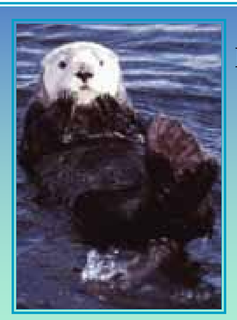
CYTOCHROME P4501A AS A BIOINDICATOR OF EXPOSURE OF SEA OTTERS TO RESIDUAL *EXXON VALDEZ* OIL



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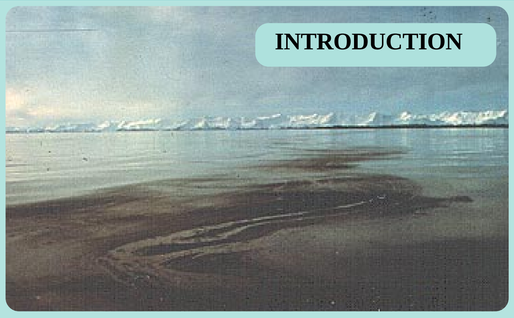
ABSTRACT

Sea otters in western Prince William Sound, Alaska, were exposed to large quantities of oil following the 1989 *Exxon Valdez* spill. In areas which received the greatest oil exposure, a high proportion (estimated at close to 90%) of the sea otters died as a result of oil. Almost a decade later, abundance of sea otters in some areas remains well below the estimated pre-spill number, indicating population recovery is not complete. To assess whether or not continued exposure to residual oil has been a factor limiting recovery of sea otters, we measured induction of cytochrome P4501A (CYP1A), a biomarker of hydrocarbon exposure. In 1996-98, CYP1A levels in sea otters from the heavily oiled area were compared to those from a nonoiled reference area in western Prince William Sound. CYP1A induction was assessed using a reverse-transcriptase PCR assay to quantify mRNA for CYP1A in peripheral blood lymphocytes. In all three years, mean CYP1A levels were significantly higher in the oiled area than in the nonoiled, indicating continued exposure to oil for sea otters in the oiled area. Similar results have been obtained for several other top-level predator species inhabiting the same areas. Residual oil from the 1989 spill is the most likely cause of the observed differences, although we cannot rule out other petroleum or organochlorine contaminants in the environment. Our results are consistent with the hypothesis that continued hydrocarbon exposure may be affecting recovery of sea otters. However, on an individual animal basis, we have not detected any relation between CYP1A induction and our limited measures of sea otter health.

INTRODUCTION



The 1989 *Exxon Valdez* oil spill released about 42 million liters of crude oil into the waters of Prince William Sound, Alaska, a small portion of which penetrated into sediments and has persisted at least 8 years post-spill. Residual oil includes unweathered fractions, which are most toxic to wildlife.

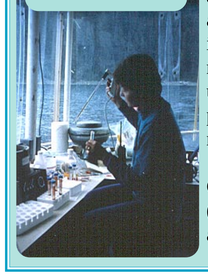
Many birds, mammals, fishes, and marine invertebrates suffered high mortality from acute oil exposure. Additionally, both sub-lethal exposure to initial high concentrations and prolonged exposure to lower levels of residual oil potentially have delayed population recovery. Although longer-term effects of oil have been difficult to document, lack of recovery has been apparent for several species including sea otters, river otters, some seabirds and marine fishes.

In 1995, we implemented the Nearshore Vertebrate Predator study (NVP) to examine the recovery status of the nearshore ecosystem in Prince William Sound. A primary study objective was to assess the continued exposure of sea otters to residual EVOS oil in the nearshore environment. Oil exposure also was evaluated in river otters, pigeon guillemots, harlequin ducks, Barrow's goldeneyes and masked greenlings.

Cytochrome P450 1A (CYP1A) is a protein produced for metabolism of aromatic hydrocarbons, including the polycyclic aromatic hydrocarbons (PAH) found in crude oil. The intermediate compounds produced during metabolism may be highly reactive and more toxic than the original compounds. Consequently, elevated levels of CYP1A indicate exposure to aromatic hydrocarbons and the potential for associated deleterious effects on health of the individual.

We assessed exposure of sea otters to residual oil by measuring the expression of CYP1A and comparing populations in oiled and nonoiled areas of western PWS in 1996-98. We also evaluated individual health of sea otters through measurement of body condition, hematology, and serum chemistry.

METHODS



Sea otters were captured at Knight and Naked islands (oiled area) and at Montague Island (unoiled area; Figure 1). Animals were aged, sexed and weighed, and approximately 35 cc of blood was drawn. CYP1A induction was measured in peripheral blood mononuclear cells. The messenger RNA (mRNA) that codes for the CYP1A protein is quantified using specific cDNA probes and a quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Results are reported as the number of molecules of mRNA for CYP1A per 100 ng of total RNA.

CYP1A in other species was measured using either IHC (immunohistochemical) or EROD (ethoxyresorufin O-deethylase activity) assays.

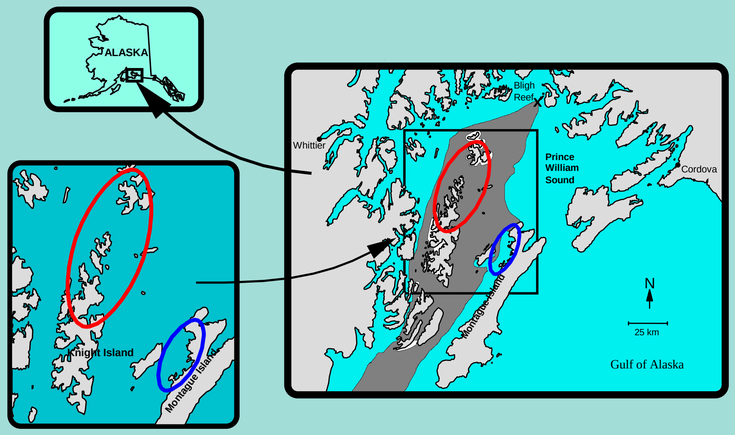
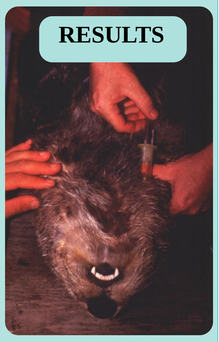


Figure 1. Map of Prince William Sound, showing study areas (oiled within red oval; unoiled within blue oval) and path of spilled oil (hatched area).

RESULTS



In all three years, CYP1A induction was higher in sea otters from the oiled area relative to otters in the unoiled area. The oiled mean was 27.3 x 10^6 molecules of CYP1A mRNA per 100 ng total RNA, vs. an unoiled mean of 1.5 x 10^6, and there was little overlap of values from the two areas. ANOVA on ranks of values showed area to be significant ($P<0.001$), whereas age, sex, and year were not ($P>0.05$). Distribution of CYP1A values pooled across years is shown in Figure 2. No relations were detected between CYP1A and blood parameters or body condition.

The other study species all showed significantly greater CYP1A induction in oiled compared to unoiled areas (Figure 3). Differences in CYP1A between areas were greatest for sea otters, harlequin ducks, Barrow's goldeneyes and masked greenlings, all of which consume benthic invertebrates, and less for river otters and pigeon guillemots, which consume primarily fish.

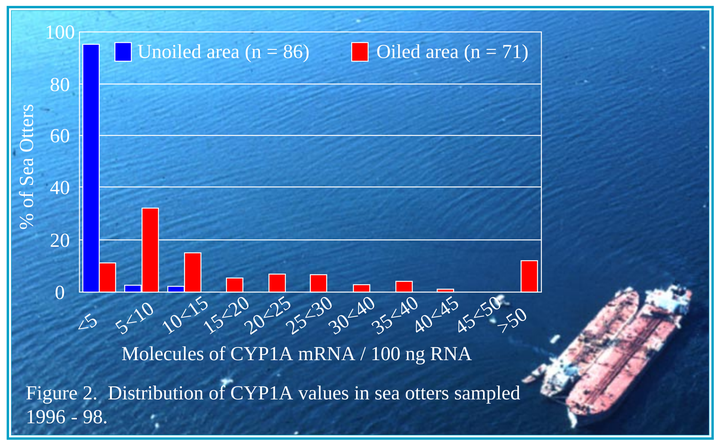


Figure 2. Distribution of CYP1A values in sea otters sampled 1996 - 98.

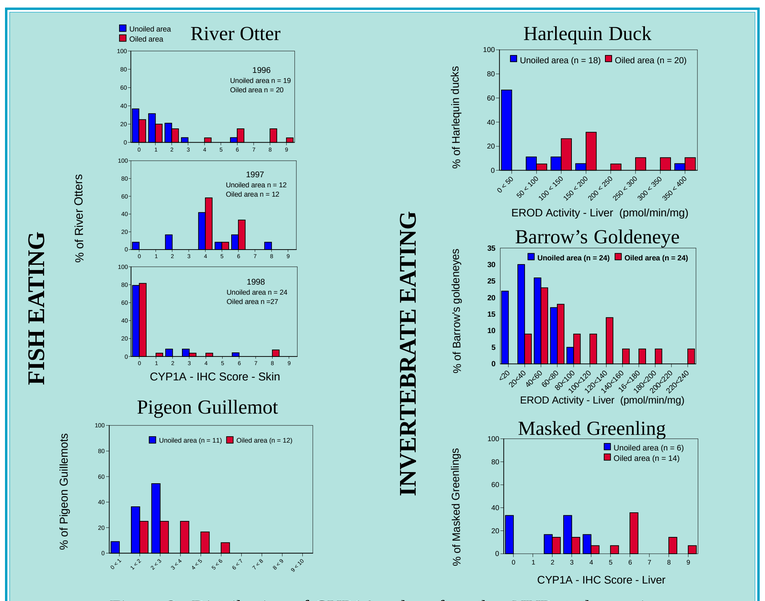


Figure 3. Distribution of CYP1A values for other NVP study species.

DISCUSSION

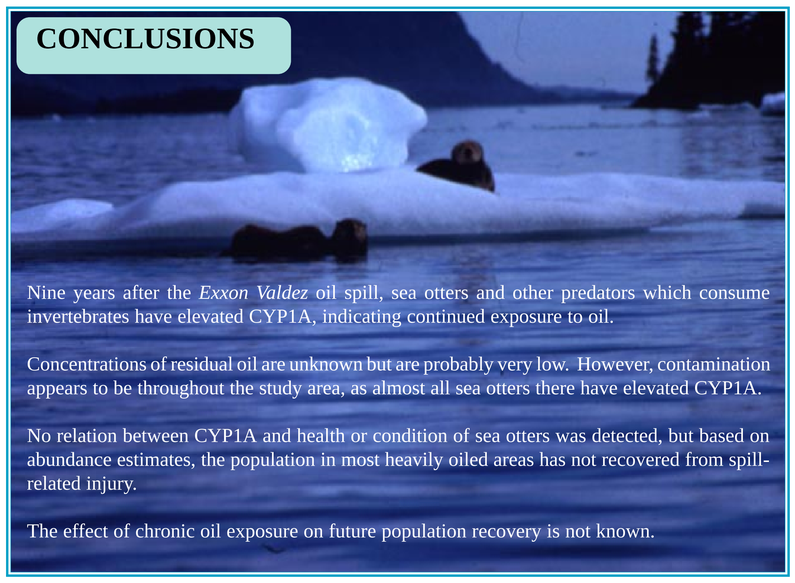
The greater induction of CYP1A in sea otters from the oiled area, and in the other species examined, is strong evidence of persistent oil contamination of nearshore areas through at least 1998 and suggests that injury from oil toxicity could extend well beyond acute spill effects.

The contaminants inducing CYP1A are almost certainly PAH from the spill. Subsurface oil on beaches still contains toxic PAH which could be released back into intertidal areas. Background hydrocarbons and PCB have both been considered as alternate contaminants that might elicit a CYP1A response, but appear unlikely to be contributing to induction.

Route of oil exposure can be evaluated by considering life histories. Sea otters, harlequin ducks, Barrow's goldeneyes and masked greenlings all forage on benthic invertebrates. In general, invertebrates lack the ability to metabolize hydrocarbons and will bioaccumulate them. River otters and pigeon guillemots both consume primarily fish, which do metabolize hydrocarbons, so opportunity for exposure should be less. The greater differences seen between areas for species which consume invertebrates are consistent with prey, and perhaps exposure to hydrocarbons in sediments as well, as a route of exposure.

Body condition, hematology and serum chemistry values of otters with elevated CYP1A generally were normal. However, sea otters in the most heavily oiled areas remain well below estimated prespill abundance and are not increasing. Blood data suggest organ damage from oil toxicity is no longer prevalent, although GGT, a serum enzyme associated with liver dysfunction, remains elevated in some otters from oiled areas. GGT levels have declined over time, however, suggesting individuals with liver damage gradually are being lost from the population, and remaining concentrations of oil in the environment are low. Whether or not continuing chronic exposure is sufficient to affect recovery of sea otters is unknown.

CONCLUSIONS



Nine years after the *Exxon Valdez* oil spill, sea otters and other predators which consume invertebrates have elevated CYP1A, indicating continued exposure to oil.

Concentrations of residual oil are unknown but are probably very low. However, contamination appears to be throughout the study area, as almost all sea otters there have elevated CYP1A.

No relation between CYP1A and health or condition of sea otters was detected, but based on abundance estimates, the population in most heavily oiled areas has not recovered from spill-related injury.

The effect of chronic oil exposure on future population recovery is not known.

ACKNOWLEDGEMENTS

Data on Barrow's goldeneyes, harlequin ducks, masked greenlings, pigeon guillemots and river otters were collected by our co-principal investigators on the NVP study, including R.T. Bowyer, T.A. Dean, D. Esler, G. Golet, S. Jewett and J. Stegeman. We appreciate their contributions and those of the many additional individuals who assisted with data collection and analyses as well as K.A. Kloecker and M.E. Whalen for graphics support.