Exxon Valdez Oil Spill State/Federal Natural Resource Damage Assessment Final Report

Aliphatic and Polycyclic Aromatic Hydrocarbons in Eggs, Livers and Stomach Contents of Black-legged Kittiwakes in Prince William Sound, Alaska, After the Exxon Valdez Oil Spill

# Bird Study 8 Final Report

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# Aliphatic and Polycyclic Aromatic Hydrocarbons in Eggs, Livers and Stomach Contents of Black-legged Kittiwakes in Prince William Sound, Alaska, After the Exxon Valdez Oil Spill

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**Study History:** Bird Study 8 was initiated in the summer of 1989 as part of the natural resource damage assessment following the *Exxon Valdez* oil spill to investigate injury to black-legged kittiwakes nesting in Prince William Sound. This report presents results of kittiwake tissue samples collected during 1989 and 1990 for analysis of aliphatic and polycylic aromatic hydrocarbon concentrations. Prior status reports for Bird Study 8 have not presented analysis and discussion of the hydrocarbon analyses of kittiwake tissues.

**Abstract**: Aliphatic and polycyclic aromatic hydrocarbon (PAHs) concentrations were determined in eggs (n=5), livers (n=24), and stomach contents (n=14) of black-legged kittiwakes (*Rissa tridactyla*) in Prince William Sound, Alaska, following the March 1989 *Exxon Valdez* oil spill. Due to the small sample size of eggs, we could not determine a linkage between oil on eggs and the reduced reproductive success of kittiwakes noted after the spill. The single egg collected in 1989 from an oiled colony was contaminated with diesel oil, and the four eggs collected in 1990 from an unoiled colony were not contaminated. None of the eggs had petroleum hydrocarbons in the egg contents. Kittiwake livers did not contain PAH concentrations above the method detection limit, but concentrations of phytane and unresolved complex mixture (UCM) in the livers suggested that kittiwakes ingested oil in 1989. Stomach contain PAH residues, almost exclusively napthalenes. The source of this napthalene pattern is unknown. "Fingerprinting" of the oil ingested by kittiwakes was not possible, and the significance of oil ingestion to the kittiwakes could not be determined.

Key Words: Alaska, black-legged kittiwake, diesel, eggs, Exxon Valdez, livers, oil spill, petroleum hydrocarbons, Prince William Sound, Rissa tridactyla, stomach contents.

**Project Data**: Hydrocarbon residue data collected for this project are available on the Exxon Valdez Oil Spill Research & Restoration Information Project CD-ROM in the State/Federal Trustee Council Hydrocarbon Database. The hydrocarbon database is an application developed using Visual Basic. The CD-ROM is available from the Exxon Valdez Oil Spill Restoration Office, 645 G St., Suite 401, Anchorage, Alaska, 99501, 907-278-8012, fax 907-276-7178, cdsupport@oilspill.state.ak.us.

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#### EXECUTIVE SUMMARY

## Introduction

Analyses of aliphatic and polycyclic aromatic hydrocarbon (PAH) concentrations in black-legged kittiwake (*Rissa tridactyla*) eggs (n=5), livers (n=24), and stomach contents (n=14) collected as part of Bird Study 8, which examined the effects of the *Exxon Valdez* oil spill on the size and productivity of kittiwake colonies within Prince William Sound, are presented. Black-legged kittiwakes are the most abundant colonial seabird in Prince William Sound. About 20,000 pairs nest at 27 colonies. Oil from the *Exxon Valdez* oil spill passed through waters adjacent to 10 of the kittiwake colonies in Prince William Sound, and the oil was present during the period when some birds were attending their colonies, but before nest building had begun. Productivity of kittiwakes nesting at the oiled colonies in 1989 was lower than expected, due to a reduced laying rate, reduced hatching success, or perhaps both.

Kittiwakes were less likely than diving birds to be affected by direct mortality of oil because kittiwakes spend less time in contact with the surface of the water. Therefore, studies of hydrocarbon concentrations in kittiwake tissues were important to determine if pathways for secondary effects were present. Kittiwakes were most likely to have been affected by incidental contact with oil and by ingestion of oil through preening or consuming prey.

#### Objective

The objective of this study was to determine if petroleum hydrocarbons were present in eggs, livers and stomach contents of black-legged kittiwakes in Prince William Sound after the *Exxon Valdez* oil spill.

#### Methods

Five kittiwake eggs were collected--one from an oiled colony in 1989, and four from an unoiled colony in 1990. Forty kittiwakes were collected in 1989, and 60 were collected in 1990. Half of the birds collected in each year were collected from oiled areas, and half were collected from unoiled areas within Prince William Sound. Due to funding limitations, samples from only 14 birds collected in 1989 and 10 birds collected in 1990 were analyzed, and only livers and stomach contents were analyzed. Collected samples were submitted to the Geochemical and Environmental Research Group at Texas A&M University for analysis using standard GC-MS methods. Method detection limits (MDL) at the 99% confidence level were determined using 7 replicate samples of spiked oyster (*Crassostrea virginica*). Concentrations of each analyte on a dry weight basis were determined and compared to the MDL calculated for each sample. The presence of phytane, unresolved complex mixture (UCM) and PAHs at levels above the MDL were considered to be indicators of petroleum hydrocarbon contamination.

# **Results and Discussion**

The single egg collected in 1989 from an oiled colony was contaminated by diesel oil, most likely from boating traffic related to spill cleanup operations. Four eggs collected in 1990 from an unoiled colony did not have PAHs on the eggshell. None of the eggs had aliphatic hydrocarbons or PAHs in the egg contents.

The regular presence of phytane and UCM in the livers of kittiwakes collected in 1989, in contrast with the much lower levels of phytane and the complete absence of UCM in the livers of kittiwakes collected in 1990, indicated that the 1989 birds ingested oil, or they had different diets in 1989 and 1990. We concluded that the differing patterns were most likely due to oil ingestion because diets of Prince William Sound kittiwakes in recent years have been consistently composed of herring and sand lance.

The absence of PAHs from livers of kittiwakes that ingested oil was not unexpected because birds metabolize hydrocarbon pollutants using the hepatic mixed-function oxidase (MFO) system, and uptake and clearance of these compounds is usually quite rapid (within 24 hours).

The stomach contents samples were characterized by high concentrations of pristane and UCM, and the absence of phytane. Polycyclic aromatic hycrocarbon compounds present in the stomach contents samples at concentrations above the MDL were the napthalenes; other PAH compounds were generally absent. The source of this napthalene signal is unknown.

## Conclusions

With only one kittiwake egg collected from the year of the spill, we could not determine any linkage between reduced nesting success of black-legged kittiwakes nesting at colonies within the spill path and contamination by *Exxon Valdez* oil. Concentrations of aliphatic hydrocarbons in the livers of kittiwakes collected in 1989, when compared to concentrations found in kittiwakes collected in 1990, suggested that kittiwakes ingested oil during the year of the spill. The lack of PAHs in kittiwake livers was not unexpected, given the ability of birds to quickly metabolize these compounds. Stomach contents of kittiwakes, presumably fish, did not have an aliphatic signal indicative of oil, but did contain napthalenes; the source and significance of this pattern is unknown. The significance of oil ingestion by kittiwakes to their population or productivity could not be established based on these limited studies. Future studies of spill impacts on marine birds using oil residue analysis should focus on residues on eggshells. Any analysis of tissues should focus on finding evidence of oil ingestion through MFO induction, rather than on documenting residues in liver and other tissues.

# **INTRODUCTION**

On March 24, 1989, the T/V *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound, Alaska, spilling over 11 million gallons of North Slope crude oil (Galt et al. 1991). As part of the natural resources damage assessment (NRDA) for the *Exxon Valdez* oil spill, the U.S. Fish and Wildlife Service (USFWS) undertook studies to estimate direct mortality of marine birds and to document effects that oil ingestion or oil contact might have had on nesting birds. A critical component of the NRDA studies was analysis of tissue samples for aliphatic and polycyclic aromatic hydrocarbon (PAH) compounds indicative of petroleum. The residue analysis was intended to provide a definitive link between oil from the *Exxon Valdez* and any observed effects of the spill.

The majority of the samples taken related to the bird studies were eggs that failed to hatch or eggshell fragments. Adult birds with oil on their plumage could transfer the oil to eggs during incubation, and one of the most likely effects of the spill on birds was reduced hatching success due to the toxic effects of oil on eggs (review in Hoffman 1990). The USFWS was also concerned about effects associated with oil ingestion and therefore collected birds to determine if they had residues in internal tissues. In some cases, prey items or stomach contents were also collected to identify pathways of ingestion.

Here, we present analyses of aliphatic and PAH concentrations in black-legged kittiwake (*Rissa tridactyla*) eggs (n=5), livers (n=24), and stomach contents (n=14) collected as part of Bird Study 8, which examined the effects of the spill on the size and productivity of kittiwake colonies within Prince William Sound (Irons 1996).

Black-legged kittiwakes, members of the gull family, are year-round residents of Prince William Sound. Kittiwakes nest in colonies on cliffs and feed at or near the surface of the water. Black-legged kittiwakes are the most abundant colonial seabird in Prince William Sound. About 20,000 pairs nest at 27 colonies (Irons 1996). In Prince William Sound, kittiwakes start to attend their colonies in March and nest building begins in mid- to late May.

Relatively few dead kittiwakes were recovered from the beaches and waters of Prince William Sound immediately following the spill (Piatt et al. 1990). Direct mortality of kittiwakes from the spill was probably relatively low, because as surface-feeding birds they likely avoided becoming heavily oiled better than diving birds (King and Sanger 1979). Oil from the *Exxon Valdez* oil spill passed through waters adjacent to 10 of the kittiwake colonies in Prince William Sound, and the oil was present during the period when some birds were attending their colonies, but before nest building had begun. Irons (1996) found that productivity of kittiwakes nesting at the oiled colonies in 1989 was lower than expected. Unfortunately, few observations were made during the beginning of the breeding season, so it was impossible to determine whether the low productivity was due to a reduced laying rate or reduced hatching success.

Hydrocarbon concentrations in kittiwake eggs, livers and stomach contents were determined as part of the efforts to determine effects of the *Exxon Valdez* oil spill on kittiwakes. Because kittiwakes were less likely than other seabirds to be affected by direct exposure to oil, studies of hydrocarbon concentrations in kittiwake tissues were important to determine if pathways for secondary effects were present. Kittiwakes were most likely to have been affected by ingestion of oil through preening or by consuming oiled prey. Kittiwakes could contact oil while feeding in oiled waters and when collecting oiled vegetation for nest

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building. Oiling of birds which does not affect the bird's ability to shed water and thus maintain body temperature may not be acutely toxic. However, birds with oil on their breast feathers, or birds building nests with oiled materials, would be expected to have lower nesting success because even minute quantities of oil on the eggshell can be fatal to eggs (Hoffman 1990). In addition, oiled birds could ingest oil through preening resulting in chronic injury to the bird or even acute toxicity.

The other likely mechanism for impacts to kittiwakes was ingestion of oiled prey. Kittiwakes feed by seizing prey at or immediately below the surface of the water. Primary prey species are small forage fish (Sanger 1987). Within Prince William Sound, the most important prey species include Pacific herring (*Clupea harengus*), Pacific sand lance (*Ammodytes hexapterus*), capelin (*Mallotus villosus*) and walleye pollock (*Theragra chalcogramma*) (Irons 1992, Irons and Suryan 1996). To determine if kittiwakes ingested oiled prey, birds were collected and analyzed for hydrocarbon concentrations in their livers and stomach contents.

# **OBJECTIVE**

The objective of this study was to determine if petroleum hydrocarbons were present in eggs, livers and stomach contents of black-legged kittiwakes in Prince William Sound after the *Exxon Valdez* oil spill.

#### **METHODS**

#### Study Area

Prince William Sound, the location of this study, is a fjord-type estuarine system located off the Gulf of Alaska (Fig. 1). Collections were made in the general vicinities of kittiwake colonies in central Prince William Sound within the path of the oil (Eleanor Island, Knight Island) and the vicinities of colonies in northern Prince William Sound which were not in the path of the oil (Shoup Bay, Gull Island, Passage Canal).

#### Sample Collection

One black-legged kittiwake egg that failed to hatch was collected from the oiled Bay of Isles colony in Prince William Sound in 1989 (Fig. 1). Four eggs were collected from the unoiled Shoup Bay colony located near Valdez in 1990. Eggs were removed from nests with aluminum foil rinsed with acetone then hexane and immediately wrapped in the rinsed foil. Eggs were stored in coolers until they could be transported to the USFWS office in Anchorage, where they were refrigerated.

Eggshells and contents were analyzed separately. Egg contents were separated from the eggshell by removing a cap off one end of the shell and pouring the contents into a sterilized I-Chem jar. Eggshells were placed in separate jars.

Forty kittiwakes were collected by shotgun in August 1989, and 60 kittiwakes were collected in August 1990. Birds were collected in August to avoid collection of birds during the active breeding season. In each year, half of the birds were collected from areas within the

path of the oil, and half the birds were collected from areas outside the path of the oil. All birds were collected from within Prince William Sound (Fig. 1).

Once retrieved, birds were wrapped in foil and immediately frozen. Birds were transported to the USFWS office in Anchorage for necropsy and removal of tissues. Due to funding limitations, not all birds were necropsied. Of the birds collected in 1989, samples from 14 birds, all collected from oiled areas, were submitted for analysis. Of the birds collected in 1990, samples from 10 birds, 5 from oiled areas and 5 from unoiled areas, were submitted for analysis. Approximately 2.0 g samples of liver, brain, kidney and breast muscle tissue were removed from the necropsied birds using cleaned dissecting tools. Only the liver samples were submitted for analysis; the other tissue samples were frozen at subzero temperatures and remain archived.

Stomachs were removed from 4 of the kittiwakes collected in 1989 and 10 of the birds collected in 1990. The stomachs were cut open, and contents were placed in a glass jar and frozen.

#### Laboratory Analysis

All samples were sent to the Geochemical and Environmental Research Group at Texas A&M University, College Station, Texas, for analysis, and general methods used in the analysis are described by Short et al. (1996). Wet weights of tissue samples used for the analysis were generally about 1 g, except for the stomach contents samples which ranged from 8 to 43 g. After the addition of internal standards (surrogates) and 50 grams of anhydrous  $Na_2SO_4$ , the tissue samples were macerated using a tissuemizer and extracted three times with dichloromethane. The stomach contents were freeze-dried and extracted with dichloromethane by sonication. A 20 ml sample was removed from the total solvent volume and concentrated to one ml for gravimetric lipid determination. The remaining extract (280 ml) was concentrated to approximately 20 ml in a flat-bottomed flask equipped with a three-ball Snyder condenser. The extract was then transferred to Kuderna-Danish tubes, which were heated in a water bath (60°C) to concentrate the extract to a final volume of 2 ml. During concentration of the solvent, dichloromethane was exchanged for hexane.

The extracts were fractionated by alumina:silica (80-100 mesh) open column chromatography. Silica gel was activated at 170°C for 12 hours and partially deactivated with 3% (v/w) distilled water. Twenty grams of silica gel were slurry packed in dichloromethane over ten grams of alumina. Alumina was activated at 400°C for four hours and partially deactivated with 1% distilled water (v/w). The dichloromethane was replaced with pentane by elution, and the extract was applied to the top of the column. The extract was sequentially eluted from the column with 50 ml of pentane (aliphatic fraction) and 200 ml of 1:1 pentanedichloromethane (aromatic fraction). The fractions were then concentrated to 1 ml using Kuderna-Danish tubes heated in a water bath at 60°C.

The aromatic fraction was further purified by HPLC to remove lipids. The lipids were removed by size exclusion using dichloromethane as an isocratic mobile phase (7 ml/min) and two 22.5 x 250 mm Phenogel columns (Krahn et al. 1988). The purified aromatic fraction was collected from 1.5 minutes prior to the elution of 4,4'-dibromooctafluorobiphenyl to 2 minutes after the elution of perylene. The retention times of the two marker peaks were checked prior

to the beginning and at the end of a set of ten samples. The purified aromatic fraction was concentrated to 1 ml using Kuderna-Danish tubes heated in a water bath at  $60^{\circ}$ C.

Quality assurance for each set of ten samples included a procedural blank and a sample spiked with all calibration analytes (matrix spike) which were carried through the entire analytical scheme. In addition, a laboratory reference oil from the *Exxon Valdez* was used to check the instrument quality control of each sample set. All internal standards (surrogates) were added to the samples prior to extraction and were used for quantification.

Aliphatic hydrocarbons (n-C12 to n-C34 including pristane and phytane) were separated by gas chromatography in the split-less mode using a flame ionization detector (FID). A 30-m x 0.32-mm I.D. fused silica column with DB-5 bonded phase (J&W or equivalent) was used with the chromatographic conditions providing baseline resolution of the n-C17/pristane and n-C18/phytane peak pairs. The five calibration solutions were in the range of 1.25 to 50  $\mu$ g/ml. The internal standards (surrogates) for the aliphatic hydrocarbon analysis were deuterated nalkanes with 12, 20, 24, and 30 carbons, and were added at approximately 10x the method detection limit. Analyte amounts were calculated using the surrogate standards. To monitor the recovery of aliphatic surrogates, gas chromatography internal standard deuterated n-C16 was added just prior to GC-FID analysis.

Aromatic hydrocarbons were separated and quantified by gas chromatography-mass spectrometry (GC-MS) (HP5890-GC and HP5970-MSD). The samples were injected in the splitless mode onto a 0.25 mm x 30 mm (0.32  $\mu$ m film thickness) DB-5 fused silica capillary column (J&W Scientific Inc.) at an initial temperature of 60°C and temperature programmed at 12°C/min to 300°C and held at the final temperature for 6 minutes. The mass spectral data were acquired using selected ions for each of the PAH analytes. The GC-MS was calibrated by injection of a standard component mixture at five concentrations ranging from 0.01 ng/ $\mu$ l to 1 ng/ $\mu$ l. Sample component concentrations were based on correct retention time of the quantitation ion (molecular ion) for the specific analyte and confirmed by the ratio of the confirmation ion.

A calibration check standard was run three times during the sample runs (beginning, middle, end) with no more than 6 hours between calibration checks. The calibration check was confirmed to maintain an average response factor within 10% for all analytes, with no one analyte greater than 25% of the known concentration. With each set of samples a laboratory reference sample (oil spiked solution) was analyzed to confirm GC-MS system performance. The internal standards (surrogates) for the PAH analysis were d8-napthalene, d10-acenapthalene, d10-phenanthrene, d12-chrysene, and d12-perylene, and were added at concentrations similar to that expected for the analytes of interest. To monitor the recovery of the PAH surrogates, gas chromatography internal standards d10-fluorene and d12-benzo(a)pyrene were added just prior to GC-MS analysis.

#### Data Analysis

Data for the kittiwake samples were obtained from the PWSOIL database (Manen et al. in press) and checked against field notes and the GERG catalogs containing the hydrocarbon data to ensure accuracy. Sample dry and wet weights, which were not included in the PWSOIL database, were obtained from the GERG catalogs. Aliphatic and aromatic hydrocarbon concentrations, reported by GERG on a wet weight basis, were converted to ppb dry weight.

Method Detection Limit.--Using 7 replicate samples of spiked oyster tissue, GERG estimated the method detection limit (MDL) at the 99% confidence level for the procedures used in the *Exxon Valdez* NRDA hydrocarbon studies. The minimum number of ng of analyte that would have to be present in a sample to be detected at the 99% confidence level for a selected number of the analytes examined is shown in Table 1. For those analytes for which the MDL was not estimated directly, the average value for the analytes for which the MDL was not estimated directly, the average value for the analytes for each analyte in each sample by dividing the MDL in ng by the weight of the sample in g. The dry weight concentrations of each compound in each sample with the relevant MDL concentrations for the sample were plotted to determine whether the sample contained most compounds at concentrations primarily above or below the MDL. Although many researchers censor data points falling below the MDL, we chose to consider all data, using MDLs simply to provide a standard basis for comparing contamination patterns among samples (Rhodes 1981, Berthouex 1993).

Interpretation.--The literature on detection and quantification of petroleum hydrocarbon residues in bird tissues reveals no standard method of interpreting such data (Burns and Teal 1971, Snyder et al. 1973, Lawler et al. 1978a, Lawler et al. 1978b, Gay et al. 1980, Peakall et al. 1980, Lawler et al. 1981, Miller and Connell 1980, Belisle et al. 1981, Boersma 1986, Llorente et al. 1987, Hall and Coon 1988, Broman et al. 1990). Researchers have typically relied on comparison of chromatograms of aliphatic data, with the presence of UCM or a gaussian pattern of n-alkane peaks superimposed on the UCM often considered to be evidence of oil. However, a major problem with the interpretation of aliphatic data is that these compounds can be of recent biogenic origin, and without control data, conclusions about oiling can be erroneous (Farrington et al. 1973). Because the presence of phytane and UCM have been considered indicators of oil, we paid particular attention to these compounds.

Complex mixtures of PAHs are not known to occur normally in animal tissues. Polycyclic aromatic hydrocarbons have been found in tissues of birds fed oil (Lawler et al. 1978b), but PAHs have not been found in tissues of birds collected from pristine areas in the wild (Llorente et al. 1987, Lawler et al. 1981). Since PAHs were unlikely to be found in bird tissues unless the bird had been recently exposed to oil, we defined samples containing PAHs at concentrations generally above the MDL as contaminated.

#### RESULTS

# Eggs

The single egg collected in 1989 from the oiled Bay of Isles colony on Knight Island (#20543) contained aliphatic hydrocarbons and PAHs on the eggshell (Table 2, Appendix A). The oil on this egg did not match *Exxon Valdez* oil, but did appear to match diesel oil made from North Slope crude (J. Short, NOAA, pers. comm.) The eggshells of the four eggs collected in 1990 from the unoiled Shoup Bay colony were not contaminated with oil residues. Oil residues were not apparent in the contents of any of the 5 eggs.

# Livers

Kittiwakes collected in 1989 had higher concentrations of phytane in their livers than kittiwakes collected in 1990 (Table 3, Table 4, Appendix B; t=4.8, df=13.1, p=0.0004). Although 5 of the 10 kittiwakes forming the 1990 sample were from areas that had been oiled during 1989, none of the 1990 birds had phytane concentrations above the MDL. In addition, none of the kittiwakes collected in 1990 had UCM in their livers, while UCM was present in the livers of 12 of the 14 kittiwakes collected in 1989 (Table 3, Table 4, Appendix B). None of the kittiwake livers contained PAHs at levels consistently above the MDLs (Table 3, Appendix B).

#### Stomach Contents

The stomach content samples were characterized by high concentrations of pristane and UCM, and the absence of phytane (Table 3, Appendix C). Total PAH concentrations in the stomach contents averaged 180.5 ng/g dry weight (SD=69.8, n=13). The PAH compounds present in the stomach contents samples at concentrations above the MDL were the napthalenes; other PAH compounds were generally absent.

# DISCUSSION

## Eggs

The diesel oil found on the 1989 Bay of Isles egg most likely came from boats used in the spill cleanup, post-spill research, or general boating traffic in the area. The egg could have been contaminated during its collection, but the boats used by the crews studying the kittiwakes used gasoline, so we feel that the egg was more likely contaminated prior to collection. The lack of oil on eggs collected in 1990 from the unoiled Shoup Bay colony was not unexpected, because this site was not within the path of the oil.

Irons (1996) found that productivity of colonies in the path of the oil was reduced in 1989. Although he was unable to determine the specific cause of the low productivity, he did determine that the low productivity was due to factors acting on the egg stage. Either kittiwakes laid fewer eggs, or fewer of the eggs laid hatched. Irons (1996) observed birds with oil on their breast feathers at colonies through August 1989, and he also observed kittiwakes using oiled boom materials used in the cleanup as nesting material. Oil from the Exxon Valdez spill could have passed from adults to eggs with negative effects on hatching success, however, with only one egg collected from a colony within the path of the oil during 1989, our data do not allow us to determine whether this happened. The finding of PAHs from diesel oil on the single egg collected in 1989 tells us only that some contamination occurred, most likely related to the cleanup rather than the spill itself.

## Livers

Like other vertebrates, seabirds can metabolize hydrocarbon pollutants using the hepatic mixed-function oxygenase (MFO) system (Lee et al. 1985, Lee et al. 1986, Peakall et

al. 1987, Payne et al. 1987, Walters et al. 1987, Rattner et al. 1989). Radiocarbon labelling studies of fish, birds and seals have demonstrated that uptake and clearance of ingested oil is usually quite rapid (Lee et al. 1972, Lee 1977, Engelhardt et al. 1977, McEwan and Whitehead 1980). Concentrations of oil in livers and other tissues of gulls (*Larus argentatus*) and mallards (*Anas platyrhynchos*) fed oil returned to background levels within 24 hours of oil ingestion (McEwan and Whitehead 1980). Due to the rapidity with which higher organisms metabolize oil, it is difficult to interpret the significance of residues, particularly those in the liver. The presence of UCM may be the only indicator that oil has been present and metabolized (Lawler et al. 1981).

The regular presence of both phytane and UCM in the livers of the kittiwakes collected in 1989 in juxtaposition with the complete absence of UCM and the much lower concentrations of phytane in the livers of kittiwakes collected in 1990, is suggestive that the 1989 birds ingested oil. The absence of phytane and UCM in the 1990 birds, half of which were collected in areas oiled during 1989, suggests that by August 1990, kittiwakes were no longer ingesting more oil than they could readily metabolize.

The differing patterns of aliphatic compound concentrations found in the 1989 and 1990 birds could also be explained by changes in diet of kittiwakes between the two years, if the signal attributed to phytane was actually a biogenic aliphatic that co-elutes with or near phytane. However, we believe this was not the case. Based on foods fed to chicks and some stomach contents analyses, the primary foods of kittiwakes in Prince William Sound during the summer are herring and sand lance (Irons and Suryan 1996). We have no reason to suspect that kittiwakes in August 1990 were eating different foods than kittiwakes in August 1989, and we therefore conclude that the differing patterns in phytane and UCM concentrations between the 1989 and 1990 birds were most likely due to ingestion of oil.

The absence of PAHs in the liver tissue of birds collected 5 months after the spill was not unexpected. Previous studies have demonstrated the liver is not the best place to look for PAH residues, even in birds known to have been exposed to oil. Other tissues, including the skin and its underlying adipose tissue, typically contain much higher concentrations (Lawler et al. 1978a, Lawler et al. 1978b, Gay et al. 1980, Tarshis and Rattner 1982, Broman et al. 1990). The absence of PAH residues in livers is undoubtably related to the liver's role in hydrocarbon metabolism. Lawler et al. (1981) analyzed breast muscle and liver tissues from the carcasses of seabirds washed ashore following the *Amoco Cadiz* oil spill. They found aliphatic hydrocarbons almost identical to *Amoco Cadiz* mousse in shag (*Phalacrocorax aristotelis*) breast tissue, but no aromatic hydrocarbons. Differential uptake or metabolism is the likely cause of this de-coupling of the aliphatic and aromatic fractions.

# Stomach Contents

Kittiwakes in Prince William Sound during the summer eat small schooling fish (Irons and Suryan 1996), and the stomach contents analyzed presumably represented primarily fish meat. Fish were clearly exposed to PAHs following the *Exxon Valdez* spill (Krahn et al. 1992, Varanasi et al. 1993, Collier et al. 1996), but PAH residues in fish muscle tissue were typically not measurable or found at very low levels (< 10 ng/g) (Varanasi et al. 1993). The significance of the signal found in kittiwake stomach contents is unclear. Aliphatic indicators were absent; PAHs were present, but the only compounds present were napthalenes. This

"napthalene pattern" appeared in other tissue samples collected after the spill, but its source has not been identified (J. Short, NOAA, pers. comm.). In oil-dosed mallard ducks, the only aromatic hydrocarbons found were napthalenes (Lawler et al. 1978b, Lawler et al. 1979), and Anderson et al. (1974) reported that some marine organisms can concentrate napthalenes. We may therefore conclude that the kittiwake stomach contents showed some indication of oil contamination, but the source of the oil, and the significance of its presence in the tissues, is unknown. This "napthalene pattern" is worthy of further study.

Whether the oil in the kittiwake livers or stomach contents was from the *Exxon Valdez* spill or from other sources is impossible to determine. When oil is spilled in the marine environment, its composition immediately begins to change due to many processes collectively termed weathering. These weathering processes may include evaporation, dissolution, vertical dispersion, emulsification and sedimentation (NAS 1985). Biological processes also alter the composition of the oil by selective ingestion and degradation. These processes alter the original characteristics and chemical composition of the oil. This combination of processes make determination of the specific source of oil contamination, especially in biological samples, difficult. "Fingerprinting" of oil residues requires comparison of those compounds that are resistant to weathering or metabolism. Compounds of the oil that are least affected by weathering and microbial degradation are the compounds of choice (Hostettler and Kvenvolden 1994).

# Lessons Learned

It is unfortunate that more detailed observations were not made at kittiwake colonies during the early stages of breeding in 1989, and that more eggs were not collected for determination of petroleum hydrocarbon concentrations on eggshells. In responding to future spills, researchers should pay particular attention to what happens during the early stages of breeding, and samples of eggs and feathers that could delineate pathways of exposure to oil should be taken.

This study also suggests that determining petroleum hydrocarbon residues in livers, and perhaps other tissues, is not the best method for assessing oil exposure following spills. If residues are to be determined, the skin and its underlying adipose tissue, which in oil-dosed ducks had the highest levels of petroleum hydrocarbons (Lawler et al. 1978a,b), might be a better tissue. Even when residues are detected in tissues, it is difficult to determine their significance, as there are few data to relate the well-known physiological effects of oil with tissue concentrations. In their study of oil-dosed guillemots, Peakall et al. (1980) found effects but no residues, suggesting that one cannot even infer the absence of effects from the absence of oil residues in tissues.

Because higher vertebrates metabolize petroleum hydrocarbons quickly, we suggest that a better way to document exposure to oil is to measure MFO induction. This approach has been used to document exposure of waterbirds and other wildlife to other pollutants (Rattner et al. 1989), particularly organochlorines (Ellenton et al. 1985, Boersma et al. 1986, Hoffman et al. 1987, Rattner et al. 1993), and we suggest that future studies of oil spill effects on marine birds should take a similar approach. Initial studies of MFO activity in seabirds have been conducted (Knight and Walker 1982a,b, Peakall et al. 1987, Walters et al. 1987). Studies to establish baseline levels of MFO activity in seabirds residing in areas with a high vulnerability to oil spills would be worthwhile.

# CONCLUSIONS

With only one kittiwake egg collected from the year of the spill, we could not determine any linkage between reduced nesting success of black-legged kittiwakes nesting at colonies within the spill path and contamination by *Exxon Valdez* oil. Concentrations of aliphatic hydrocarbons in the livers of kittiwakes collected in 1989, when compared to concentrations found in kittiwakes collected in 1990, suggested that kittiwakes ingested oil during the year of the spill. The lack of PAHs in kittiwake livers was not unexpected, given the ability of birds to quickly metabolize these compounds. Stomach contents of kittiwakes, presumably fish, did not have an aliphatic signal indicative of oil, but did contain napthalenes; the source and significance of this pattern is unknown. The significance of oil ingestion by kittiwakes to their population or productivity could not be established based on these limited studies. Future studies of spill impacts on marine birds using oil residue analysis should focus on residues on eggshells. Any analysis of tissues should focus on finding evidence of oil ingestion through MFO induction, rather than on documenting residues in liver and other tissues.

# ACKNOWLEDGMENTS

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# LITERATURE CITED

- Anderson, J.W., J.M. Neff, B.A. Cox, H.E. Tatem, and G.M. Hightower. 1974. The effects of oil on estuarine animals: toxicity, uptake and depuration, respiration. pp. 285-310 in F.J. Vernberg and W.B. Vernberg, editors. Pollution and Physiology of Marine Organisms. Academic Press, New York.
- Belisle, A.A., M.L. Gay, and N.C. Coon. 1981. Comparison of two extraction methods for the analysis of petroleum hydrocarbon residues in mallard duck eggs by GC and GC-MS. Chemosphere 10:1197-1203.
- Berthouex, P.M. 1993. A study of the precision of lead measurements at concentrations near the method limit of detection. Water Environ. Res. 65:620-629.

- Boersma, D.C., J.A. Ellenton, and A. Yagminas. 1986. Investigation of the hepatic mixedfunction oxidase system in herring gull embryos in relation to environmental contaminants. Environ. Toxicol. Chem. 5:309-318.
- Boersma, P.D. 1986. Ingestion of petroleum by seabirds can serve as a monitor of water quality. Science 231:373-376.
- Broman, D., C. Naf, I. Lundbergh, and Y. Zebuhr. 1990. An in situ study on the distribution, biotransformation and flux of polycyclic aromatic hydrocarbons (PAHs) in an aquatic food chain (seston-Mytilus edulis L.-Somateria mollissima L.) from the Baltic: An ecotoxicological perspective. Environ. Toxicol. Chem. 9:429-442.
- Burns, K.A., and J.M. Teal. 1971. Hydrocarbon incorporation into the salt marsh ecosystem from the West Falmouth oil spill. Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, Technical Report, Reference No. 71-69. 14pp + figures.
- Collier, T.K., C.A. Krone, M.M. Krahn, J.E. Stein, S.-L. Chan, and U. Varanasi. 1996. Petroleum exposure and associated biochemical effects in subtidal fish after the *Exxon* Valdez oil spill. pp. 671-683 in S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright, editors. Proceedings of the Exxon Valdez oil spill symposium. American Fisheries Society Symposium 18.
- Ellenton, J.A., L.J. Brownlee, and B.R. Hollebone. 1985. Aryl hydrocarbon hydroxylase levels in herring gull embryos from different locations on the Great Lakes. Environ. Toxicol. Chem. 4:615-622.
- Engelhardt, F.R., J.R. Geraci, and T.G. Smith. 1977. Uptake and clearance of petroleum hydrocarbons in the ringed seal, *Phoca hispida*. J. Fish. Res. Board. Can. 34:1143-1147.
- Farrington, J.W., J.M. Teal, J.G. Quinn, T. Wade, and K. Burns. 1973. Intercalibration of analyses of recently biosynthesized hydrocarbons and petroleum hydrocarbons in marine lipids. Bull. Environ. Contam. and Toxicol 10:129-136.
- Galt, J.A., W.J. Lehr. and D.L. Payton. 1991. Fate and transport of the Exxon Valdez oil spill. Environ. Sci. and Technol. 25:202-209.
- Gay, M.L., A.A. Belisle, and J.F. Patton. 1980. Quantification of petroleum-type hydrocarbons in avian tissue. J. Chromatogr. 187:153-160.
- Hall R.J. and N.C. Coon. 1988. Interpreting residues of petroleum hydrocarbons in wildlife tissues. U.S. Fish and Wildl. Serv., Biol. Rep. 88(15). 8pp.
- Hoffman, D.J. 1990. Embryotoxicity and teratogenicity of environmental contaminants to bird eggs. Rev. Environ. Contam. Toxicol. 115:39-89.

- \_\_\_\_\_, B.A. Rattner, L. Sileo, D. Docherty, and T.J. Kubiak. 1987. Embryotoxicity, teratogenicity, and aryl hydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. Environ. Res. 42:176-184.
- Hostettler, F.D. and K.A. Kvenvolden. 1994. Geochemical changes in crude oil spilled from the *Exxon Valdez* supertanker into Prince William Sound, Alaska. Org. Geochem. 21:927-936.
- Irons, D.B. 1992. Aspects of foraging behavior and reproductive biology of the black-legged kittiwake. Ph.D. Dissertation, University of California, Irvine, Irvine, California. 143 pp.
- \_\_\_\_\_\_. 1996. Size and productivity of black-legged kittiwake colonies in Prince William Sound, Alaska, before and after the *Exxon Valdez* oil spill. pp. 738-747 in S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright, editors. Proceedings of the Exxon Valdez oil spill symposium. American Fisheries Society Symposium 18.
- and R.M. Suryan. 1996. Kittiwakes as indicators of change in forage fish. Appendix E-1 *in* D.C. Duffy, compiler. *Exxon Valdez* oil spill restoration project annual report. APEX: Alaska Predator Ecosystem Experiment. Restoration Project 95163, Annual Report. Alaska Natural Heritage Program, University of Alaska Anchorage, Anchorage.
- King, J.G., and G.A. Sanger. 1979. Oil vulnerability index for marine oriented birds. pp. 227-239 in J.D. Bartonek and D.N. Nettleship, editors. Conservation of marine birds of northern North America. U.S. Fish and Wildlife Service, Wildl. Res. Rept. 11:1-319.
- Knight, G.C., and C.H. Walker. 1982a. A study of the hepatic microsomal monooxygenase of sea birds and its relationship to organochlorine pollutants. Comp. Biochem. Physiol. 73C:211-221.
- \_\_\_\_\_, and \_\_\_\_\_. 1982b. A study of the hepatic microsomal epoxide hydrolase in sea birds. Comp. Biochem. Physiol. 73C:463-467.
- Krahn, M.M, L.K. Moore, R.G. Bogar, C.A. Wigren, S-L. Chan, and D.W. Brown. 1988. High performance liquid chromatographic method of isolating organic compounds from tissue and sediment extracts. J. Chromatogr. 437:161-175.
- \_\_\_\_\_, D.G. Burrows, G.M. Ylitalo, D.W. Brown, C.A. Wigren, T.K. Collier, S.-L. Chan, and U. Varanasi. 1992. Mass spectrometric analysis for aromatic compounds in bile of fish sampled after the *Exxon Valdez* oil spill. Environ. Sci Technol. 26:116-126.

- Lawler, G.C., W. Loong, and J.L. Laseter. 1978a. Accumulation of saturated hydrocarbons in tissues of petroleum-exposed mallard ducks (*Anas platyrhynchos*). Environ. Sci. Technol. 12:47-51.
- \_\_\_\_\_, \_\_\_\_, and \_\_\_\_\_. 1978b. Accumulation of aromatic hydrocarbons in tissues of petroleum-exposed mallard ducks (*Anas platyrhynchos*). Environ. Sci. Technol. 12:51-54.
- J.P. Holmes, B.J. Fiorito, R.C. Szaro, and J.L. Laseter. 1979. Quantification of petroleum hydrocarbons in selected tissues of male mallard ducklings chronically exposed to South Louisiana crude oil. pp. 584-612 in Proceedings of the Conference of Assessment of Ecological Impacts of Oil Spills (1978). American Institute of Biological Sciences.
- , \_\_\_\_, D.M. Adamkiewicz, M.I. Shields, J.-Y. Monnat, and J.L. Laseter. 1981.
  Characterization of petroleum hydrocarbons in tissues of birds killed in the Amoco Cadiz oil spill. pp. 573-583 in Amoco Cadiz: Fates and effects of the oil spill. Proc. Int. Symposium. 19-22 Nov., 1979. Centre oceanologique de Bretagne. Brest (France).
- Lee, R.F. 1977. Accumulation and turnover of petroleum hydrocarbons in marine organisms. pp. 60-70 In Fate and Effects of petroleum hydrocarbons in marine organisms and ecosystems. D.A. Wolfe, editor. Pergamon Press, New York.
- , R. Sauerheber, and G.H. Dobbs. 1972. Uptake, metabolism, and discharge of polycyclic aromatic hydrocarbons by marine fish. Mar. Biol. 17:201-208.
- Lee, U.-Z, F.A. Leighton, D.B. Peakall, R.J. Norstrom, P.J. O'Brien, J.F. Payne, and A.D. Rahimtula. 1985. Effects of ingestion of Hibernia and Prudhoe Bay crude oils on hepatic and renal mixed-function oxidase and detoxification activities in nestling herring gulls (*Larus argentatus*). Environ. Res. 36:248-255.
- P.J. O'Brien, J.F. Payne, and A.D. Rahimtula. 1986. Toxicity of petroleum crude oils and their effect on xenobiotic metabolizing enzyme activities in the chick embryo in ovo. Environ. Res. 39:153-163.
- Llorente, G.A., A. Farran, X. Ruiz, and J. Albaiges. 1987. Accumulation and distribution of hydrocarbons, polychorobyphenyls, and DDT in tissues of three species of Anatidae from the Ebro Delta (Spain). Arch. Environ. Contam. Toxicol. 16:563-572.
- Manen, C.A., J.R. Price, S. Korn, and M.G. Carls. in press. Natural resource damage assessment: Database design and structure. NOAA Tech. Memo.

Tissue Type, Location and Sample #	Date	Matching Liver/ Stomach Sample #	Wet Wt. (g)	Dry Wt. (g)	Phytane (ppb)	UCM (ppb)	Total PAHs (ppb)	Oil ?
PWS								
#28041	8/90	#28037	33.10	8.47	6	0	53	yes

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- McEwan, E.H., and P.M. Whitehead. 1980. Uptake and clearance of petroleum hydrocarbons by the glaucous-winged gull (*Larus glaucescens*) and the mallard duck (*Anas platyrhynchos*). Can. J. Zool. 58:723-726.
- Miller, G.J. and D.W. Connell. 1980. Occurrence of petroleum hydrocarbons in some Australian seabirds. Aust. Wildl. Res. 7:281-293.

National Academy of Science. 1985. Oil in the sea: input, fates, effects. Washington, D.C.

- Payne, J.F., L.L. Fancey, A.D. Rahimtula, and E.L. Porter. 1987. Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. Comp. Biochem. Physiol. 86C:233-245.
- Peakall, D.B., D. Hallett, D.S. Miller, R.G. Butler, and W.B. Kinter. 1980. Effects of ingested crude oil on black guillemots: a combined field and laboratory study. Ambio 9:28-30.
- \_\_\_\_\_, D.A. Jeffrey and D. Boersma. 1987. Mixed-function oxidase activity in seabirds and its relationship to oil pollution. Comp. Biochem. Physiol. 88C:151-154.
- Piatt, J.F., C.J. Lensink, W. Butler, M. Kendziorek, and D.R. Nysewander. 1990. Immediate impact of the *Exxon Valdez* oil spill on marine birds. Auk 107:387-397.
- Rattner, B.A., D.J. Hoffman, and C.M. Marn. 1989. Use of mixed-function oxygenases to monitor contaminant exposure in wildlife. Environ. Toxicol. and Chem. 8:1093-1102.
- M.J. Melancon, T.W. Custer, R.L. Hothem, K.A. King, L.J. LeCaptain, J.W. Spann,
  B.R. Woodin, and J.J. Stegeman. 1993. Biomonitoring environmental contamination with pipping black-crowned night heron embryos: induction of cytochrome P450.
  Environ. Toxicol. Chem. 12:1719-1732.
- Rhodes, R.C. 1981. Much ado about next to nothing, or what to do with measurements below the detection limit. pp. 157-162 in Environmetrics 81: Selected papers, SIAM-SIMS Conference Series No. 8., Philadelpia, PA.
- Sanger, G.A. 1987. Trophic levels and trophic relationships of seabirds in the Gulf of Alaska. Pages 229-257 in J.P. Croxall, ed. Seabirds: feeding ecology and role in marine ecosystems. Cambridge University Press, Cambridge, England.
- Short, J.W., T.J. Jackson, M.L. Larsen, and T.L. Wade. 1996. Analytical methods used for the analysis of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the Natural Resources Damage Assessment of the Exxon Valdez oil spill. pp. 140-148 in S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright, editors. Proceedings of the Exxon Valdez oil spill symposium. American Fisheries Society Symposium 18.

- Snyder, S.B., J.G. Fox and O.A. Soave. 1973. Mortalities in waterfowl following bunker C fuel exposure: An examination of the pathological, microbiological and oil hydrocarbon residue findings in birds that died after the San Francisco Bay oil spill. Div. of Laboratory Animal Medicine, Stanford Medical Center, Stanford, CA. 27 pp.
- Tarshis, I.B. and B.A. Rattner. 1982. Accumulation of C14-naphthalene in the tissues of redhead ducks fed oil-contaminated crayfish. Arch. Environ. Contam. Toxicol. 11:155-159.
- Varanasi, U., D.W. Brown, T. Hom, D.G. Burrows, C.A. Sloan, L.J. Field, J.E. Stein, K.L. Tilbury, B.B. McCain, and S-L. Chan. 1993. Volume 1: Survey of Alaskan subsistence fish, marine mammal, and invertebrate samples collected 1989-91 for exposure to oil spilled from the *Exxon Valdez*. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-12, 110 p.
- Walters, P., S. Khan, P.J. O'Brien, J.F. Payne, and A.D. Rahimtula. 1987. Effectiveness of a Prudhoe Bay crude oil and its aliphatic, aromatic and heterocyclic fractions in inducing mortality and aryl hydrocarbon hydroxylase in chick embryo in ovo. Archives of Toxicology 60:454-459.

Table 1.Method detection limits (MDLs) in ng for aliphatic and polycyclic aromatic<br/>hydrocarbons (PAHs) analyzed in animal tissues by the Texas A&M University,<br/>Geochemical and Environmental Research Group as part of the Exxon Valdez oil<br/>spill state/federal natural resources damage assessment. MDLs based on 7<br/>replicate analyses of spiked tissue of oysters (Crassostrea virginica) from the<br/>Gulf of Mexico. MDL in ng indicates the minimum number of ng of analyte<br/>needed in a sample to be detected at the 99% confidence level.

Analyte	MDL in Ng
Aliphatic Hydrocarbons	
C10	124.6
C11	50.9
C12	48.9
C15	101.0
C16	54.8
C17	40.8
Pristane	61.7
C18	35.9
C19	16.6
C20	31.9
C21	30.9
C22	23.3
C23	12.8
C24	30.2
C24	35.9
C26	31.8
C27	26.4
C28	25.0
C29	46.1
C30	30.1
C32	48.9
C34	80.2

Analyte	MDL in N
mean	44.9
PAHs	
Naphthalene	29.4
Biphenyl	19.5
Acenaphthylene	27.3
Acenaphthene	13.0
Fluorene	16.3
Phenanthrene	14.3
Anthracene	11.8
Fluoranthene	8.2
Pyrene	11.7
Benz(a)anthracene	32.4
Chrysene	24.2
Benzo(b)fluoranthene	25.5
Benzo(k)fluoranthene	24.9
Benzo(e)pyrene	25.2
Benzo(a)pyrene	28.1
Perylene	12.9
Ideno(1,2,3-cd)pyrene	29.4
Dibenzo(a,h)anthracene	25.7
Benzo(g,h,i)perylene	20.0
2-methylnaphthalene	46.5
1-methylnaphthalene	32.8
2,6-dimethylnaphthalene	33.4
2,3,5-trimethylnaphthalene	28.6
1-methylphenanthrene	37.7
mean	24.1

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Tissue Type and Sample ID#	Date	Location	Wet Wt. (g)	Dry Wt. (g)	Phytane (ppb)	UCM (ppb)	Total PAHs (ppb)	Oil ?
Egg Contents								
20380	8/6/89	Bay of Isles	1.11	.21	0	13,743	561	no
24429	7/30/90	Shoup Bay	5.01	2.18	32	3,677	36	no
24431	7/30/90	Shoup Bay	5.02	1.55	2	98,457	206	no
24433	7/30/90	Shoup Bay	5.00	1.96	1	32,653	172	no
24435	7/30/90	Shoup Bay	5.02	1.38	1	199,345	642	no
Eggshells								
20543	8/6/89	Bay of Isles	2.81	2.04	309	38,293	1,572	yes
24430	7/30/90	Shoup Bay	2.37	1.60	24	28,440	79	no
24432	7/30/90	Shoup Bay	2.22	1.37	11	8,750	69	no
24434	7/30/90	Shoup Bay	2.91	1.85	21	8,337	62	no
24436	7/30/90	Shoup Bay	3.22	2.06	20	2,814	50	no

Table 2.Phytane, unresolved complex mixture (UCM), and polycyclic aromatic hydrocarbon (PAH) concentrations (ppb dry<br/>wt.) in egg contents and eggshells of black-legged kittiwakes in Prince William Sound, Alaska, after the Exxon<br/>Valdez oil spill.

1 41402, 01								
Tissue Type, Location and Sample #	Date	Matching Liver/ Stomach Sample #	Wet Wt. (g)	Dry Wt. (g)	Phytane (ppb)	UCM (ppb)	Total PAHs (ppb)	Oil ?
Livers								
Eleanor Is.								
#20386	8/12/89	n/a	2.09	.68	267	22,160	178	yes
#20390	8/12/89	n/a	2.14	.73	1261	46,904	251	yes
#20391	8/12/89	n/a	2.03	.66	652	32,818	207	yes
#20393	8/12/89	n/a	2.02	.65	1,218	41,581	248	yes
#20394	8/12/89	n/a	2.01	.70	280	12,347	232	yes
#28361	8/12/89	#28365	1.94	.64	351	14,853	165	yes
#28397	8/12/89	#28401	2.08	.71	662	103,121	217	yes
#38433	8/12/89	#28437	1.82	.53	336	0	261	yes
#28043	8/20/90	#28047	1.86	.67	25	0	155	no
#28055	8/20/90	#28059	1.86	.62	36	0	260	no
#28073	8/20/90	#28077	1.56	.51	30	0	241	no
#28079	8/20/90	#28083	1.74	.54	0	0	229	no
Bay of Isles								
#20411	8/12/89	n/a	2.01	.64	382	22,613	219	yes

Table 3.Phytane, unresolved complex mixture (UCM), and polycyclic aromatic hydrocarbon (PAH) concentrations (ppb dry<br/>wt.) in livers and stomach contents of black-legged kittiwakes in Prince William Sound, Alaska, after the Exxon<br/>Valdez oil spill.

Tissue Type, Location and Sample #	Date	Matching Liver/ Stomach Sample #	Wet Wt.	Dry Wt. (g)	Phytane (ppb)	UCM (ppb)	Total PAHs (ppb)	Oil ?
#20413	8/12/89	n/a	2.04	.68	230	17,340	212	yes
#20414	8/12/89	n/a	1.94	.58	216	0	244	yes
#20419	8/12/89	n/a	2.00	.63	930	7,619	263	yes
#20420	8/12/89	n/a	1.98	.69	0	6,026	168	no
#28391	8/12/89	#28395	1.96	.55	544	18,887	210	yes
#28067	8/20/90	#28071	1.21	.37	58	0	357	no
Shoup Bay								
#28025	8/23/90	#28029	1.81	.61	43	0	261	no
#28031	8/23/90	#28035	2.06	.63	30	0	161	no
Gull Island								
#28049	8/21/90	#28053	1.88	.55	51	0	146	no
Passage Canal								
#28109	8/21/90	#28113	1.83	.56	53	0	249	no
PWS								
#28037	8/90	#28041	1.54	.48	24	0	207	no

# Stomach Contents

Eleanor Island

Tissue Type, Location and Sample #	Date	Matching Liver/ Stomach Sample #	Wet Wt. (g)	Dry Wt. (g)	Phytane (ppb)	UCM (ppb)	Total PAHs (ppb)	Oil ?
#28365	8/12/89	#28361	18.64	5.48	85	14,626	154	yes
#28401	8/12/89	#28397	10.88	3.37	68	7,103	217	yes
#28437	8/12/89	#28433	14.3	3.80	69	7,903	291	yes
#28047	8/20/90	#28043	17.17	4.89	15	702	225	yes
#28059	8/20/90	#28055	15.06	4.74	16	4,130	267	yes
#28065	8/20/90	#28061	8.06	2.50	54	3,869	496	yes
#28083	8/20/90	#28079	8.29	2.59	19	18,244	205	yes
#28077	8/20/90	#28073	43.48	10.52	3	425,294	95	yes
Bay of Isles								
#28071	8/20/90	#28067	23.19	7.86	11	0	88	yes
#28395	8/12/89	#28391	24.76	10.60	118	156,268	166	yes
Shoup Bay								
#28029	8/23/90	#28025	10.59	3.77	14	0	180	yes
#28035	8/23/90	#28031	11.76	3.48	28	0	256	yes
Gull Island								
#28053	8/21/90	#28049	30.75	8.24	12	2,239	150	yes
Passage Canal								
#28113	8/21/90	#28109	9.41	2.68	31	5,969	No data	no?

·			
	<u>n</u>	mean	std
Phytane			
1989	14	535.5	367.7
1990	10	35.0	16.3
UCM			
1989	14	24,733.5	25,638.6
1000	10	<u> </u>	
1990	10	0.0	0.0

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Table 4.Average phytane and unresolved complex mixture (UCM) concentrations in<br/>livers of black-legged kittiwakes collected in Prince William Sound, Alaska, in<br/>1989 and 1990, following the Exxon Valdez oil spill

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Fig. 1. Prince William Sound, Alaska, showing the path of surface oil from the *Exxon Valdez* oil spill and black-legged kittiwake colonies from which eggs or kittiwakes were collected for study of aliphatic and polycyclic aromatic hydrocarbon concentrations.

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# APPENDIX A

Aliphatic and polycyclic aromatic hydrocarbon concentrations (ng/g dry weight) in egg contents and eggshells of black-legged kittiwakes in Prince William Sound, Alaska, in 1989, after the *Exxon Valdez* oil spill.

Abbreviations: Aliphatics--10 through 34=carbon number of n-alkanes; PRI=pristane, PHY=phytane, UCM=unresolved compound mixture. Aromatics--in order from left to right--N=naphthalene, 1=C1-naphthalene, 2=C2-naphthalene, 3=C3-naphthalene, 4=C4naphthalene, B=biphenyl, A=acenaphthylene, A=acenaphthene, F=fluorene, 1=C1fluorene, 2=C2-fluorene, 3=C3-fluorene, 4=C4-fluorene, P=phenanthrene, A=anthracene, 1=C1-phenanthrene, 2=C2-phenanthrene, 3=C3-phenanthrene, 4=C4-phenanthrene, D=dibenzothiophene, 1=C1-dibenzothiophene, 2=C2-dibenzothiophene, 3=C3dibenzothiophene, F=fluoranthene, P=pyrene, F=methyl fluroranthene-pyrene, B=benz(a)anthracene, C=chrysene, 1=C1-chrysene, 2=C2-chrysene, 3=C3-chrysene, 4=C4-chrysene, b=benzo(b)fluoranthene, k=benzo(k)fluoranthene, e=benzo(e)pyrene, a=benzo(a)pyrene, P=perylene, i=ideno(1,2,3-cd)pyrene, D=dibenzo(a,h)anthracene, B=benzo(g,h,i)perylene, 2=2-methylnaphthalene, 1=1-methylnaphthalene, 2=2,6dimethylnaphthalene, 2=2,3,5-trimethylnaphthalene, 1=1-methylphenanthrene.



Sample #24435 -+- Estimated MDL



Sample #20380 --- Estimated MDL

Black—legged Kittiwake Egg Contents Shoup Bay 7/30/90



A-4


A-5

Black—legged Kittiwake Egg Contents Shoup Bay 7/30/90



A-6

Black—legged Kittiwake Eggshell Knight I. 8/6/89



Black—legged Kittiwake Eggshell Shoup Bay 7/30/90





Black-legged Kittiwake Eggshell Shoup Bay 7/30/90



A-10

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A-11

#### APPENDIX B

Aliphatic and polycyclic aromatic hydrocarbon concentrations (ng/g dry weight) in livers of black-legged kittiwakes in Prince William Sound, Alaska, in 1989, after the *Exxon Valdez* oil spill.

Abbreviations: Aliphatics--10 through 34 = carbon number of n-alkanes; PRI=pristane, PHY=phytane, UCM=unresolved compound mixture. Aromatics--in order from left to right--N=naphthalene, 1=C1-naphthalene, 2=C2-naphthalene, 3=C3-naphthalene, 4=C4naphthalene, B=biphenyl, A=acenaphthylene, A=acenaphthene, F=fluorene, 1=C1fluorene, 2=C2-fluorene, 3=C3-fluorene, 4=C4-fluorene, P=phenanthrene, A=anthracene, 1=C1-phenanthrene, 2=C2-phenanthrene, 3=C3-phenanthrene, 4=C4-phenanthrene, D=dibenzothiophene, 1=C1-dibenzothiophene, 2=C2-dibenzothiophene, 3=C3dibenzothiophene, F=fluoranthene, P=pyrene, F=methyl fluroranthene-pyrene, B=benz(a)anthracene, C=chrysene, 1=C1-chrysene, 2=C2-chrysene, 3=C3-chrysene, 4=C4-chrysene, b=benzo(b)fluoranthene, k=benzo(k)fluoranthene, e=benzo(e)pyrene, a=benzo(a)pyrene, P=perylene, i=ideno(1,2,3-cd)pyrene, D=dibenzo(a,h)anthracene, B=benzo(g,h,i)perylene, 2=2-methylnaphthalene, 1=1-methylnaphthalene, 2=2,6dimethylnaphthalene, 2=2,3,5-trimethylnaphthalene, 1=1-methylphenanthrene.



































B-16









## Black-legged Kittiwake Liver Shoup Bay 8/23/90



### Black-legged Kittiwake Liver Shoup Bay 8/23/90



#### Black-legged Kittiwake Liver Gull Island 8/21/90



#### Black-legged Kittiwake Liver Passage Canal 8/21/90



#### Black-legged Kittiwake Liver Prince William Sound 8/90



#### APPENDIX C

Aliphatic and polycyclic aromatic hydrocarbon concentrations (ng/g dry weight) in stomach contents of black-legged kittiwakes in Prince William Sound, Alaska, in 1989, after the *Exxon Valdez* oil spill.

Abbreviations: Aliphatics--10 through 34=carbon number of n-alkanes; PRI=pristane, PHY=phytane, UCM=unresolved compound mixture. Aromatics--in order from left to right--N=naphthalene, 1=C1-naphthalene, 2=C2-naphthalene, 3=C3-naphthalene, 4=C4naphthalene, B=biphenyl, A=acenaphthylene, A=acenaphthene, F=fluorene, 1=C1fluorene, 2=C2-fluorene, 3=C3-fluorene, 4=C4-fluorene, P=phenanthrene, A=anthracene, 1=C1-phenanthrene, 2=C2-phenanthrene, 3=C3-phenanthrene, 4=C4-phenanthrene, D=dibenzothiophene, 1=C1-dibenzothiophene, 2=C2-dibenzothiophene, 3=C3dibenzothiophene, F=fluoranthene, P=pyrene, F=methyl fluroranthene-pyrene, B=benz(a)anthracene, C=chrysene, 1=C1-chrysene, 2=C2-chrysene, 3=C3-chrysene, 4=C4-chrysene, b=benzo(b)fluoranthene, k=benzo(k)fluoranthene, e=benzo(e)pyrene, a=benzo(a)pyrene, P=perylene, i=ideno(1,2,3-cd)pyrene, D=dibenzo(a,h)anthracene, B=benzo(g,h,i)perylene, 2=2-methylnaphthalene, 1=1-methylnaphthalene, 2=2,6dimethylnaphthalene, 2=2,3,5-trimethylnaphthalene, 1=1-methylphenanthrene.



#### Black-legged Kittiwake Stomach Contents Eleanor Island (Clove Triangle) 8/12/89



# Black-legged Kittiwake Stomach Contents Eleanor Island (Clove Triangle) 8/12/89

Sample #28437 ---- Estimated MDL

### Black-legged Kittiwake Stomach Contents Eleanor Island (Clove Triangle) 8/20/90










# Black-legged Kittiwake Stomach Contents Bay of Isles (Knight Is.) 8/20/90



## Black-legged Kittiwake Stomach Contents Bay of Isles (Knight Is.) 8/12/89



#### Black-legged Kittiwake Stomach Contents Shoup Bay 8/23/90



#### Black-legged Kittiwake Stomach Contents Shoup Bay 8/23/90



#### Black-legged Kittiwake Stomach Contents Gull Island 8/21/90



#### Black-legged Kittiwake Stomach Contents Passage Canal 8/21/90



# Aromatic data--sample lost in processing.

Black-legged Kittiwake Stomach Contents Prince William Sound 8/90

