

River Otters as a Sentinel Species: Effect and Detection of Crude Oil on the Fur of River Otters

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Abstract

River otters (*Lutra canadensis*) have been used as a sentinel species in pollution studies throughout North America. A modified wipe test of river otter fur was developed to detect the presence of residual crude oil on the fur of river otters inhabiting Prince William Sound, Alaska. River otter pelts (both tanned and untanned) were used as models and exhibited differences in hair structure and absorption of crude oil. Immunochemical detection, as well as detection by mass spectrometry, after a methanol extraction of crude oil from the fur were compared. Our results showed that an immunoassay provides an inexpensive and reliable test for oil at concentrations greater than 1 ppm. Crude oil on the fur after extraction with methanol could also be detected in the 1 ppm range. Using the immunoassay wipe test, 17 river otters from oiled and nonoiled areas of Prince William Sound sampled in the summer of 1996 showed no detectable oil > 1 ppm. Mass spectrometry can be used to increase the sensitivity of detection.

Introduction

River otters (*Lutra canadensis*) are widely distributed along coastal shores of Prince William Sound, Alaska (Ben-David et al, 1997; Testa et al, 1994). As nearshore foragers on marine fishes and invertebrates in intertidal and subtidal zones (Bowyer et al, 1994), they are potentially excellent indicators of pollution (Baker et al, 1981; Clark et al, 1981) such as that from the Exxon Valdez oil spill of March, 1989. In that spill, over 39,000 metric tons of North Slope crude oil spread over > 3,500 km of shoreline in Prince William Sound. We previously documented (Duffy et al, 1993; 1994a) that 2 years after the oil spill and an extensive effort to clean shorelines, river otters from a heavily

oiled area had lower body mass as well as elevated levels of blood haptoglobins and liver enzymes than did otters inhabiting a nonoiled area. We hypothesized that elevated haptoglobin levels over so long a period could indicate chronic inflammation and liver injury after acute exposure to oil. Further sampling over large areas of Prince William Sound supported a hypothesis that river otters inhabiting oiled areas could be discriminated from those living in nonoiled areas using biomarkers, such as liver enzymes (Duffy et al, 1994a; 1994b) and fecal profiles of porphyrins (Blajeski et al, 1996). Moreover, there were corresponding ecological effects from the spill including changes in diet, habitat selection, and sizes of home ranges for river otters (Bowyer et al, 1994; 1995).

Although these studies indicate that the health of river otters in Prince William Sound may be recovering, environmental assessments continue to show indications that some river otters in the oiled area of Prince William Sound still may be exposed to crude oil 7 years after the spill. Subsurface oil patches have been reported on beaches within the spill region. A simple assay was needed to determine continued exposure of the river otter to oil. Since Mazet et al (1997) had developed a field wipe assay, in 1996, we surveyed river otters for exposure to this oil using an alcohol swipe test of their fur. In this study we report these results which used an immunochemical detection system as well as laboratory studies using a GC-MS detection system.

Subjects and Methods

Approximately 8 years after the Exxon Valdez oil spill, river otters were trapped from the shoreline in both oiled (Knight Island) and nonoiled

(Jack Pot Bay) areas of Prince William Sound. River otters were captured at latrine sites from May to August 1996 using either Hancock or #11 Sleep Creek double jaw leg-hold traps (Blundell et al, 1997). Once captured, 17 animals were wiped with gauze, which had been presoaked in isopropanol. After wiping both the sides and the underside of the body of a river otter, the gauze was wrapped in foil and frozen until analyzed in the laboratory in Fairbanks, Alaska.

The presence of polycyclic aromatic hydrocarbons (PAHs) were used as an indicator of the presence of crude oil on the fur. The tests used to quantify the concentrations of polycyclic aromatic hydrocarbons were performed using the Quantix Portable WorkStation. This test involves an immunoassay where the PAHs bind to an anti-oil antibody. After an extracted sample was added to the detector, an enzyme substrate was added which bound to any remaining antibody. The enzyme substrate produced a color that was interpreted by the detector, and was inversely proportional to the concentration of PAH in the sample. A QuantiMeter hand-held spectrometer was used to read the detector. The low range of PAH detection was 50 - 820 ppb. Each sample was analyzed in this manner and data were recorded. This test was repeated using a Millipore PAH immunochemical detection assay with 1ppm detection limit.

To validate the reliability of this methodology, a dose-response experiment was conducted. Tanned and raw river otter pelts were used and a varying number of droplets of North Slope Crude Oil approximately 50 μ l each (0, 1, 3, 5, and 10 drops) were placed in a 2-cm² area on each pelt. Each area was then wiped using the same methodology that was used in the field. The gauze was first soaked in isopropanol and then the area was wiped 10 times back and forth. The oil in the gauze was then extracted using 3 mL of methanol. This extract was filtered and analyzed by an PAH immunoassay (Millipore Corp). In this assay, the extract was placed in a test tube that was lined with PAH antibody. An enzyme conjugate was then added and the absorbance of each sample was then measured. Again, the extent of absorbance is inversely proportional to the amount of PAH present in the sample. Each sample was analyzed in this manner and the data were recorded. Also hair samples from river otter fur were viewed on an ISI-40 scanning electron microscope. Before view-

ing, the hair from tanned (detergent extracted) and untanned hides, was mounted on an aluminum stub with a resin. The specimens were then gold coated in an ISI PS-2 sputter coating unit for three minutes. Each of the specimens were then viewed at various magnifications using a 10KeV electron beam.

Results and Discussion

All 17 river otters had exposures of < 1 ppm, which was the detection level for the Millipore method. The more sensitive Quantix method however, showed one river otter to have 74 ppb. This one result confirms utility of the wipe test previously reported (Mazet et al, 1997) and demonstrates that a swipe test can be a good screening method for fur bearing mammals, especially if a sensitive detection system is employed. For example, these wipes could be analyzed by a more sensitive (and expensive) GC-mass spectrometer. Table 1 summarizes the results from GC-MS analysis. In the GC-MS analysis, pentadecane concentrations were calculated based on a tridecane standard curve. In most of the wipe samples, phenol, pentadecane and hexadecane were detected by a GC-MS. Using tridecane as a calibration standard, it was found that the concentrations of phenol and hexadecane were relatively consistent with that of pentadecane.

As a follow up to the field study, we obtained both a tanned river otter hide and an untanned hide. Using the Millipore assay, the absorption and removal of the crude oil from the two different pelts were compared with crude oil volumes ranging from 50 to 500 μ l.

The tanned hide had very little background extractable PAH while the raw hide had more PAH cross-reacting oils as indicated by the lower absorbance. Figure 1 compares the extraction results for tanned and raw fur followed by immunochemical detection. The raw hide shows that at low doses, the PAHs can be detected, whereas a five fold greater amount of oil is needed for detection on the tanned fur. This difference is most likely due to the reabsorption of oil by the tanned fur. EM analysis of the fur showed small differences in the ultrastructure of the tanned and raw fur.

This study shows that with either of two immunoassay tests, the wipe method of oil detection gives reasonable results. If there is oil on the pelt, it will be absorbed by the wiping method and can

Table 1: Concentration of penta decane on fur wipes analyzed by GC-MS.

| Jackpot Bay, PWS | | Herring Bay, PWS | |
|------------------|------------------------|------------------|------------------------|
| Sample: | ppb ± S _c : | Sample: | ppb ± S _c : |
| 02 | 169 ± 24 | 16 | 260 ± 30 |
| 10 | 223 ± 27 | 11 | 278 ± 32 |
| 14 | 183 ± 25 | 18 | 199 ± 26 |
| 19 | 131 ± 23 | 09 | 452 ± 49 |
| mean: | 176 ± 25 | mean: | 297 ± 34 |

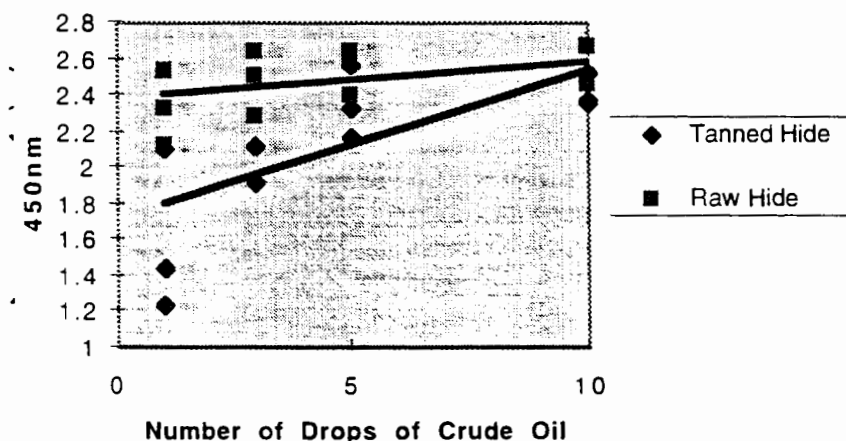


Figure 1. A Comparison Between Tanned and Raw Otter Hide Oil Absorptions.

be used for quantitation if the assay is sensitive enough or the dose is high. This study also implies that the river otters in the Knight Island area of Prince William Sound are not being exposed to acutely toxic concentrations of crude oil, that is greater than 1 ppm.

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