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Harbor Seals

State-Federal Natural Res[illegible] Damage Assessment

for April 1989-December 1990

1990 Status Report

Marine Mammals Study Number 5:

Assessment of Injury to Harbor Seals

in Prince William Sound, Alaska, and Adjacent Areas

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EXECUTIVE SUMMARY

The goal of this project is to determine whether the Exxon Valdez oil spill (EVOS) has had a measurable impact on harbor seals, *Phoca vitulina richardsi*, in Prince William Sound (PWS) and adjacent areas. Harbor seals are one of the most abundant species of marine mammals in PWS. They are resident throughout the year, occurring primarily in the coastal zone where they feed and haul out to rest, bear and care for their young, and molt. Some of the largest haulouts in PWS, and waters adjacent to these haulouts, were directly impacted by substantial amounts of oil during the EVOS. Oil impacted harbor seal habitat in the Gulf of Alaska (Gulf) at least as far to the southwest as Tugidak Island. The impacts of the EVOS on harbor seals are of particular concern since trend count surveys have indicated that the number of harbor seals in PWS declined by 40% from 1984 to 1988, and similar declines have been noted in other parts of the northern Gulf.

During the EVOS, harbor seals were exposed to oil both in the water and on land. In the early weeks of the spill they swam through oil and inhaled aromatic hydrocarbons as they breathed at the air/water interface. On haulouts in oiled areas, seals crawled through and rested on oiled rocks and algae throughout the spring and summer. Pups were born on haulouts in May and June, when some of the sites still had oil on them, resulting in pups becoming oiled. Many also nursed on oiled mothers. At haulouts throughout the oiled areas, seals were exposed to greatly increased human activity in the form of air and boat traffic and cleanup activities.

This study was designed to investigate and quantify, as possible, the effects of oil and the disturbance associated with cleanup on distribution, abundance, and health of harbor seals in the affected area. There were five major field components: 1) small boat work was conducted in order to observe seals on oiled and unoiled haulouts and to classify them by presence and extent of oil; 2) searches were made of the coastline by project personnel and others and the carcasses of any dead harbor seals were documented, necropsied, and if in suitable condition, samples obtained for toxicological and histopathological analyses; 3) harbor seals that were oiled to various degrees were collected in order to conduct necropsies and to obtain samples for histopathological and toxicological analysis; 4) aerial surveys were conducted in June in order to count the number of non-pups and the number of pups at haulout sites in oiled and unoiled areas; and 5) aerial surveys were conducted during the molt in September to count seals at 25 trend count sites, for comparison of trends in abundance at oiled and unoiled sites.

During small boat operations in 1989, we saw no oiled seals in unoiled areas, and few oiled seals in intermediate areas. In

oiled areas over 70% of the seals seen in May were oiled, most of them heavily. By early September, when seals older than pups were molting, less than 20% were oiled. Seal pups born in oiled areas became oiled shortly after birth. In Bay of Isles and Herring Bay, 89-100% of all seal pups seen were oiled. In April and June 1990 there was no sign of external oiling observed on any seals.

Abnormal behavior by oiled harbor seals in oiled areas was observed on many occasions in April-June 1989. Oiled seals were reported to be sick, lethargic, or unusually tame. Helicopters and other aircraft often approached at 80 m altitude without causing those seals to flee into the water, and on several occasions investigators were able to approach on foot to within a few meters. In September 1989 and April 1990 seals were noticeably more wary and difficult to approach.

In the first few months after the EVOS, we were notified of 19 harbor seals that were found dead or died in captivity. Fifteen of these were externally oiled and 13 were pups. They were examined and sampled as possible.

In 1989, 20 harbor seals were collected in order to obtain complete, high-quality tissue samples for histopathology and toxicology. Of these, 11 were heavily oiled, 3 were lightly or moderately oiled, and 6 were not externally oiled. In April 1990 six seals were collected; all were collected in areas that had been heavily oiled, but none showed external signs of oiling. Two "control" animals were collected in the Ketchikan area in August 1990.

Fluorimetric analyses of bile are complete for all specimens except the two control seals. Levels of phenanthrene and napthalene in the bile clearly indicated that most seals from oiled areas had been exposed to and had assimilated hydrocarbons. Mean values for harbor seals from oiled areas of PWS were 8-12 times higher than those from the Gulf. The highest bile values for individual oiled seals were over 1000 times higher than for unexposed seals. One year after the spill, average values from PWS seals were 5-6 times higher than the 1989 values from the Gulf. A pregnant female collected at this time had the fourth highest bile values of any seal that was analyzed. The values for her unborn fetus were low, but did indicate exposure. Since elevated levels of hydrocarbons in bile indicate recent exposure (i.e. within 2-4 weeks), the elevated levels found in spring 1990 suggest that seals were still encountering oil in the environment or that they were metabolizing stored fat reserves that had elevated levels of hydrocarbons.

All seals collected from the Gulf of Alaska had non-detectable or very low parts per billion (ppb) levels of polycyclic aromatic hydrocarbons (PAHs) in liver, blubber, muscle, and brain tissue.

PAH values in PWS seals from oiled areas were also non-detectable or low for all tissues except blubber. Total PAH values in blubber were greater than 100 ppb and ranged as high as 800 ppb in 7 of 12 seals that were collected from oiled areas of PWS in April-June 1989, and 1 of 6 collected in April 1990. Two of the 1989 seals with high PAH values were a mother-pup pair. Milk from the pup had the highest PAH value (1200 ppb) of any tissue in any seal that we analyzed. Health implications of these toxicological findings are unknown. There is little information available on the effects on seals of exposure to hydrocarbons.

Preliminary microscopic examination of seal tissues to detect any damage caused by exposure to oil is complete. Severe debilitating lesions (intramyelinic edema and axonal degeneration) were found in the thalamus of the brain of a heavily oiled seal collected in Herring Bay 36 days after the spill. Similar but milder lesions were found in five other seals collected three or more months after the spill. Such lesions were not present in either of the control seals. The thalamus is responsible for relaying impulses of sensory systems, and any interference with transmission of impulses may interfere with respiration and predispose a seal to drowning.

Results of aerial surveys conducted during June 1989 to compare pup production in oiled and unoiled areas indicated no significant difference in the ratio of pups to non-pups. In 1990, however, the ratio of pups to non-pups was significantly higher at oiled sites than at unoiled sites. Together with the dead fetuses and pups found following the spill, this suggests that pup mortality was higher than normal in oiled areas in 1989. Pupping surveys for at least one additional year are necessary to support this conclusion.

Prior to the EVOS, seals in PWS had declined between 1984 and 1988. The magnitude of the decline was similar at oiled and unoiled sites (37% versus 36%). From 1988 to 1990, however, the decline in seals at oiled sites was much greater than at unoiled sites (35% versus 13%). Orthogonal contrasts from a repeated measures ANOVA clearly indicated that the difference between oiled and unoiled areas was significant. The overall change in the number of seals counted at intermediate areas (-41%) was similar to that in oiled areas, but due to small sample size it was not significantly different from unoiled areas.

In order for the objectives of this project to be fully met, the following tasks must be completed:

1. Aerial surveys during pupping should be conducted for at least one additional year, in June 1991.
2. Aerial surveys during the fall molt should be conducted for at least one additional year, in August-September 1991.

3. Remaining blubber and milk tissues should be analyzed, as well as blood samples from select seals.
4. Final analysis and interpretation of histopathology slides should be completed.

As part of restoration and monitoring natural recovery, we recommend that the number of harbor seals at trend count areas in PWS be monitored during pupping and molting for the next three years and at intervals thereafter, as necessary. In addition we recommend that a study be initiated to satellite tag harbor seals in PWS in order to better understand habitat use, and facilitate protection and management of important harbor seal habitat.

OBJECTIVES

1. To describe the characteristics and persistence of oiling of harbor seal pelage that resulted from contact with oil in the water and on haulouts.

2. To test the hypothesis that harbor seals found dead in the area affected by the EVOS died due to oil toxicity.

3. To test the hypothesis that seals exposed to oil from the EVOS assimilated hydrocarbons which resulted in harmful pathological conditions.

4. To test the hypothesis that pup production was lower in oiled than in unoiled areas, or than in years not affected by the EVOS.

5. To test the hypothesis that the number of harbor seals on the trend count route during pupping and molting decreased in oiled areas of PWS as compared to unoiled areas.

6. To identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

METHODS

Methodology used in this study was described in detail in the methods section and Protocols A and B of the detailed study plan for this project submitted in October 1989, and in the preliminary status report dated January 12, 1990. The description of methodology for 1989 field work is not repeated in this report.

Field work conducted in 1990 included observations and collections of seals from small boats, aerial surveys during the pupping season, and aerial surveys during the molt.

Small boat observations were conducted in Herring Bay and Bay of Isles during April 10-14 and May 29-June 30. The number of adults and pups was counted, and seals and haulouts were inspected for the presence of oil.

Six harbor seals were collected in April 1990 at and adjacent to sites in PWS that were impacted by the EVOS, and where externally oiled seals had been collected in June 1989. Since seals had molted the previous autumn it was not possible to select oiled seals based on their pelage. Two seals were collected near Ketchikan, Alaska in August 1990 to serve as control animals from an unoiled area. The location where these seals were collected was more than 1000 km from the region impacted by the EVOS. Each animal was necropsied as soon as possible after death by a qualified veterinary pathologist (Dr. Terry Spraker, Colorado State University), and measurements and samples were taken as described in the study plan, Protocols, and preliminary report.

Aerial surveys were flown in PWS in June and September along the previously established trend count route (Calkins and Pitcher 1984; Pitcher 1986, 1989). The trend count route covered 25 haulout sites and included 6 sites that were impacted by the EVOS (Agnes, Little Smith, Big Smith, Seal, and Green islands, and Applegate Rocks), 16 unoiled sites, and 3 intermediate sites that were not heavily oiled but were adjacent to oiled areas. Visual counts were made of seals at each site and photographs were taken of large groups to facilitate counting, as described in Protocol B of study plan. During June surveys, separate counts were made of pups and non-pups.

Several analyses were performed on the 1989-90 pupping data. For each year a one-way analysis of co-variance (COANOVA) (Neter and Wasserman 1974) was performed on the square roots of the trimeans (Hoaglin et al. 1985) of pup counts, using the square roots of non-pup trimean counts as the covariate. The square root transformation was used to correct for non-constant variation of the count data (Snedecor and Cochran 1980). Linear contrasts (Neter and Wasserman 1974), where the average number of pups was adjusted to a common number of non-pups, were used to test the following hypotheses:

1) H_o: Average number of pups was greater in oiled areas than in unoiled areas;
   H_a: Average number of pups was less in oiled areas than in unoiled areas.

2) H_o: Average number of pups was greater in intermediate areas than in unoiled areas.
   H_a: Average number of pups was less in intermediate areas than in unoiled areas.

The contrasts used to test the hypotheses were as follows:

C1: $N_{oiled90} - N_{unoiled90}$

C2: $N_{intermediate90} - N_{unoiled90}$

where N = the average number of pups counted.

For between year comparisons of pup production, a one-way COANOVA was performed on the square root of the paired differences (1990-89) for the trimeans of pup counts using the square root of the difference in non-pup trimean counts as the covariate. The hypotheses were:

H_o: There was an increase in the number of pups, adjusted for the number of non-pups, in oiled areas compared to unoiled areas from 1989-90;

H_a: There was a decrease in the number of pups, adjusted for the number of non-pups, in oiled areas compared to unoiled areas from 1989-90.

This hypothesis was tested using the contrast:

C1: $(N_{oiled90} - N_{oiled89}) - (N_{unoiled90} - N_{unoiled89})$

where N = average pup count adjusted for the number of non-pups.

If a significant increase in birth rate occurred from 1989 to 1990, the contrasts would show large positive values. Values for unoiled areas were used in the contrasts to adjust for any differences due to non-EVOS caused trends in pup production. The same comparisons were also done for intermediate compared to unoiled areas.

We also analyzed differences between oiled and unoiled areas for non-pup counts during the pupping period. A one-way COANOVA was performed on the square roots of the paired differences (1990-89) in non-pup trimean counts, to test the following hypothesis:

H_o: There was a decrease from 1989 to 1990 in the number of non-pups in oiled areas compared to unoiled areas;

H_a: There was an increase from 1989 to 1990 in the number of non-pups in oiled areas compared to unoiled areas.

This hypothesis was tested using the contrast:

C1: $N_{oiled} - N_{unoiled}$

where N = average number of non-pups counted.

The same comparison was done for intermediate compared to unoiled areas.

Overall trends in abundance during the autumn molt were examined using a repeated measures ANOVA (Winer 1971) performed on the trimean (Hoaglin et al. 1985) of the site count data for September surveys. The trimean statistic was transformed with the square root transformation to correct for non-constant variation (Snedecor and Cochran 1980) and, because sphericity assumptions were violated, the F statistics were adjusted using the Greenhouse-Geisser parameter (Fleiss 1986). The hypotheses that were tested, using orthogonal contrasts derived from the ANOVA, were:

1) H_o: Average counts in oiled areas, compared to the unoiled areas, were $\geq$ the historical difference;
   H_a: Average counts in oiled areas, compared to the unoiled areas, were < the historical difference.

2) H_o: Average counts in intermediate areas, compared to the unoiled areas, were $\geq$ the historical difference;
   H_a: Average counts in intermediate areas, compared to the unoiled areas, were < the historical difference.

Hypothesis 1 was tested using the contrast:

$$C1: \{ 0.5 \times (N_{Oiled90} - N_{Unoiled90} + N_{Oiled89} - N_{Unoiled89}) - 0.5 \times (N_{Oiled84} - N_{Unoiled84} + N_{Oiled88} - N_{Unoiled88}) \}$$

where N = average number of seals counted.

Hypothesis 2 was tested in a similar manner by substituting intermediate area values for oiled area values.

RESULTS

A. Observations of seals and haulouts

Oiling

During the EVOS harbor seals contacted oil both in the water and on haulouts. It is impossible to identify all of the specific areas used as haulouts in PWS and the Gulf of Alaska, but major areas are fairly well known. An indication of the degree of oiling of harbor seal haulouts in PWS is shown in Table 1. This is based on m[illegible]ing conducted by the Alaska Department of Environmental C[illegible]rvation as well as on-site observations by ADF&[illegible] personne[illegible] [illegible]able 1 includes all locations in PWS where [illegible]eals wer[illegible] by project personnel, and also provides an indication of the range in number of seals and percent that were oiled during the April-July 1989 observation period.

Boat-based observations of the degree of oiling of seals were begun in mid-May. Initially we worked throughout eastern and central PWS in both oiled and unoiled areas. We found no evidence of oiled seals in unoiled areas, and relatively few oiled seals in intermediate areas (Table 2). In oiled areas over 70% of the seals were oiled, most of them heavily.

Subsequent boat observations focused on oiled areas. Three areas, Seal Island, Bay of Isles, and Herring Bay, were particularly suitable because they contained adequate numbers of seals that could be approached closely enough to examine and classify (Table 3). The degree of oiling of seals differed among areas. From 49%-89% of seals older than pups were classified as oiled at Seal Island, with fewer seals oiled in late June and July than in May. This area was surrounded by oil during the spill and was one of the first high priority seal haulouts identified for cleanup. Some of the gross contamination was removed from haulouts on Seal Island prior to May 15. Possible explanations for the progressive decrease in oiled seals on Seal Island include: 1) immigration of clean seals into the area; 2) emigration of oiled seals away from the area; 3) mortality of oiled seals; or 4) natural cleaning of oiled seals. Based on radio-tagging studies in Alaska and elsewhere, harbor seals are known to show considerable site fidelity (Pitcher and McAllister 1981; Yochem, et al. 1987) and we think it unlikely that immigration or emigration of seals was responsible for the decrease in the percent of oiled seals. We saw no oiled seals at unoiled sites and have no reason to think that unoiled seals would have moved to oiled sites. For example, Lower Herring Bay which was unoiled and undisturbed during April and May is only 15 km south of Herring Bay which was heavily oiled and the site of extensive cleanup activity. During small boat observations in Lower Herring Bay in mid-May we saw no evidence of oiled seals there that might have moved in to avoid oil or disturbance in adjacent areas. A simple field experiment did demonstrate that oiled seal hide that was soaked and agitated in clean sea water for several days became visibly cleaner. Since much of the heaviest oil on the Seal Island haulouts was removed in May, seals were not continually exposed to heavy oil and they may have becme cleaner with time.

In Herring Bay all seal haulouts were contaminated and they were treated by cleanup crews at various times through September 15. Virtually 100% of all seals seen through mid-July were oiled, suggesting that any natural cleaning was offset by continued exposure to oil on the rocks and algae at haulouts. Circumstances in Bay of Isles, where some but not all haulouts were heavily oiled, were intermediate. Treatment by cleanup crews in Bay of Isles was not complete until August. When observations were made in Bay of Isles and Herring Bay on September 4, over 80% of the seals other than pups appeared unoiled. This was probably due to molting which occurs annually in late August and September.

Pups born in all three areas became oiled, but this was especially true in Herring Bay and Bay of Isles (Table 3). Some pups only 1-2 days old (as evidenced by a bright pink umbilicus) were seen to be heavily oiled. Pups do not molt during their first summer of life and many were therefore still oiled during the September observation period.

The seals that were seen during small boat work conducted in April and June 1990 did not appear to be externally oiled. Observations were made at Herring Bay, Bay of Isles, and Seal Island, where oiled seals were seen repeatedly in 1989. Haulout sites were examined for evidence of oil. No significant amounts of oil were detected on the surface of rocks or on the algae.

Behavior

During field work project personnel made qualitative observations of the behavior of harbor seals in PWS. Harbor seals are generally quite difficult to approach, especially in PWS, and go into the water if aircraft fly over at low altitude or boats pass by close to haulout areas.

In 1989 there were many observations of "strange acting" harbor seals reported by biologists and others accustomed to observing harbor seals (Table 4). Oiled seals were variously reported as sick, lethargic, or unusually tame. On several occasions, investigators were able to approach on foot to within a few meters of oiled seals without causing the animals to flee. During the weeks immediately following the spill it was often possible to fly over hauled out seals in a helicopter at less than 80 m altitude and not cause them to go into the water. In areas such as Herring Bay, seals continued to haul out despite very extensive boat and aircraft traffic.

On multiple occasions we saw heavily oiled pups nursing on heavily oiled females. The hair around the mammary glands was noticeably cleaner, appearing as two light circles on an otherwise black abdomen.

During field work in 1990, harbor seals were noticeably more wary and difficult to approach by boat than they were in 1989.

B. Salvage of dead animals

Nineteen harbor seals were found recently dead, or died in captivity, and were necropsied between early April and early July

1990 (Appendix A). Several other partial carcasses were also found and examined, but all were judged to be from seals that had died before the oil spill. Of the 19 fresh carcasses, 9 were heavily oiled, 4 were unoiled, and the remaining 6 were light-to-moderately oiled. One of the unoiled animals was a seal taken by hunters from Tatitlek for subsistence purposes, and submitted for sampling. Thirteen were pups, including two oiled pups that were captured alive in early May and died after approximately one month in captivity. Four dead, prematurely born pups were found during April. The remaining 7 dead pups were found after commencement of the normal pupping period, from mid-May through early July. Two of these were unoiled, 1 was lightly oiled and 4 were heavily oiled.

Toxicology samples were taken from all beach-found carcasses and histopathology samples were collected when the condition of tissues allowed.

No dead harbor seals were reported to or located by project personnel in 1990.

C. Collection of seals

During the period from April 29, 1989 through April 14, 1990 ADF&G personnel collected 28 harbor seals in PWS and adjacent portions of the Gulf that were impacted by the EVOS (Appendix B). Two were collected under authority provided in Section 109(h) of the Marine Mammal Protection Act which allows government officials to take moribund animals for their own welfare. The remaining 26 animals were collected under authority of NMFS Scientific Permit Number 584, issued to the National Marine Mammal Laboratory.

Twelve seals were collected in PWS during April-June. All were oiled, most of them very heavily. Seven seals were collected in June-July in the Gulf. Two of them were obviously oiled. In October-November two seals were collected, one in PWS and one in the Gulf. Six seals were collected in PWS in April 1990. None of these seals showed signs of external oiling, but they were collected in areas that had been heavily oiled during the EVOS. Two seals were collected near Ketchikan, over 1000 km from the area impacted by the spill, in July 1990.

Complete sets of specimens for toxicology and histopathology were collected from all seals, with one exception (AF-HS-1). All necropsies were conducted by the same veterinary pathologist, again with the exception of AF-HS-1. This ensured a high degree of consistency in examinations and sampling of tissues. Together the tissues from the 28 seals represent the most complete and carefully collected samples ever obtained from oiled and unoiled harbor seals.

D. Toxicology

Toxicological analyses have been completed for at least some tissues from all harbor seals that were collected and/or found (Appendix C). All bile samples that were collected have been analyzed, except those from the two control seals collected near Ketchikan in July 1990. Liver, blubber, muscle, and brain tissue has also been analyzed for all 26 seals collected through April 1990, as well as some samples from seals found dead following the EVOS.

Fluorometric analysis of bile was performed by the Environmental Conservation Division, Northwest and Alaska Fisheries Center, NOAA/NMFS. Results for individual seals collected at various times and locations, in oiled and unoiled areas, indicated a wide range in values for the aromatic hydrocarbons naphthalene (NPH) and phenanthrene (PHN) (Appendix D). The values for oiled seals from PWS that were collected in 1989, and for those seals not obviously oiled but collected in the same areas of PWS in 1990, were markedly higher than for seals from the Gulf (Table 5).

A comparison of seals collected in June-July 1989 indicated that bile values were 8 to 12 times higher for NPH and PHN in the PWS samples than in those from the Gulf. The averages for the 10 PWS seals were 63,420 parts per billion (ppb) NPH and 35,850 ppb PHN, compared to 8,317 ppb NPH and 2,822 ppb PHN for the Gulf (Table 5). Two seals collected in PWS in late April-early May 1989 had even higher values. Maximum values in PWS were 360,000 ppb NPH and 220,000 ppb PHN, compared to maximum values in the Gulf of 14,000 ppb NPH and 8,000 ppb PHN.

Levels of NPH and PHN in bile from seals collected in oiled areas of PWS in 1990, one year after the EVOS, were 5-6 times higher than 1989 values for the Gulf (Table 5). Since elevated levels in bile are thought to indicate recent (within 2-4 weeks) exposure to petroleum hydrocarbons, the elevated levels in the spring 1990 sample suggest that seals were still encountering oil in the environment (through direct exposure or ingestion of contaminated prey) or that they were metabolizing stored fat reserves that had elevated levels of hydrocarbons. A single seal collected in November 1989 at the northeast edge of the oiled area had levels that were intermediate between Gulf seals and most other PWS seals.

The highest aromatic contaminant values in bile were found in the two seals collected in Herring Bay in April-May 1989 (one pregnant and one subadult female); a nursing pup collected from Bay of Isles in June 1989; and of particular interest, a pregnant female from Eleanor Island that was collected in April 1990, a year after the EVOS. Values for premature pups from spring 1989, and the fetus of the heavily contaminated female from spring 1990 were low, as were those for a subadult male from eastern PWS (out of the EVOS area) and several seals from the Gulf (Table 5).

There was a marked change with time in the ratio of NPH:PHN in seals from PWS. The NPH:PHN ratio was 1.8 in spring/summer 1989 (range 0.8-3.8) compared to 3.8 (range 2.5-6.7) in spring 1990. The significance of this change is unknown. The NPH:PHN ratio for summer 1989 Gulf seals was intermediate at 2.9 (range 1.7-7.7). 7

Samples of liver, skeletal muscle, blubber, brain, and in some cases kidney, from most seals that were collected have been analyzed for the presence of polycyclic aromatic hydrocarbons (PAHs). Laboratory analyses of most liver, muscle, and blubber were conducted by the Environmental Conservation Division, Northwest Fisheries Center, NOAA/NMFS. Brain and some of the other tissues were analyzed by the Geochemical and Environmental Research Group, Texas A&M University. Results are reported for low molecular weight aromatic compounds (LACs) and high molecular weight aromatic compounds (HACs) for individual seals in Appendix E, and for sample groups in Table 5.

All seals from the Gulf had very low levels of PAHs in liver, blubber, and muscle tissues. In over half the samples, PAHs were not detected. With a single exception, the maximum concentration was 5 ppb. The blubber of one seal collected in October 1989 had an LAC value of 21 ppb. Values for LACs in the brain of Gulf seals ranged from 21-61 ppb.

Seals found or collected in oiled areas of PWS in spring 1989 contained 14-156 ppb LACs and lower but detectable levels of HACs (4-32 ppb) in the liver. All other liver samples from PWS seals had either very low or undetectable levels of both LACs and HACs.

Blubber tissue from a premature pup found on Applegate Rocks in April 1989 had 408 ppb LACs. Blubber samples from the June 1989 PWS seals had average LAC concentrations of 194 ppb (range 18-738), compared to 38 ppb (range 19-86) in April 1990. HACs were substantially lower, ranging from undetectable to 39 ppb.

Analysis of skeletal muscle indicated low PAH concentrations in all samples (0-10 ppb). LACs were detectable in only 7 of 24 samples, and 5 of those were from the June 1989 collection. HACs were detectable in 8 of 24 samples, always at concentrations of less than 2 ppb.

Brain tissue was analyzed from 24 seals. Mean LAC values for PWS 1989 (23 ppb), Gulf 1989 (41 ppb), and PWS 1990 (27 ppb) collections were similar. HAC values were uniformly low and within the range of processing blanks.

Three mother-pup or mother-fetus pairs were available for analysis. In all three pairs, PAH levels in blubber and brain tissue were similar in mother and pup/fetus. The two highest PAH values of any seals were in PWS mother and pup TS-HS-7 and 8. Bile values were markedly different in mothers and pup/fetus for all pairs. The spring 1990 fetus had much lower PHN and NPH levels than its mother. The fluorometric peaks did, however, indicate that it had been exposed. Both 1989 pups had significantly higher bile PHN and NPH levels than did their mothers.

Mammary tissue and/or milk was analyzed from three adult females and two pups. Total PAHs in mammary tissue ranged from 34-71 ppb, and in mother's milk from 44-58 ppb. Milk for female TS-HS-7 was not available. However, milk from the stomach of her pup (TS-HS-8) had the highest PAH value (1200 ppb) of any tissue in any seal that we analyzed.

E. Histopathology

Preliminary histopathological analysis is complete for all seals. The primary pathology was to the brain of some seals (Table 6). Lesions causing intramyelinic edema were detected in the posterior ventral aspect of the thalamus, specifically in the ventral caudal lateral and ventral caudal medial nuclei of the thalamus, and to some degree in the lateral nuclear area and the reticular nucleus of the thalamus. A few other nuclei had mild lesions. However, these were considered minor and probably reversible. There were also lesions in the nerves of the vibrissae of several seals. These mild lesions in the facial nerve of the vibrissae help to confirm the importance of the thalamic lesions.

Intramyelinic edema was present in six seals. It was mild in four seals from PWS and one in the Gulf. Intramyelinic edema was acute in only one seal, TS-HS-1, which was collected in April 1989. In this seal, axonal degeneration was also present, indicating damage to the seal's brain. No sign of intramyelinic edema was present in either control seal. The thalamic nuclei where the edema was present relay impulses of sensory systems except olfaction to the cerebrum. The specific nuclei affected are primarily sensory to the head and body, with some influence on respiration.

Intramyelinic edema occurs when there is swelling within the myelin sheaths of the nerve axons. The myelin is rich in lipids, and this may attract toxic, fat-soluble hydrocarbons. The swelling causes diffusion of the electrical impulse and reduces the ability of the axon to transmit neural impulses. Since these nerves are located in the part of the brain responsible for non-visual perception of the environment, poor transmittal of nerve impulses could result in confusion relative to orientation in the environment, and therefore predispose the seal to drowning. Seals breath voluntarily, in contrast to terrestrial mammals, and if they become confused about where they are, breathing may not be triggered at the appropriate time.

In other mammals, the highly volatile C5-C8 hydrocarbons are acutely toxic. They cause central nervous system damage, axonal degeneration, and cerebral edema (Cornish 1980). There is a complete parallel between the intramyelenic edema in TS-HS-1 and that present in humans who die from inhaling solvents.

In the opinion of the pathologist, toxicity for seals caused by volatile aromatics would be acute and would occur within 1-2 months. It was his opinion that seal TS-HS-1 would not have survived. The seals sampled in the June-July collections showed only mild lesions and in fact were all survivors of the spill that had not experienced the same degree of acute toxicity.

Two other pathologic conditions occurred commonly in seals collected in PWS and the Gulf in June-November 1989. Those were mild rhabdomyolosis (degeneration of muscle cells) of the nostrils and acanthosis and hyperkeratosis of the skin (dry, scaly, thickened skin) (Table 6). Both of these conditions were likely to have been due to exposure to hydrocarbons, but the lesions that resulted were minor and not likely to have caused death in any of the seals examined.

Tissues from seals found dead were generally not suitable for histopathological analysis. This was especially true of brain tissue, where autolysis begins soon after death and may obscure lesions that might be caused by exposure to hydrocarbons.

Some additional histopathological analyses are ongoing. Special stains are required to more clearly differentiate some lesions. Second opinions are being sought to confirm diagnosis of some lesions.

F. Aerial surveys

Don't use this

Pupping

Aerial surveys during the pupping period were flown for the first time in PWS in 1989, and again in 1990. The 25 trend count haulout sites were surveyed during May 25-June 27, 1989 and June 7-15, 1990. In each year six to nine counts from each haulout site were suitable for use in the analysis (Appendices F and G).

For 1989 results the COANOVA indicated that there was no difference in the number of pups, after adjustment for differences in the number of non-pups, at oiled compared to unoiled sites (p = 0.973). There were 25.8 pups/100 non-pups at the oiled sites compared to 24.5 pups/100 non-pups at the unoiled sites, with fewer pups (14.8 pups/100 non-pups) at the three intermediate sites (Table 7). In 1990, however, results of the COANOVA indicated that there were significantly more pups at oiled sites than at unoiled sites (p = 0.009). There were 33.8 pups/100 non-pups at oiled sites compared to 21.4 pups/100 non-pups at unoiled sites.

We performed between-year tests to compare the 1989-90 differences in pup counts in the oiled areas, after adjustment for differences in non-pup counts, to differences in unoiled areas. Results indicated that there was a significant increase (p = 0.013) in the number of harbor seal pups born in oiled areas in 1990 versus 1989, after adjusting for the number of non-pups and changes in population levels (Table 8). Intermediate areas showed no significant trend.

A between-year comparison of the numbers of adults seen on June surveys indicated that there was no significant difference in adult counts in 1990 versus 1989 in oiled (p = 0.160) or intermediate (p = 0.726) areas, after adjusting for changes suggested by the difference in counts in unoiled areas.

Molting

During the annual molt, aerial surveys of some or all of the 25 trend count sites were flown on 10 days during September 3-16, 1989 (Appendix H). Initial inspection of the 1989 data, based on trimean values, indicated that the difference in the average counts of seals in 1989, compared to previous years, declined substantially more at oiled sites than at unoiled sites (Figure 1, Table 9). Between 1988 and 1989, the average counts of seals at oiled sites declined 45%, compared to 16% at unoiled sites. In contrast, between 1984 and 1988, the proportional decline at the two groups of sites was similar: 37% in the oiled group and 38% in the unoiled group, or approximately 9-10% per year. Thus, following the EVOS, the decrease in the number of seals at oiled sites was disproportinately greater than the decrease at those same sites between 1984 and 1988, and greater than the decrease at unoiled sites in all survey years.

Fall molting surveys were conducted again on 8 days during August 28 through September 11, 1990 (Appendix I). There was a moderate increase (17%) in the number of seals at oiled sites in 1990, and a substantial decline (49%) at adjacent intermediate sites, suggesting that there may have been some displacement of seals in 1989 followed by a return to the oiled sites in 1990. The number of seals at unoiled sites, as well as the overall total for the whole PWS trend count route, did not change substantially between 1989 and 1990.

Statistical comparisons of pre-spill (1984 versus 1988) and post-spill (1989 versus 1990) counts from oiled and unoiled sites clearly indicated that the difference between oiled and unoiled sites was significant (Table 10). Overall, from 1988 to 1990 there was a 35% decline in the number of seals in oiled areas compared to a 13% decline in unoiled areas.

The overall (1988-1990) decline in the number of seals counted in intermediate areas (41%) was similar to that in oiled areas. However, ANOVA values indicated that due to the small sample size (only 3 intermediate sites) and within-site variation in trend, the decrease in intermediate areas was not significant when compared to unoiled areas ($p = 0.135$).

Based on the percent declines between 1988 and 1990, we estimated the number of missing seals in oiled areas that could be attributed to the EVOS. In order to do so, it was necessary to apply the rate of decline from 1988 to 1990 for unoiled seals to the oiled sites and produce an expected number of seals for 1990. We considered this a valid procedure since the decline from 1984 to 1988 was similar in both oiled and unoiled areas. The actual number of seals counted at oiled sites in 1990 was then subtracted from the expected number to determine the numerical impact of the EVOS in the trend count area. Expressed as a formula, the calculations were as follows:

$$\text{Missing in oiled trend count area} = (1 - (\text{Unoiled}_{88} - \text{Unoiled}_{90})/\text{Unoiled}_{88}) \times \text{Oiled}_{88} - \text{Oiled}_{90}$$

Substitution of values from Table 9 indicates that 91 more seals were missing in oiled areas than could be accounted for by the ongoing decline.

No systematic aerial survey data were available for oiled haulouts outside the trend count area. To estimate the number of seals in those areas, we summed the maximum counts obtained for those haulouts during small boat operations in May-July 1989 (see Table 1). This total of 277 seals is undoubtedly conservative since: 1) not all oiled areas were counted; and 2) some counts were made over 3 months after the spill, by which time most mortality would already have occurred (according the the pathologist). To estimate the number of seals missing in these oiled areas, the rate of decline from oiled trend count sites, corrected for the ongoing decline, was applied as follows:

$$\text{Missing other oiled areas of PWS} = \text{Missing}_{\text{oiled trend}} \times (\text{Seals}_{\text{oiled other PWS}} / \text{Seals}_{\text{oiled trend}})$$

Substitution of values gives an estimate of 109 seals missing in oiled parts of PWS other than the trend count area.

Therefore, the total number of seals missing in PWS due to the EVOS would be:

$$\text{Total missing}_{\text{oiled PWS}} = \text{Missing}_{\text{oiled trend}} + \text{Missing}_{\text{other PWS}}$$

Substitution of values gives an estimate of 200 seals missing from PWS due to the EVOS. This is a very conservative estimate since no correction has been made for seals present but not hauled out. Pitcher and McAllister (1981) found that radiotagged harbor seals in a study at Tugidak Island in the Gulf of Alaska were hauled out on average 41% of the time, with an individual range from 16%-80%

It is not possible to state definitively that the missing seals died. Since the majority of dead seals would sink rather than float, the number of carcasses found is not a valid index of mortality. Furthermore, because of tissue degradation in seals found dead it was usually not possible to positively ascertain the cause of death.

The most likely alternate explanation, that seals did not die but were displaced elsewhere beyond the study area, is not supported by any of the available information. When we conducted small boat observations in May 1989 we saw a few oiled seals at intermediate trend count sites adjacent to oiled areas, but no oiled seals at unoiled trend count sites in eastern or northern PWS (Table 2). The same pattern was evident in western and southwestern PWS outside of the trend count area, where unoiled areas only a few kilometers from heavily oiled and highly disturbed areas did not contain any oiled seals. This strongly suggests that whatever movements of oiled seals occurred were very local. Heavily oiled and highly disturbed areas like Herring Bay were not abandoned by seals. Counts there were similar in mid-May and mid-September 1989. Following the EVOS oiled seals were observed to be very lethargic and reluctant to enter the water. It is unlikely that seals in this condition would swim long distances to other areas. Radiotagging studies of harbor seals at Tugidak Island, Alaska (Pitcher and McAllister 1981) give some indication the normal movements patterns of unoiled seals. Seals followed in that study showed considerable fidelity to a particular haulout site, and movements to other haulouts were usually to the nearest adjacent location.

The 17% increase in the number of seals at oiled trend count sites between 1989 and 1990 is consistent with the possibility that some short-term displacement occurred in 1989. In 1990, the number of seals at oiled sites was still 35% lower than pre-spill, compared to a 13% difference in unoiled areas. There was no increase at unoiled trend count sites that would suggest that oiled seals had moved into these areas, and remained.

There have been a number of studies of the effects of disturbance on harbor seals (e.g. Renouf et al. 1981, Allen et al. 1984, Weber 1990). These studies show that seals will respond to a variety of disturbance sources including people on foot, airplanes, and boats. In most cases seals respond by going into the water, then hauling out after the disturbance has gone or on the next tidal cycle. When distubance occurs consistently, seals may alter their behavior patterns in order to haul out at times when they are less likely to be disturbed (Paulbitsky 1975). Long term displacement has not been documented, with the exception of Newby (1971) who attributed abandonment of a site in Puget Sound partly to increased boat activity.

STATUS OF INJURY ASSESSMENT

Observations to describe characteristics and persistence of oiling of harbor seal pelage (Objective 1) showed that harbor seals continued to utilize heavily oiled haulouts, even when unoiled sites were available nearby; that they gave birth and cared for their pups on heavily oiled haulouts; and that the pelage of pups and adults became oiled when seals used oiled haulouts or contacted oil in the water. The pelage did become cleaner with time if the seals were not continually exposed to oiled substrate. No oil was seen on the pelage of seals examined in April and June 1990.

Histological and toxicological analyses of tissues from all seals collected or found dead are nearing completion. Small numbers of toxicology samples, particularly blubber and blood, remain to be analyzed, as do all tissues from the two control seals collected near Ketchikan. Final interpretation of histopathology slides will be complete within the next few months.

Values for NPH and PHN in bile clearly indicate that most seals collected in oiled areas were exposed to and assimilated hydrocarbons and that values, on average, were substantially higher in PWS, even one year after the EVOS, than in the Gulf. Aromatic hydrocarbon values (LACs and HACs) for most tissues were generally in the low ppb range. In seals where several tissues were analyzed, the highest values were in the blubber and milk.

It is not possible at this time to determine, based on the toxicology data, whether seals found dead in spring 1989 died because of the EVOS (Objective 2). The health implications of toxicological results are unknown. The hydrocarbon levels in seal tissue were low in comparison to levels found in invertebrates from oiled areas of PWS. Since seals metabolize hydrocarbons very efficiently, the levels remaining in tissues when they were sampled may underestimate the actual degree of exposure and assimilation. Essentially no information is available on the likely effects of hydrocarbons on seals for anything other than short-term experimental exposure. It is important to note that toxicological analyses did not measure the most volatile and acutely toxic C5-C8 carbons, which have been documented to cause mortality in humans.

Histopathologic investigations demonstrated that seals exposed to oil did develop harmful pathological conditions (Objective 3). Severe brain lesions (intramyelinic edema and axonal degeneration) were present in one seal collected 36 days after the spill, and milder lesions were found in five other seals from oiled areas. These lesions are the same as those found in the brains of humans that die from inhalation of fumes from C5-C8 solvents. It is the opinion of the veterinary pathologist that such lesions would predispose a seal to drowning, and in all likelihood would result in mortality within a few weeks of severe exposure. It is likely that seals collected in June-July 1989 which had mild lesions were either recovering from a survivable level of exposure or had not been exposed to the most toxic volatile components.

Data from two field seasons supports the hypothesis that pup production was lower in oiled areas during the year of the EVOS than it was one year later (Objective 4). Counts made during pupping in June 1989 and 1990 indicated that significantly more pups/100 non-pups were present at oiled sites in 1990 than in 1989. At unoiled sites, there was not a significant difference between years. This, together with the fact that several dead fetuses and pups were found prior to and during pupping in 1989, suggests that pup mortality occurred and that the proportion of pups at oiled sites was significantly lower than normal because of the EVOS. In order to confirm this, it will be necessary to conduct aerial surveys during pupping for at least one additional year to determine whether 1990 reflects normal pupping at the oiled sites.

Aerial surveys during the fall molt substantiate the hypothesis that the number of harbor seals decreased more in oiled areas of PWS than in unoiled areas (Objective 5). Following the EVOS, there were far fewer seals present on the six oiled haulouts on the trend count route than were present at those sites in 1988. The decline in numbers was significantly greater than occurred in unoiled parts of PWS. The fact that numbers were low at oiled sites in 1990 as well as 1989 suggests that mortality, rather than displacement, was responsible for the decline. This conclusion will be strengthened by a third year of data to reinforce the trends in abundance in oiled and unoiled areas.

The fact that the number of harbor seals in PWS was declining prior to the EVOS makes it even more important that efforts be made to restore the population. However, in the case of seals, the options available for the restoration of use, populations, or habitat (Objective 7) are limited. Vigorous protection of habitat should be encouraged. NRDA studies and previous work have identified the terrestrial areas used as haulouts. Information is needed on marine areas that are important for feeding. A study to gather this information by attaching satellite transmitters to seals has been proposed as part of the restoration program.

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Table 4. Observations of unusual behavior by oiled harbor seals in Prince William Sound, 1989.

Date	Location	Observer	# Seals	Observation
4/12/89	Agnes Island	KP	8	Some heavily oiled; did not go into water when approached at very close range by helicopter.
4/13/89	Smith Island	KP	14	Stayed on rocks through 2 low passes (60m) by helicopter; landed 50m away and walked to within 12m without spooking seals.
4/15/89	Smith Island	LL	13	No reaction by seals when helicopter circled 4 times at 80m; seals oiled.
4/17/89	Smith Island	LL	13	Seals heavily oiled; seals did not spook when helicopter landed; approached closely on foot.
4/17/89	Green Island	LL	10	At least 6 oiled; very reluctant to go into the water; stayed on rocks until circled closely within 30m at 25m altitude.
4/19/89	Smith Island	LL	11	Reluctant to go into water; some heavily oiled.
4/19/89	Applegate Rocks	LL	59	Most heavily oiled; 2/3 of seals stayed hauled out when helicopter circled 5 times at 60m.
4/21/89	Herring Bay	LL, KF	24	All heavily oiled; none went into water until circled down to 60m, 8 stayed up until circled down to 25m.
4/21/89	Smith Island	KP		Seals spooked by helicopter but rehauled immediately when helicopter was present; extremely tame; seals oiled.

Table 4. Continued.

Date	Location	Observer	# Seals	Observations
4/27/90	Northwest Bay	RS	10	Did not move when helicopter flew to within 200m at 30m altitude.
5/10/89	S. Applegate Rocks	KP	30	Remained hauled out in presence of large cleanup crew and heavy helicopter traffic.
5/11/89	S. Applegate Rocks	LL	10	Seals remained hauled out in presence of circling helicopter and Twin Otter.
5/15/89	Herring Bay	LL, KF	1	Heavily oiled seal; squinty eyes; did not move when approached by boat.
5/24/89	Seal Island	LL, KF	2	Oiled pup of unoiled female; very lethargic.
5/26/89	Herring Bay	KF	10+	Heavily oiled seals; allowed approach on foot to within 3-5m; another group stayed on rocks until Whaler within 20m.
6/8/89	Applegate Rocks	KF	1	Heavily oiled adult; hauled out very high on beach; allowed approach to within 2m. Appeared very ill; mucous nasal discharge, tattered nostril edges.
6/10/89	Herring Bay	KF, LL	13	Two of the pups in this group not very responsive; walked to within 2m of one lightly oiled pup.
6/24/89	Herring Bay	LL, KF	6	Stayed on rocks when large H3 helicopter flew over at 60m.
6/26/89	Evans I. NE	LL, KF	1	Did not move when boat approached very close; very tame; left eye very runny.

KP = Ken Pitcher; LL = Lloyd Lowry; KF = Kathy Frost;
RS = Richard Shideler

Table 1. Oiling of harbor seals and harbor seal haulouts in Prince William Sound, 1989.

Haulout	Degree of Oiling	Observation period	# Seals	% Oiled
Trend count haulouts				
Agnes Island	light	April-July	15-40	5-66
Applegate Rocks	heavy	April-July	26-204	51-81
Channel Island	light	May	18-32	11-66
Green Island	moderate	April	10	60
Little Green I.	unoiled	May	40	20
Little Smith I.	heavy	April-July	12-23	83-100
Port Chalmers	unoiled	May	19	5
Seal Island	heavy	May-July	15-74	33-77
Smith Island	heavy	April-July	10-25	25-56
Stockdale Harbor	unoiled	May	1	100
Other PWS haulouts				
Bay of Isles	mod.-heavy	May-July	5-42	87-100
Crafton Island	mod.-heavy	June-July	17-33	76-83
Dick Island	heavy	May	1	100
Evans Island	light	June	43	35
Fleming Island	light	June	2	50
Foul Pass/Ingot I.	heavy	May	5-6	100
Herring Bay	heavy	April-July	10-58	98-100
Junction Island	mod.-heavy	June-July	14-28	36-56
Lone Island	moderate	July	4	25
Northwest Bay	heavy	April-July	1	100
Peak Island	heavy	July	7	14
Perry Island SE	moderate	July	22	23
Rua Cove/Marsha Bay	mod.-heavy	May	5	75
Upper & Lower Pass	heavy	May-June	10-25	100

Table 2. Percent of seals that were oiled, as determined from boat-based observations in Prince William Sound, May 1989.

Date	Area type	Number of seals classified	Percent in category		
			Heavily oiled	Oiled	Unoiled
15-18 May	oiled	185	86.5	9.7	3.8
	intermediate	51	9.8	3.9	86.3
	unoiled	34	0.0	0.0	100.0
23-27 May	oiled	408	44.6	28.9	26.5
	intermediate	72	18.1	22.2	59.7
	unoiled	124	0.0	0.0	100.0

Table 3. Percent of seals and seal pups that were oiled at Seal Island, Bay of Isles, and Herring Bay in Prince William Sound, Alaska during May-September 1989.

Date	Seal Island % oiled		Bay of Isles % oiled		Herring Bay % oiled	
	non-pups	pups	non-pups	pups	non-pups	pups
16-18 May	89.5	--	85.7	50.0	98.0	--
24-26 May	74.3	100.0	93.5	91.2	100.0	100.0
8-9 June	70.3	80.0	90.9	90.0	100.0	100.0
16-19 June	77.3	64.3	90.9	100.0	100.0	100.0
24-28 June	49.1	42.9	100.0	100.0	100.0	100.0
11-13 July	61.5	100.0	86.8	88.9	100.0	100.0
4 September	--	--	17.2	--	15.5	100.0

Table 5. Results o HPLC fbrometric analysis of harbor seal bile for the presence of the fluorscent amatic hydrocarbons phenanthrene (PHN) and napthalene (NPH), and GCMS nalysis f seal liver (Li) and blubber (Bl) for the presence of low (LAC) andhigh (HA molecular weight aromatic contaminants. Results are given in parts per bilbn (= ng/g). N is the number of animals in each sample. Dashes inicate tlt no samples were analyzed; nd means the compound was not detected.

		Bile			Aromatic hydrocarbons				
						LAC		HAC	
Area/sample	Date	N	PHN	NPH	N	Li	Bl	Li	Bl
PRINCE WILLIAM SOUN									
unoiled area-juvenie	Apr 89	1	220	1,700	-	-	-	-	
oiled area-fetus/prmature	or-May 89	2	1,035	7,650	6	33	-	19	-
oiled area-adult/juenile	or-May 89	2	94,500	190,000	2	100	-	6	-
oiled area-all ages	Jun 89	10	35,850	63,420	10	nd	194	nd	6
oiled area-adult	Nov 89	1	6,200	20,000	1	nd	21	nd	3
oiled area-fetus	Apr 90	1	1,800	3,300	1	nd	20	nd	4
oiled area-adult/juenile	Apr 90	6	13,933	53,500	6	2	38	nc	11
GULF of ALASKA									
oiled area-adult/juenile	an-Jul 8	6	2,822	8,317	6	3	1	<1	<1
oiled area-juvenile	Nov	1	170	140	1	nd	21	nd	2