02-18	·	NEW	7/23/2002	F	A	5	23.6
SO-02-19	·	NEW	7/23/2002	M	A	4	30.2
SO-02-20	·	NEW	7/23/2002	M	A	7	32.3

Table 3. Histological findings[a] on liver biopsies collected from sea otters at northern Knight (KNI) and Montague (MON) islands, Prince William Sound, summer 2002.

Animal	Area	Inflammation	Vacuolar and Fatty Change	Necrosis	Eosinophilic foci	Pigment	Other
SO-01-24	KNI	0	1	0	0	0	
SO-01-27	KNI	1	0	0	1	0	
SO-01-28	KNI	0	0	0	0	0	fibrosis
SO-02-01	KNI	1	1	0	0	0	
SO-02-03	KNI	2	1	0	0	0	
SO-02-04	KNI	1	1	0	0	0	
SO-02-05	KNI	0	1	0	0	0	
SO-02-06	KNI	1	1	0	0	0	
SO-02-07	KNI	0	1	0	0	0	
SO-02-08	KNI	0	0	0	0	0	
SO-02-09	KNI	0	2	0	0	0	
SO-02-22	KNI	0	2	0	0	0	
SO-02-23	KNI	0	1	0	1	0	
SO-02-24	KNI	0	2	0	0	0	
SO-02-25	KNI	1	1	0	0	0	
SO-02-26	KNI	1	0	0	0	0	
SO-02-27	KNI	0	1	0	0	0	fibrosis
SO-02-28	KNI	0	2	0	1	0	
SO-02-31	KNI	2	1	1	0	0	
SO-02-32	KNI	0	0	0	0	0	
SO-02-33	KNI	0	0	0	0	0	
SO-96-08	KNI	2	1	0	0	0	
SO-97-21	KNI	1	2	0	0	0	
SO-97-25	KNI	1	1	0	0	0	
SO-97-98	KNI	0	2	0	0	0	
SO-98-29	KNI	0	2	0	1	2	
SO-98-38	KNI	1	1	0	1	0	
SO-02-10	MON	0	2	0	0	0	
SO-02-11	MON	0	2	0	0	0	
SO-02-12	MON	1	3	0	0	2	
SO-02-13	MON	3	0	0	1	0	
SO-02-14	MON	0	3	0	0	0	fibrosis
SO-02-15	MON	0	2	0	0	1	
SO-02-16	MON	0	1	0	1	0	
SO-02-17	MON	1	0	0	0	0	
SO-02-19	MON	0	1	0	0	0	
SO-02-20	MON	1	1	0	0	0	

[a]0 = none 1 = minimal 2 = slight 3 = moderate 4 = severe

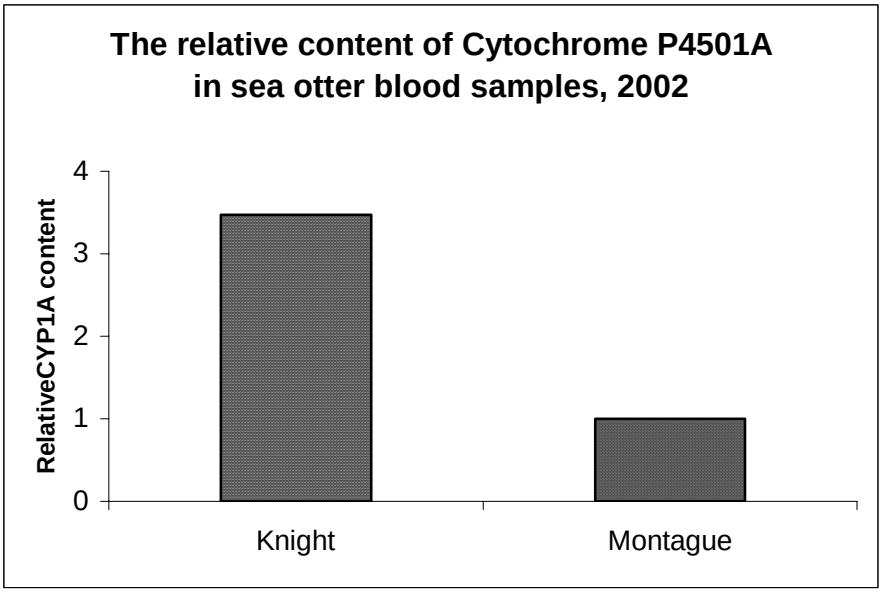

Figure 1. Relative CYP1A content in peripheral blood mononuclear cells collected from sea otters at Northern Knight island (oiled area) and Montague Island (unoiled area) of western PWS, 2002.