MM 5

State-Federal Natural Resource Damage Assessment

for April 1989-November 1991

1991 Status Report

Marine Mammals Study Number 5:

Assessment of Injury to Harbor Seals

in Prince William Sound, Alaska, and Adjacent Areas

Principal Investigator

Kathryn J. Frost
Alaska Department of Fish and Game
1300 College Road
Fairbanks, Alaska 99701

Assisted by

Lloyd Lowry, Ken Pitcher, Dennis McAllister,
Don Calkins, Tom Loughlin, Beth Sinclair,
Earl Becker, Dan Reed, Rob DeLong,
Terry Spraker, and Ramona Haebler

20 November 1991

EXECUTIVE SUMMARY

The goal of this project has been 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 that were not near or adjacent to oiled sites. 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 18 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. Seven were unsuitable for necropsy because they were either scavenged or severely autolyzed. Three dies for reasons clearly unrelayed to oiling. For eight others, cause of death could not be determined; histology was of limited value due to autolysis.

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. 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 7-13 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.

Microscopic examination of seal tissues to detect any damage caused by exposure to oil (histopathology) 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 and 1991, 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.

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 1991, however, the decline in seals at oiled sites was much greater than at unoiled sites (31% decline at oiled sites versus 2% increase at unoiled sites). Orthogonal contrasts from a repeated measures ANOVA clearly indicated that the difference between oiled and unoiled areas was significant. Further analyses indicated that the decline occurred between 1988 and 1989 and that since then seals at both oiled and unoiled areas have increased at rates that are not statistically different (20% and 18%). The overall decline in the number of seals counted at intermediate areas between 1988 and 1991 (-22%) was not significantly different from unoiled areas due to small sample size and within-site variability.

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

1. Final analyses of aerial survey data must be completed.
2. Analyses of remaining blubber, milk, and blood samples must be completed by Texas A & M University and the results incorporated into data analyses completed to date.
3. A final report of the analysis and interpretation of histopathology slides must be completed by Dr. Terry Spraker and submitted as an appendix to the final report.
4. Results of haptoglobin analyses must be analyzed and reported.

As part of restoration and monitoring natural recovery, we strongly recommend that the number of harbor seals at trend count areas in PWS be monitored during the fall molt for the next three years and at intervals thereafter, as necessary. In addition, we recommend that the ongoing restoration study to satellite tag harbor seals in PWS be continued in order to better understand habitat use, and to 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

Project personnel observed harbor seals on oiled and unoiled haulouts from the time of the EVOS until early September when the annual molt occurred. Small boats were used to closely approach hauled out seals. Seals were counted and examined using 7-10 power binoculars and a 25 power spotting scope. Behavior of seals was observed, and any unusual behavior was recorded. Haulout sites were inspected for presence of oil or dead animals. Where possible each seal we counted was classified as to the degree of pelage oiling (heavy, oiled but not heavy, or unoiled). Seals classified as heavily oiled were those that were totally oiled and appeared a uniform dark chocolate brown or black. The oiled but not heavy group included all seals that were partially oiled on any parts of their bodies, but did not appear a uniform dark color. Early in the sampling period, this category was subdivided into lightly and moderately oiled groups, but as the summer progressed and the oil weathered on the animals, the distinction was sometimes difficult to make. Thus the lightly and moderately oiled categories were combined.

During the months following the EVOS, searches of the coastline were conducted by project personnel and other people using helicopters and boats. Any sick or dead seals were documented and their condition was noted. Carcasses that were in suitable condition were necropsied by trained biologists, veterinarians, or pathologists, and samples were obtained and preserved for toxicological and histopathological examination according to the protocol described in Appendix A. Care was taken to ensure that tissues were collected only from carcasses that were suitable fresh and carefully handled. Searches did not include all areas of PWS that are used by harbor seals, nor were they likely to detect all carcasses since some seals would sink when they died and large daily tidal fluctuations would be likely to wash dead animals off the rocks.

Harbor seals from PWS and the Gulf of Alaska were collected by ADF&G under authorization of a permit issued to National Marine Fisheries Service, National Marine Mammal Laboratory, in order to conduct gross necropsies and to obtain samples for histopathological and toxicological analyses. Seals were collected at and adjacent to sites impacted by the EVOS and were selected, as possible, according to the degree of oiling, age (pup or non-pup), and sex. Animals were humanely killed by shooting in the head or neck with a high-powered rifle. Each animal was necropsied as soon as possible after death by a qualified veterinary pathologist. All necropsies were conducted by the same veterinary pathologist, with the exception of AF-HS-1. This ensured a high degree of consistency in examinations and sampling of tissues. Together the tissues from these 28 seals represent the most complete and carefully collected samples ever obtained from oiled and unoiled harbor seals.

Collected animals were weighed, measured, and photographed; time, date, location, and circumstances of collection were noted; and any gross abnormalities were recorded. Blood samples for serum, plasma, and whole blood analyses were taken. Complete sets of specimens for toxicology and histopathology were collected from all seals, with the exception of AF-HS-1, according to the protocol specified in Appendix A. Chain of custody was maintained for all samples. Histopathology samples were analyzed by Dr. Terry Spraker, a veterinary pathologist at Colorado State University. Reference histology slides will be archived at the Armed Forces Institute of Pathology. Triplicate toxicology samples were frozen and stored in a central ADF&G holding facility in Anchorage. Some samples were sent to the National Marine Fisheries Service (NMFS), Northwest and Alaska Fisheries Center Laboratory for toxicological analysis in conjunction with Economic Uses Study No. 6. Other priority samples were sent to Texas A & M University for analysis under Technical Services Study No. 1.

Aerial surveys were flown in PWS in June and August/September along a 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), 3 intermediate sites that were not heavily oiled but were adjacent to oiled areas, and 16 unoiled sites that were north, east, and south of the primary area impacted by oil (Table 1). There was an adequate sample of both oiled and unoiled areas.

Surveys were conducted from a single engine fixed-wing aircraft (Cessna 180 or 185) on either wheels or floats. Haulout sites were flown over at an altitude of 200-300 meters. Visual counts were made of seals at each site, usually with the aid of 7 power binoculars, and photographs were taken of large groups to facilitate counting. Photographs were taken using a hand held 35-mm camera with a 70-210-mm zoom lens and high speed film (ASA 400). Color slides were commercially developed and the seals were counted from images projected on a white surface. During June surveys, separate counts were made of pups and non-pups.

In order to conduct surveys at a time when a relatively large and consistent proportion of the population is hauled out and can be counted, it is necessary to consider factors that may affect haulout patterns. These include weather, seasonal behavior patterns, tidal stage, and disturbances. Maximum numbers of harbor seals are known to haul out during the pupping period (May-June) and during the molt (August-September) (Pitcher and Calkins 1979, Calambokidis et al. 1987). Within these periods, more animals are usually hauled out at lower stages of the tide, since availability of most haulout sites is limited by tidal stage. Consequently, our surveys were conducted within biological time windows imposed by the pupping and molting periods and were timed to begin within two hours before daylight low tides and to finish within two hours after low tide.

Results of previous harbor seal trend counts have indicated that it is desirable to obtain 7-10 counts during a survey period in order to provide statistically valid estimates of the average number of seals hauled out in a trend count area(Pitcher 1986, 1989). In practice, the number of counts is almost always limited by the number of days within the survey window that are suitable for flying. During pupping, the survey window cannot be extended to accommodate sample size needs, since as pups grow and are weaned they become increasingly difficult to differentiate from the air. Similarly, during the molt it is necessary to confine surveys to the period when maximum numbers are thought to haul out.

Aerial surveys of harbor seals do not estimate the total number of seals present since they do not account for seals that are in the water or seals hauled out at locations not on the trend count route. Surveys provide indices of abundnce based on the number of hauled out seals counted on the trend count route. Interpretation of trend count surveys relies on the assumption that counts of harbor seals on select haulouts are valid linear indices of local abundance. We have assumed that within a given biological window, such as the pupping or molting period, haul out behavior remains the same from one year to the next, and counts can thus be compared. Standardization of procedures minimizes the efects of variables such as tide and weather that could influence the number of seals hauled out on a given day. If there was reason to suspect that a particular count was not valid (e.g., haulout empty with a boat nearby) it was not included in the analysis.

Data Analysis

The trimean statistic (Hoaglin et al. 1985) was used as a measure of central tendency for both spring and fall survey data because sets of counts at a single location sometimes showed bimodal distributions or included extreme variations. If the number of repetitive counts was less than three, a trimean could not be calculated. The median was used instead which, like the trimean, is considered an "L" estimator (Hoaglin et a. 1983, p306) and produces similar results.

Several analyses were performed on the 1989-91 pupping data. For each year a one-way analysis of co-variance (COANOVA) (Neter and Wasserman 1974) was performed on the trimeans (Hoaglin et al. 1985) of pup counts, using the non-pup trimean counts as the covariate. 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:

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

The contrasts used to test the hypothesis were as follows:

C1: $N_{oiled} - N_{unoiled}$ (or $N_{intermediate} - N_{unoiled}$)

where N = the average number of pups counted.

For between year comparisons of pup production, a Repeated Measures Analysis (Winer 1971), a specialized ANOVA, was performed on the paired differences (1991-89) for the trimeans of pup counts using 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 1989-91;
- 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 1989-91.

This hypothesis was tested using the contrast:

$$C1: (N_{oiled91} - N_{unoiled91}) - (N_{oiled89} - N_{unoiled89})$$

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

If a significant increase in birth rate occurred from 1989 to 1991, 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.

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 August/September surveys in 1983, 1984, and 1988-1991. The trimean was transformed with the square root transformation to correct for non-constant variation (Snedecor and Cochran 1980) and, because sphericity assumptions were violated (Winer 1971), 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:

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

The pre-EVOS historical data set included 1984 and 1988 counts. Post-EVOS data included 1989-1991 counts.

The hypotheses were tested using the contrast:

$$C1: \{ 0.333 \times [(N_{Oiled91} - N_{Unoiled91}) + (N_{Oiled90} - N_{Unoiled90}) + (N_{Oiled89} - N_{Unoiled89}) - 0.5 \times (N_{Oiled84} - N_{Unoiled84}) + (N_{Oiled88} - N_{Unoiled88}) \}$$

where N = trimean of the number of seals counted.

The same comparison was done for intermediate areas by substituting those values for oiled area values.

Orthogonal contrasts derived from a specialized ANOVA were also used to determine whether there was evidence of recovery following the declines that occurred in the year of the EVOS. As for pre-EVOS comparisons, this was done by assuming that the magnitude and direction of change in numbers in the unoiled areas was "normal" and that, without the EVOS, a similar trend should be evident at the oiled sites. Any differences would be attributable to the EVOS. The following hypotheses were tested:

H_o: Average counts in oiled areas, compared to the unoiled areas, declined or remained constant following the EVOS.

H_a: Average counts in oiled areas, compared to the unoiled areas, increased following the EVOS.

This hypothesis was tested using the following contrast:

$$C1: (N_{Oiled91} - N_{Unoiled91}) - (N_{Oiled89} - N_{Unoiled89}).$$

Under the null hypothesis of no recovery following the EVOS, we would expect C1 to be zero, indicating no change, or negative, indicating further decline. If recovery from the EVOS had occurred, we would expect C1 to be positive. The same comparison was done for intermediate compared to unoiled areas.

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 2. This is based on mapping conducted by the Alaska Department of Environmental Conservation as well as on-site observations by ADF&G personnel. Table 2 includes locations in PWS where seals were seen by project personnel during boat-based observations, and also provides an indication of the range in number of seals and percent that were oiled during the April-July 1989 observation period.

Systematic boat-based observations of the degree of oiling of seals were begun in mid-May 1989. Initially work was conducted throughout eastern and central PWS in both oiled and unoiled areas.

During May observations, only 1% of the seals in unoiled areas were oiled (2 of 182) and 8%-40% in intermediate areas (Table 3). In oiled areas over 70% of the seals were oiled, most of them heavily so.

Subsequent boat observations focused on oiled areas. Three of these, 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 4). The degree of oiling of seals differed among areas. From 49%-90% 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 during the summer when pupping and molting take place (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 almost 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 become cleaner with time.

In Herring Bay all seal haulouts were contaminated and they were treated by cleanup crews at various times through September 15. Through mid-July, 98%-100% of all seals seen were oiled, suggesting that any natural cleaning was offset by continued exposure to oil on 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 4). 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 year and many were therefore still oiled during the September observation period.

Small boat observations were conducted in Herring Bay, Bay of Isles, and Seal Island during April 10-14 and May 29-June 30, 1990. The number of adults and pups was counted, and seals and haulouts were inspected for the presence of oil. No seals appeared to be externally oiled. 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 5). 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 a black abdomen.

During field work in 1990 and 1991, harbor seals were noticeably more wary and difficult to approach by boat than they were in 1989.

B. Salvage and necropsy of dead animals

Eighteen harbor seals were found recently dead or died in captivity, and were necropsied between early April and early July 1989 (Table 6). Several other partial carcasses were found and examined, but all were judged to be from seals that had died before the oil spill. Of the 18 fresh carcasses, 9 were heavily oiled, 3 were unoiled, and the remaining 6 were light-to-moderately oiled. 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. One other was seal shot by a subsistence hunter from Tatitlek and provided to us for necropsy and sampling. This animal appeared to be completely normal during necropsy.

Seven of the carcasses that were recovered were unsuitable for complete necropsies. They were either scavenged, with major parts of the body and internal organs missing, or autolyzed. None-the-less, toxicology samples were taken from all beach-found carcasses and histopathology samples were collected when the condition of tissues allowed.

Necropsies suggested that two of the seals that were found dead had probably died because of collisions with boats. Both had fractured ribs, perforated diaphragms, and free blood in the body cavity. Both seals were light to moderately oiled. Their deaths may have been completely unrelated to the EVOS, or it is possible that behavioral changes caused by oiling (as described in Table 5) may have made these seals less able to avoid boats. Toxicological samples for these seals have not been analyzed.

One old, adult female was captured alive by a sea otter rescue crew and died en route to a rehabilitation facility. This seal had a severe pyometra and peritonitis that was judged to be secondary to the loss of a fetus, either through in utero mortality or abortion.

One heavily oiled pup had a severe purulent dermatitis, necrotic myositis, and a severe systemic bacteremia. There were many small raised bumps on the skin, the hair easily came off when scraped, and there were pustular lesions under these bumps. Blubber and muscle layers beneath the skin necrosis were also necrotic. Most lymph nodes were enlarged and many were necrotic.

Of the remaining seven seals, one was an unoiled seal pup with no apparent abnormalities and no indication of cause of death. Four other pups and one subadult had free blood in the abdominal cavity and another pup had free blood in the pleural cavity; all were oiled to some degree (or had been prior to being held in captivity). It is common for stillborn fetuses to have free abdominal blood (T. Spraker, pers. commun.). Several showed hemorrhage in kidneys, lungs, and gastric mesenteries. The cause of hemorrhage is unknown.

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 (Table 7). 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 in July 1990 near Ketchikan, Alaska, to serve as control animals from an unoiled area. The location where these seals were collected was over 1000 km from the area impacted by the EVOS.

D. Toxicology

Toxicological analyses have been completed for at least some tissues from all harbor seals that were collected and/or found (Appendix B). All bile samples that were collected have been analyzed. Liver, blubber, muscle, and brain tissue has been analyzed for all 26 seals collected through April 1990, as well as some samples from seals found dead following the EVOS. A few remaining blubber, milk, mammary, and blood samples have been submitted and are awaiting analysis at Texas A & M.

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) (Table 8). 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 seven seals from the Gulf, two from Ketchikan, and ten ringed seals collected near Barrow (Table 9).

A comparison of seals collected in June-July 1989 indicated that bile values were 7 to 13 times higher for NPH and PHN in the PWS samples than in those from the Gulf. The averages for the 10 PWS seals were 62,420 parts per billion (ppb) NPH and 35,850 ppb PHN, compared to 8,300 ppb NPH and 2,800 ppb PHN for the Gulf (Table 9). Two seals collected in PWS in late April-early May 1989 had even higher values. Maximum values in PWS were 365,000 ppb NPH and 215,000 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 9). 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 NPH and PHN 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.

There was a marked difference among samples in the ratio of NPH:PHN in bile. Seals (not including fetuses) from oiled areas of PWS had a mean NPH:PHN ratio of 2.1:1 in April-June 1989 (range 0.9-3.8) compared to 5.3:1 (range 2.5-6.7) in April 1990. Seals collected in June-July 1989 from the Gulf of Alaska had a mean NPH:PHN ratio of 4.3:1 (range 1.7-7.7). The ratio for two seals from the Ketchikan area was 7.5:1 (6.8-8.2). For a sample of ten ringed seals (Phoca hispida) from Barrow (mean PHN = 880 and NPH = 11,500) the ratio was 12.7:1 (P. Becker, unpublished data). The significance of these differences is unknown, but it is clear that NPH:PHN ratios are lowest in the seals that were the most heavily and most recently exposed to oil and highest in those collected farthest from the spill.

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 C, and for sample groups in Table 9.

All seals from the Gulf had very low levels of PAHs in liver, blubber, and muscle. In over half the samples, PAHs were not detected. With one exception, the maximum concentration was 5 ppb. The blubber of one seal collected in October 1989 had an LAC value of 21 ppb. 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, with 5 of those were from June 1989. 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 females and two pups. Total PAHs in mammary tissue were 34-71 ppb, and in mother's milk, 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

Histopathological analysis is complete for all seals. The primary pathology was to the brain of some seals (Table 10). Lesions most likely to be associated with oil toxicity included intramyelinic edema of the large myelinated axons of the midbrain (especially the thalamus, corpus callosum, internal capsule, and crus cerebri), neuronal swelling and necrosis (especially within the thalamus and particularly in the ventral caudal lateral and ventral caudal medial nuclei of the thalamus), and axonal degeneration (particularly in the thalamus, corpus callosum, internal capsule, and crus cerebri).

Intramyelinic edema was present in six seals. It was severe in one seal, TS-HS-1, which was collected in April 1989, and was most prominent in the caudal ventral lateral and caudal ventral medial nuclei of the thalamus and within large myelinated fibers of the thalamus, corpus callosum, crus cerebri and internal capsule. Intramyelinic edema is a sensitive but reversible indicator of brain damage. Intramyelinic edema was present but milder in five other seals (TS-HS-2,3,7,11, and 17). No sign of intramyelinic edema was present in either control seal.

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. 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.

Neuronal swelling with loss of Nissl substance was also most severe in TS-HS-1 and occurred primarily in the thalamus. Mild neuronal swelling was found in eight other seals (TS-HS 2, 3, 5, 11, 14, 16, 17, 19). Neuronal swelling is also an extremely sensitive and acute but reversible change caused by neurotoxins. Neuronal necrosis and dropout is a severe, nonreversible change. Neuronal necrosis was most evident in the ventral caudal lateral and ventral caudal medial nuclei of the thalamus. These lesions were moderate to severe in six seals (TS-HS-1, 7, 11, 14, 16, 17) and mild in three (TS-HS-3, 5, 10).

Axonal swelling and degeneration may be associated with neuronal degeneration, secondary lesions following myelin damage, or primary lesions. Axonal swelling/degeneration was found in three seals; it was severe in TS-HS-1 and mild in TS-HS-11 and 17.

Since the thalamus is a primary relay station for many incoming impulses, damage to the thalamus could result in failure of these impulses to reach the cerebral or cerebellar cortex. This failure would be most troublesome during diving. These lesions in the ventral caudal lateral and ventral caudal medial nuclei of the thalamus would primarily alter peripheral proprioception. They could account for behavioral changes such as decreased flight distance, disorientation, and increased amount of time spent hauled out that were observed in oiled harbor seals following the spill. If forced to swim or dive, affected seals would probably be incapable of performing these normal tasks and thus would be markedly predisposed to drowning. Seals breathe voluntarily, in contrast to terestrial mammals, and if they become confused about where they are, breathing may not be triggered at the appropriate time. These seals probably were suffering from severe pain due to edema of the brain.

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 that probably had little effect on them, with the possible exception of TS-HS-17 which had a severe degree of neuronal necrosis within the caudal ventral lateral and caudal ventral medial nuclei of the thalamus.

Three other pathologic conditions occurred commonly in seals collected in PWS and the Gulf in June-November 1989 (Table 11). There were lesions in the nerves of the vibrissae of three seals, mild rhabdomyolosis (degeneration of muscle cells) of the nostrils, and acanthosis and hyperkeratosis of the skin (dry, scaly, thickened skin). All 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.

Chronic effects of oiling were not found in this study.

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.

F. Aerial surveys

Pupping

Aerial surveys during the pupping period were flown for the first time in PWS in 1989, and again in 1990 and 1991. The 25 trend count haulout sites were surveyed during May 25-June 27, 1989, June 7-15, 1990, and June 6-20, 1991. In each year six to ten counts from each haulout site were suitable for use in the analysis (Appendices D, E, and F).

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 12). In 1990, however, results of the COANOVA indicated that there were significantly more pups at oiled sites than at unoiled sites. There were 33.8 pups/100 non-pups at oiled sites compared to 21.4 pups/100 non-pups at unoiled sites. In 1991, this was also true, with 35.3 pups/100 non-pups at oiled sites and 23.6 pups/non-pups at unoiled sites.

We performed between-year tests to compare the 1989-1990 and 1989-91 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<0.01) 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 leve ls (Table 13). This was also true for 1991. 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 or intermediate areas, after adjusting for changes suggested by the difference in counts in unoiled areas. In 1991, however, the number of non-pup seals counted at oiled sites was about 6% greater than in 1990, compared to decreases of over 30% at unoiled and intermediate areas. Thorough analysis of the 1991 pupping data has not been completed and reasons for this difference between sites is unknown. We do know, however, that significant disturbance occurred near one of the major pupping locations in the unoiled area and the counts at that site were markedly lower than in previous years.

Molting

Aerial surveys were conducted during the annual molt in August-September 1989-1991. Some or all of the 25 trend count sites were flown on 10 days during September 3-16, 1989 (Appendix G); 8 days during August 28-September, 1990 (Appendix H); and 10 days during August 22-September 1, 1991 (Appendix I).

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 for which there were survey data (1984 and 1988), declined substantially more at oiled sites than at unoiled sites (Table 14). 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 during 1990 indicated a moderate increase (+17%) in the number of seals at oiled sites 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. In 1991, counts during the fall molt increased in all sample groups as follows: oiled - +6%, unoiled - +18%, and intermediate - +32%.

Statistical comparisons of pre-spill (1984 and 1988) and post-spill (1989-1991) counts from oiled and unoiled sites clearly indicated that the difference between oiled and unoiled sites following the EVOS was significant (Table 15). Results of the repeated measures ANOVA ($p = 0.0111$) and a negative contrast statistic (-1.609) indicated an EVOS-caused reduction in the number of harbor seals at oiled sites. From 1988 to 1991 there was a 31% decline in the number of seals in oiled areas compared to a 2% increase in unoiled areas. This is in marked contrast to counts prior to the EVOS, when the two sample groups declined at a similar rate. There was also an overall decline from 1988 to 1991 in the number of seals counted in intermediate areas (-21%). 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 ($p = 0.0546$).

Further examination of the data from oiled areas indicated that the EVOS-related decline in counts, as detectable by aerial surveys, was confined to the year of the spill. In the subsequent two years, counts increased in both sample groups. However, from 1989 to 1991, there was no evidence in the oiled areas of recovery from the EVOS above and beyond the "normal" increase that occurred in the unoiled areas ($p=0.3646$). In fact, the increase at the oiled sites was considerably less than at unoiled sites.

The number of seals missing in oiled areas that could be attributed to the EVOS was calculated in two ways: by comparing percent declines between fall 1988 and fall 1989, and also by comparing fall 1988 and fall 1990. Comparisons based on 1989 counts assume that all EVOS-caused mortality took place within the first five months after the spill and that mortality, not a short-term difference in distribution, was responsible for any decreases in counts. Since it was not possible to determine whether either of these assumptions was correct, we also estimated the number of missing seals based on percent declines between 1988 and 1990. By fall 1990 surveys, residual effects of oiling that caused more seals to die would be reflected, distribution should have returned to normal, and any reduction in pup production would be evident in the count.

Calculation of missing seals was done by applying the rate of decline from 1988 to either 1989 or 1990 for unoiled seals to the oiled sites to produce an expected number of seals for the post-EVOS year. 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 1989 or 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:

$$\text{Missing in oiled trend count area} = \{1 - (\text{Unoiled}_{88} - \text{Unoiled}_{89/90}) / \text{Unoiled}_{88}\} \text{ X } \text{Oiled}_{88} - \text{Oiled}_{89/90}$$

Substitution of values from Table 14 indicates that 120 more seals were missing in oiled areas than could be accounted for by the ongoing decline in 1989; in 1990, the calculated number of missing seals was 91.

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 2). This total of 296 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:

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

Substitution of values gives an estimated number of seals missing in oiled parts of PWS other than the trend count area of 154 using 1989 data and 117 using 1990 data.

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 either 274 (1989) or 208 (1990) seals missing from PWS due to the EVOS. These estimates are likely to be conservative 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 sites adjacent to oiled areas, but no oiled seals at unoiled sites in eastern or northern PWS (Table 2). The same pattern was evident in western and southwestern PWS, 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. Two harbor seals that were radio-tagged at Seal Island in April 1991 remained near there throughout the spring and early summer.

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.

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. Results are expected in the very near future. 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.

Histological analyses of tissues from all seals collected or found dead and final interpretation of histopathology slides are complete. 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 three 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 1989-91 indicated that significantly more pups/100 non-pups were present at oiled sites in 1990 and 1991 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.

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 and 1991 as well as 1989 suggests that mortality, rather than displacement, was responsible for the decline.

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 pilot study to gather this information by attaching satellite transmitters to seals has been initiated as part of the restoration program. It is important to continue this study in order to learn more about the movements, site fidelity and diving behavior of harbor seals in PWS. We also strongly recommend that aerial surveys during the fall molt are conducted as part of future restoration studies. The final fall surveys conducted under this NRDA study in 1991 indicated that counts of seals were increasing throughout the PWS trend count area, but that the increase was substantially less in areas that had been oiled. It is important to continue to monitor trends in abundance in order to determine how long residual effects of the EVOS persist.

LITERATURE CITED

Allen, S. G., D. G. Ainley, G. W. Page, and C. A. Ribic. 1984. The effect of disturbance on harbor seal haul out patterns at Bolinas Lagoon, California. Fish. Bull. 82:493-500.

Calkins, D., and K. Pitcher. 1984. Pinniped investigations in southern Alaska: 1983-84. Unpubl. Rep. ADF&G, Anchorage, AK. 16pp.

Cornish, H. H. 1980. Solvents and vapors. Pages 468-496 in: J. Doull, C. D. Klaassen, and M. O. Amdur (eds). Casarett and Doull's Toxicology. MacMillan Publ. Col. New York, N. Y. 478 p.

Fleiss, J. L. 1986. The design and analysis of clinical experiments. John Wiley & Sons. New York, N.Y. 432pp.

Hoaglin, D. C., F. Mosteller and J. W. Tukey. 1985. Exploring data tables, trends, and shapes. John Wiley & Sons. New York, N.Y. 527 pp.

Neter, J., and W. Wasserman. 1974. Applied linear statistical models. Irwin Inc. Homewood, IL. 842pp.

Newby, T. C. 1971. Distribution, population dynamics and ecology of the harbor seal of southern Puget Sound, Seattle. MS Thesis, University of Puget Sound, Tacoma, WA. 75pp.

Paulbitsky, P. 1975. The seals of Strawberry spit. Pac. Discovery 28:12-15.

Pitcher, K. W. 1986. Harbor seal trend count surveys in southern Alaska, 1984. Unpubl. Rep. ADF&G, Anchorage, AK. 10pp.

Pitcher, K. W. 1989. Harbor seal trend count surveys in southern Alaska, 1988. Final Rep. Contract MM4465852-1 to U.S. Marine Mammal Commission, Washington, D.C. 15pp.

Pitcher, K. W. and D. C. McAllister. 1981. Movements and haulout behavior of radio-tagged harbor seals, Phoca vitulina. Can. Field-Natur. 95: 292-297.

Renouf. D., L. Gaborko, G. Galway, and R. Finlayson. 1981. The effect of disturbance on the daily movements of harbour seals and grey seals between the sea and their hauling grounds at Miquelon. Appl. Anim. Ethol. 7:373-379.

Snedecor, G. W. and W. G. Cochran. 1980. Statistical Methods. Iowa State University Press, Ames, IO. 507 pp.

Weber, R. A. 1990. Factors affecting haul-out of _Phoca vitulina_ on three substrates in Massachusetts. MS Thesis, Northeastern University, Boston, MA. 86pp.

Winer, B. J. 1971. Statistical principle in experimental design. 2nd Ed. McGraw-Hill, New York, N. Y. 907 pp.

Yochem, P. K., B. S. Stewart. R. L. Delong, and D. P. DeMaster. 1987. Diel haul-out patterns and site fidelity of harbor seals (_Phoca vitulina richardsi_) on San Miguel Island, California, in Autumn. Mar. Mamm. Sci. 3:323-332.

Table 1. Prince William Sound harbor seal trend count route.

Site #	Site name	Oiling status
1	Sheep Bay	unoiled
2	Gravina Island	unoiled
3	Gravina Rocks	unoiled
4	Olson Bay	unoiled
5	Porcupine Point	unoiled
6	Fairmount Island	unoiled
7	Payday	unoiled
8	Olsen Island	unoiled
9	Point Pellew	unoiled
10	Little Axel Lind Island	unoiled
11	Storey Island	peripheral
12	Agnes Island	oiled
13	Little Smith Island	oiled
14	Big Smith Island	oiled
15	Seal Island	oiled
16	Applegate Rocks	oiled
17	Green Island	oiled
18	Channel Island	peripheral
19	Little Green Island	peripheral
20	Port Chalmers	unoiled
21	Stockdale Harbor	unoiled
22	Montague Point	unoiled
23	Rocky Bay	unoiled
24	Schooner Rocks	unoiled
25	Canoe Passage	unoiled

Table 2. Oiling of harbor seals and harbor seal haulouts in Prince William Sound, 1989. Data on oiling of seals are for animals older than pups.

Haulout	Degree of Oiling on shoreline	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
Fairmount Island	unoiled	May	15	0
Gravina Island	unoiled	May	10-20	0
Gravina Rocks	unoiled	May	2-9	0
Green Island	moderate	April	10	60
Little Green I.	unoiled	May	40	20
Little Smith I.	heavy	April-July	12-23	83-100
Olsen Bay	unoiled	May	22-48	0
Olsen Island	unoiled	May	3	0
Payday	unoiled	May	3	0
Point Pellew	unoiled	May	4	0
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
Chenega Island	light	June	12	8
Crafton Island	mod.-heavy	June-July	17-33	76-83
Disk Island	heavy	May-June	1-8	100
Eshamy Bay	unoiled	June	3	0
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 3. Percent of seals older than pups that were oiled, as determined from boat-based observations in Prince William Sound, May 1989. Areas were classified as oiled if oil was present along the shoreline nearby, and as unoiled if no oil was detected along the shoreline. Intermediate unoiled areas were those near oiled sites where oil was observed in the water; they included Channel and Little Green islands and the coast of Knight Island south of Herring Bay. This terminology is consistent with classification of aerial survey trend count sites.

			Percent in category		
Date	Area type	Number of seals classified	Heavily oiled	Oiled	Unoiled
15-18 May	oiled	177	84.8	10.7	4.5
	intermediate unoiled	24	8.3	0.0	91.7
	unoiled	58	1.7	1.7	96.6
23-27 May	oiled	408	44.6	28.9	26.5
	intermediate unoiled	72	18.1	22.2	59.7
	unoiled	124	0.0	0.0	100.0

Table 4. 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	90	--	86	50	98	--
24-26 May	74	100	94	91	100	100
8-9 June	70	80	91	90	100	100
16-19 June	77	64	91	100	100	100
24-28 June	49	43	100	100	100	100
11-13 July	62	100	87	89	100	100
4 September	--	--	17	--	16	100

Table 5. Observations of unusual behavior by oiled harbor seals in Prince William Sound, 1989.

Date	Location	Observer[1]	# 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 5. 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.

[1]KP = K. Pitcher; LL = L. Lowry; KF = K. Frost; RS = R. Shideler

Table 6. Harbor seals that were found dead or died in captivity during EVOS response and damage assessment. (MH-HS-4 was killed by a subsistence hunter and turned over for sampling)

Specimen number	Date found	Location	Degree of oiling	Comments
no number	9 April	Eleanor Island, PWS	heavy	premature pup in lanugo
MH-HS-2	12 April	Eleanor Island, PWS	moderate	premature pup
MH-HS-3	19 April	Green Island, PWS	unoiled	premature pup
MH-HS-4	20 April	Tatitlek Narrows, PWS	unoiled	subsistence kill, juvenile
MH-HS-5	21 April	Applegate Rocks, PWS	heavy	premature pup, scavenged
MH-HS-7	28 April	Windy Bay, G of AK	heavy	predated or scavenged, adult
MH-HS-6	1 May	Herring Bay, PWS	heavy	captured alive and died, adult, pyometra
MH-HS-12	2 May	Herring Bay, PWS	moderate	in lanugo when caught, rehabilitated pup, died May 31 in captivity
MH-HS-13	3 May	PWS	heavy	rehabilitated pup, died May 31 in captivity
MH-HS-8	11 May	Axel Lind Island, PWS	light	adult, broken ribs
LL-HS-1	15 May	Herring Bay, PWS	light	pup
AF-HS-2	16 May	Herring Bay, PWS	unoiled	pup
KP-HS-1	20 May	Raspberry Cape, G of AK	light	subadult, broken ribs
MH-HS-9	25 May	Drier Bay, G of AK	unoiled	pup, scavenged
MH-HS-10	30/31 May	Herring Bay, PWS	heavy	pup
MH-HS-11	30/31 May	Herring Bay, PWS	heavy	pup, scavenged
MH-HS-14	22 June	Chugach Bay, G of AK	heavy	pup, badly autolyzed
GA-HS-1	25 June	Dutch Group, PWS	light	subadult
MH-HS-15	9 July	Herring Bay	heavy	pup, severe dermatitis

Table 7. Harbor seals collected by ADF&G personnel during EVOS response and damage assessment.

Specimen number	Date	Location	Degree of oiling	Comments
TS-HS-1	4/29/89	Herring Bay, PWS	very heavy	adult female, pregnant
AF-HS-1	5/16/89	Herring Bay, PWS	very heavy	subadult female
TS-HS-2	6/16/89	Bay of Isles, PWS	very heavy	adult male
TS-HS-3	6/16/89	Seal Island, PWS	heavy	adult female
TS-HS-4	6/16/89	Seal Island, PWS	heavy	pup of TS-HS-3
TS-HS-5	6/17/89	Bay of Isles, PWS	very heavy	adult female
TS-HS-6	6/17/89	Applegate Rocks, PWS	light	pup
TS-HS-7	6/17/89	Bay of Isles, PWS	very heavy	adult female
TS-HS-8	6/17/89	Bay of Isles, PWS	very heavy	pup of TS-HS-7
TS-HS-9	6/18/89	Herring Bay, PWS	very heavy	adult male
TS-HS-10	6/18/89	Herring Bay, PWS	very heavy	adult female
TS-HS-11	6/18/89	Herring Bay, PWS	very heavy	adult female
TS-HS-12	6/25/89	Perenosa Bay, Afognak Island	unoiled	adult female
TS-HS-13	6/25/89	Perenosa Bay, Afognak Island	unoiled	subadult female
TS-HS-14	6/29/89	W. Amatuli Island, Barren Islands	moderate	adult male
TS-HS-15	6/30/89	Ushagat Island, Barren Islands	unoiled	adult male
TS-HS-16	6/30/89	Ushagat Island, Barren Islands	unoiled	adult female
TS-HS-17	7/6/89	Perl Island, Chugach Islands	light	adult female
TS-HS-18	10/26/89	Big Fort Island, Gulf of Alaska	unoiled	subadult male
TS-HS-19	11/1/89	Agnes Island, PWS	unoiled	adult male
TS-HS-20	4/11/90	Herring Bay, PWS	unoiled	subadult male
TS-HS-21	4/12/90	Herring Bay, PWS	unoiled	subadult male
TS-HS-22	4/12/90	Herring Bay, PWS	unoiled	adult male
TS-HS-23	4/12/90	Eleanor Island, PWS	unoiled	adult female, pregnant
TS-HS-24	4/12/90	Herring Bay, PWS	unoiled	adult male
TS-HS-25	4/13/90	Bay of Isles, PWS	unoiled	adult male
TS-HS-26	8/15/90	Ketchikan	unoiled	adult female
TS-HS-27	8/16/90	Ketchikan	unoiled	adult male

Table 8. Preliminary results of HPLC fluorometric analysis of bile from harbor seals collected in PWS and the Gulf of Alaska in 1989-90. Values are for fluorescing aromatic hydrocarbons (FACs), expressed in parts per billion (= ng PHN (NPH) equilvalents/g bile). Values in parentheses are corrected for bile protein and are expressed as ng/mg bile protein. Degree of external oiling is indicated for seals collected prior to the fall 1989 molt (lt=light, mod=moderate, hv=heavy).

Specimen number	Phenanthrene	Napthalene	Comments
LL-HS-1	2,000 (100)	13,000 (600)	dead pup-lt oiled
MH-HS-3	4,000 (170)	51,000 (2,200)	dead pup-unoiled
MH-HS-4	2,000 (46)	33,000 (750)	subadult male-unoiled
MH-HS-6	14,000 (740)	48,000 (2,500)	adult female-hv oiled
AF-HS-1	79,000 (2,240)	180,000 (5,100)	subad female-hv oiled
TS-HS-1	110,000 (4,780)	200,000 (8,700)	preg female-hv oiled
TS-HS-2	8,800 (700)	31,000 (2,500)	adult male-hv oiled
TS-HS-3	2,700 (680)	2,300 (600)	adult female-hv oiled
TS-HS-4	25,000 (1,620)	46,000 (3,000)	pup of TS-3-hv oiled
TS-HS-5	32,500 (2,455)	54,000 (4,050)	adult female-hv oiled
TS-HS-6	3,700 (220)	7,000 (400)	pup-lt oiled
TS-HS-7	36,000 (3,310)	53,000 (4,900)	adult female-hv oiled
TS-HS-8	215,000(20,725)	365,000(35,150)	pup of TS-7-hv oiled
TS-HS-9	1,300 (20)	4,900 (100)	adult male-hv oiled
TS-HS-10	18,500 (675)	41,000 (1,500)	adult female-hv oiled
TS-HS-11	15,000 (680)	30,000 (1,400)	adult female-hv oiled
TS-HS-12	730 (40)	5,600 (300)	adult female-unoiled
TS-HS-13	2,200 (90)	7,200 (300)	subad female-unoiled
TS-HS-14	8,000 (430)	14,000 (800)	adult male-mod oiled
TS-HS-15	3,000 (100)	11,000 (400)	adult male-unoiled
TS-HS-16	800 (70)	4,400 (400)	adult female-unoiled
TS-HS-17	2,200 (160)	7,700 (500)	adult female-lt oiled
TS-HS-18	170 (10)	1,400 (100)	adult male
TS-HS-19	6,200 (290)	20,000 (900)	adult male
TS-HS-20	14,000 (320)	68,000 (1,500)	juvenile male
TS-HS-21	4,000 (240)	22,000 (1,300)	juvenile male
TS-HS-22	4,200 (180)	28,000 (1,200)	adult male
TS-HS-23	44,000 (830)	110,000 (2,100)	adult female
TS-HS-23F	1,800 (30)	3,300 (100)	fetus of TS-HS-23
TS-HS-24	5,350 (165)	34,500 (1,050)	adult male
TS-HS-25	12,000 (140)	67,000 (800)	adult male
TS-HS-26	455 (20)	3,100 (200)	adult female, unoiled
TS-HS-27	740 (110)	6,100 (900)	adult male, unoiled

Samples were analyzed by the Environmental Conservation Division, Northwest Fisheries Center, NOAA, NMFS.

Table 9. Results of HPLC fluorometric analysis of harbor seal bile for the presence of the fluorescent aromatic hydrocarbons phenanthrene (PHN) and napthalene (NPH), and GCMS analysis of seal liver (Li) and blubber (Bl) for the presence of low (LAC) and high (HAC) molecular weight aromatic contaminants. Results are given in parts per billion (= ng/g). N is the number of animals in each sample. Dashes indicate that no samples were analyzed; nd means the compound was not detected. Ringed seal samples were collected as part of the NOAA tissue archival program and were provided by P. Becker (unpubl. data).

					Aromatic hydrocarbons				
		Bile				LAC		HAC	
Area/sample	Date	N	PHN	NPH	N	Li	Bl	Li	Bl
PRINCE WILLIAM SOUND									
unoiled area-juvenile (MH-4)	Apr 89	1	2000	33,000	-	-	-	-	-
oiled area-fetus/premature (bile - MH-3, LL-1; li,bl - MH-10,12,13, AF-2, LL-1, TS-1F)	Apr-May 89	2	3,000	32,000	6	33	-	19	-
oiled area-adult/juvenile (AF-1, TS-1)	Apr-May 89	2	94,500	190,000	2	100	-	6	-
oiled area-all ages (TS-2 through 11)	Jun 89	10	35,850	62,400	10	nd	194	nd	6
oiled area-adult (TS-19)	Nov 89	1	6,200	20,000	1	nd	21	nd	3
oiled area-fetus (TS-23F)	Apr 90	1	1,800	3,300	1	nd	20	nd	4
oiled area-adult/juvenile (TS-20 through 25)	Apr 90	6	13,900	54,900	6	2	38	nd	11
GULF of ALASKA									
oiled area-adult/juvenile (TS-12 through 17)	Jun-Jul 89	6	2,800	8,300	6	3	1	<1	<1
oiled area-juvenile (TS-18)	Nov 89	1	170	1400	1	nd	21	nd	2
KETCHIKAN									
unoiled-adult (TS-26,27)	Aug 90	2	600	4,600					
BARROW									
unoiled ringed seals	Jul 88	10	880	11,500					

Table 10. Summary of lesions found in the brains of 27 harbor seals collected in Prince William Sound, the Gulf of Alaska, and Ketchikan following the *Exxon Valdez* oil spill.

Specimen number	IME	NS	NND	AS/D	ICIB	NE/BS	MC/BS
TS-HS-1	+++	+++	++	+++	-	-	-
TS-HS-2	+	+	-	-	+	-	-
TS-HS-3	+	+	+	-	+	-	-
TS-HS-4	NE	NE	NE	NE	NE	NE	-
TS-HS-5	-	+	+	-	-	-	-
TS-HS-6	-	-	-	-	-	-	-
TS-HS-7	+	-	++	-	+	-	-
TS-HS-8	-	-	-	-	+	-	-
TS-HS-9	-	-	-	-	+	-	-
TS-HS-10	-	-	+	-	+	+	-
TS-HS-11	+	+	++	+	+	-	-
TS-HS-12	NE	NE	NE	NE	NE	NE	-
TS-HS-13	-	-	-	-	+	+	-
TS-HS-14	-	+	++	-	-	+	-
TS-HS-15	-	-	-	-	-	+	-
TS-HS-16	-	+	++	-	+	-	-
TS-HS-17	+	+	+++	+	-	-	-
TS-HS-18	-	-	-	-	+	+	-
TS-HS-19	-	+	-	-	+	+	-
TS-HS-20	-	-	-	-	+	+	+
TS-HS-21	-	-	-	-	+	-	-
TS-HS-22	-	-	-	-	+	-	+
TS-HS-23	-	-	-	-	+	+	+
TS-HS-24	-	-	-	-	+	-	+
TS-HS-25	-	-	-	-	+	-	-
TS-HS-26	-	-	-	-	-	-	-
TS-HS-27	-	-	-	-	+	-	-

IME = Intramyelinic edema
NS = Neuronal swelling with loss of Nissl substance
NND = Neuronal necrosis with dropout
AS/D = Axonal swelling/degeneration
ICIB = Intracytoplasmic inclusion bodies in thalamic neurons
NE/BS = Nonsuppurative encephalitis, mild, brain stem
MC/BS = Microcavitation, mild, brain stem
NE = Not examined
+++ =Severe; ++ = Moderate; + = Mild; - = Negative

Table 11. Preliminary analysis of pathology present in harbor seals collected in oiled areas of Prince William Sound and the Gulf, 1989 and 1990. PMI is the post-mortem interval (time between death and sample) in minutes.

Specimen	PMI	Intramyelinic edema-brain	Axonal dengeneration-vibrissae	Rhabdomyolosis-nostrils	Acanthosis and hyperkeratosis-skin
1	20	+++	-	-	-
1F	120	-	-	-	-
2	30	+	-	-	+
3	60	+	+	+	++
4	30	na	na	na	-
5	60	-	-	+	+
6	90	-	+	-	-
7	10	+	-	+	++
8	120	-	-	-	+
9	120	-	-	-	+
10	30	-	-	+	-
11	30	+	-	-	++
12	30	na	na	na	-
13	40	-	-	-	+
14	60	-	-	+	+
15	60	-	-	-	+
16	90	-	-	-	-
17	45	+	+	+	+
18	30	-	-	-	-
19	30	-	-	+	-
20	40	-	-	-	-
21	15	-	-	+	-
22	25	-	-	-	-
23	40	-	-	-	-
23F	120	-	-	-	-
24	40	-	-	-	-
25	30	-	-	-	-
26	20	-	-	-	-
27	20	-	-	-	-

+++ = Severe, ++ = Moderate, + = Mild, - = Negative, na = Not available

Table 12. Trimean values for seals and seal pups in oiled, unoiled, and intermediate sample groups based on 25 trend count haulout sites in Prince William Sound surveyed during June 1989-1991.

		1989			1990			1991		
	n	non-pups	pups	pups/100 non-pups	non-pups	pups	pups/100 non-pups	non-pups	pups	pups/100 non-pups
Oiled	6	268.4	69.3	25.8	292.5	99.0	33.8	309.4	109.3	35.3
Unoiled	16	277.9	68.2	24.5	286.6	61.3	21.4	189.6	44.7	23.6
Intermediate	3	178.9	26.4	14.8	142.8	10.3	7.2	101.8	9.5	9.3
All combined	25	725.2	163.9	22.6	721.9	170.6	23.6	600.8	163.5	27.2

Table 13. Results of a one-way COANOVA conducted on the square roots of the differences in pup and non-pup trimean counts between years (1990-89), based on surveys conducted during June 1989 and 1990 in Prince William Sound.

Source of variation	COANOV DF	SS	MS
Oiled group	2	5.561	2.185
Co-variate (non-pup counts)	1	0.337	0.337
Error	22	9.764	0.514

Contrast	Hypothesis Statistics DF	MS	F	P<T
Oiled vs Unoiled	1	2.969	5.777	0.013
Intermediate vs Unoiled	1	0.698	-1.165	0.871

Table 14. Trimean values and percent change for oiled, unoiled and intermediate sample groups based on 25 trend count haulout sites surveyed by ADF&G in Prince William Sound, Alaska in September 1984, 1988, and 1989-1991.

		Year									
		1984		1988		1989		1990		1991	
Oil category	n	trimn	% change	trimn	% change	trimn	% change	trimn	% change	trimn	% change
Oiled	6	652.9	---	414.8	-36	230.3	-44	270.3	+17	287.1	+ 6
Unoiled	16	798.4	---	496.8	-38	419.1	-16	432.0	+ 3	508.2	+18
Inter-mediate	3	356.7	---	124.7	-65	136.4	+10	74.2	-46	98.0	+32
All combined	25	1808.0	--	1036.3	-43	784.8	-24	776.5	- 1	893.3	+15

Table 15. Results of a Repeated Measures ANOVA conducted on the square root of the trimean of harbor seals counts conducted in Prince William Sound during the molt in 1984, 1988, 1989-1991.

	ANOVA			
Source of variation	MS	DF	F	Pr>F
Between sites				
Oiled Group	57.21	2	1.804	0.1663
Error A*	29.59	22		
Within Site				
Year	35.62	4	11.032	0.0104
Year x Oiled Group	3.61	8	2.434	0.0499
Error B*	1.48	88		

	HYPOTHESIS STATISTICS				
Contrast	DF	Mean	M.S.	F	PR>F
Oiled vs Unoiled	1	-1.609	13.57	9.16	0.0111
Intermediate vs Unoiled	1	-1.492	6.75	4.55	0.0546
Error B*	88		1.48		

* Error A denotes between site variation. Error B denotes within site variation.

Appendix A. Methodology for collecting harbor seal tissue samples for histopathology and toxicology following the <u>Exxon Valdez</u> oil spill.

Histological Analysis

Prepare a solution of buffered formalin in a 5 gallon plastic bucket as follows:

76 grams of monobasic sodium phosphate
123 grams of dibasic sodium phosphate
1,900 cc of 37% formaldehyde
16,900 cc of tapwater

If sodium phosphate salts are not available, make the solution with nine parts of seawater and one part of 37% formaldehyde.

Collect the appropriate tissue or organ samples using clean cutting tools (new sterile, disposable surgical blades for each animal, and clean forceps). The samples should be about 2x2x1 cm, or the size of a small walnut. Place the samples in a large ziploc bag (2 gallon if available), then add formalin and labels. All tissues from the same animal can go into the same bag, but make sure that there is sufficient formalin to totally immerse the samples, with a ratio of formalin to tissue of about 10:1. After 6 to 8 hours, replace the solution with fresh formalin, then change it again every 24 hours for the next few days. Use labels that will not disintegrate in the solution. Plastic tags or waterproof field notebook paper works well. Permanent marking pens or pencil work better than ballpoint pens. Information on the label must include species, sex, date sampled, and collection location. Additional information could include time of death and condition of the carcass. Avoid contamination of the samples with oil, tar balls, etc. If an organ or tissue appears irregular or damaged, take samples of both the unhealthy tissue and normal tissue.

Tissues to be collected for histological examination (not in priority) include:

skin	brain	pituitary	stomach
liver	lung	kidney	blubber
thyroid	adrenal	bone marrow	spleen
heart	esophagus	tonsil	skeletal muscle
eyes	mammary gland		

small and large intestine with attached pancreas
gonads (epididymis, testes, prostate, uterus, ovaries)

Appendix A. continued

Toxicological Analysis

Samples taken under this protocol must be collected with care since the slightest amount of contamination may result in erroneous results. Extreme care must be taken to avoid hydrocarbon contamination. These samples must not come in contact with any plastic or other petroleum derived products.

Samples collected for this protocol should be placed in clean glass jars. Use new ICHEM jars if possible. If new ICHEM jars are not available, thoroughly wash jars with clean water, rinse them with reagent grade methylene chloride, and allow them to dry. Methylene chloride is toxic and should be handled in a hood or used out of doors. Do not breathe the fumes. If methylene chloride is not available, rinse jars with another organic solvent (acetone or ethanol). Jar lids should be lined with teflon. If jars are not available, samples may be tightly wrapped in aluminum foil. Samples of bile and milk shoul be put in amber-colored jars with teflon lids. Samples of whole blood should be put in gray-topped vacutainers or ICHEM jars.

Samples should be handled only with knives and forceps that have been cleaned with acetone, ethanol, or methylene chloride. Rinse instruments with ethanol after each sample. Be sure that the samples do not come in contact with rubber or surgical gloves. Gloves without talc are preferred. Whenever possible, take the sample from the center of the organ, avoiding possible contaminating material. Tissue samples should be about 2x2x1 cm. Fluid samples should be 5-10 cc. If adequate material is available take triplicate samples and package each separately.

Sample information should be put on the outside of the jar on a cloth label. Permanent marking pens or pencil work better than ballpoint pen. Information on the label must include species, sex, date sampled, and collection location. Immediately cool the sample, and freeze as soon as possible (-20 F if possible).

Bile, liver, blubber, and lung are the highest priority to sample. Other samples that should be taken, if they are available and time and supplies permit, include: kidney, brain, heart, skin, skeletal muscle, blood, and milk. If there are any prey or other items in the stomach, take a sample of those and clearly label them as such.

Appendix B. Harbor seal tissue samples that have been analyzed for the presence of hydrocarbon contaminants. Li=liver; Br=brain; H=heart; K=kidney; Ov=ovary; F=fat/blubber, Lu=lungs, Test=testicle; Mam=mammary; Sk=skin; M=muscle

Sample	Bile	Histo	Toxicology NMFS-Subsistence	Toxicology Texas A & M-NRDA
AF-1	X	X		Li
TS-1F	X	X		Li
TS-1	X	X	Li, F	Li, Br, Placenta
TS-2	X	X	M, Li, F	Br, Test
TS-3	X	X	M, Li, F	Br, H, K, Lung, Mam, Milk, Ov, F
TS-4	X	X	M, Li, F	Br, Milk
TS-5	X	X	M, Li, F	Br, H, K, Lu, Mam, Milk, Ov, Li, F
TS-6	X	X	M, Li, F	Br
TS-7	X	X	M, Li, F	Br, H, K, Mam, F, Li
TS-8	X	X	M, Li, F	Br, K, Lu, Milk, F, Li
TS-9	X	X	M, Li, F	Br
TS-10	X	X	M, Li, F	Br, Ov
TS-11	X	X	M, Li, F	Br
TS-12	X	X	M, Li, F, K	--
TS-13	X	X	M, Li, F, K	Br
TS-14	X	X	M, Li, F, K	Br
TS-15	X	X	M, Li, F, K	--
TS-16	X	X	M, Li, F, K	Br
TS-17	X	X	M, Li, F, K	Br
TS-18	X	X	M, Li, F	Br
TS-19	X	X	M, Li, F	Br
TS-20	X	X	M, Li, F	Br
TS-21	X	X	M, Li, F	Br
TS-22	X	X	M, Li, F	Br
TS-23	X	X	M, Li, F	Br
TS-23F	X	X	M, Li, F	Br
TS-24	X	X	M, Li, F	Br
TS-25	X	X	M, Li, F	Br
TS-26	X	X		
TS-27	X	X		
MH-2		X		
MH-3	X	X		
MH-4	X	X		
MH-5		X		F
MH-6	X	X		K, Lu, Br, F, Sk, Li, all to EPA
MH-7		X		F to EPA
MH-8		X		
MH-9		X		
MH-10				Li
MH-11				
MH-12	-			Li
MH-13	-			Li
MH-14				
MH-15				
LL-1	X	X		Li
AF-2	-			Li
KP-1	-			
GA-1				

Appendix C. Results of GCMS analysis of tissue samples from harbor seals collected in PWS and the Gulf of Alaska in 1989-1990. Values are expressed in parts per billion (=ng/g), and are given separately for replicate samples, where available. Dashes indicate that no sample was analyzed; nd means the compound was not detected. Comments are given in Appendices A and B for all specimens except TS-HS-1F, which was the fetus of TS-HS-1.

	Liver		Blubber		Muscle		Brain	
Specimen #	LAC	HAC	LAC	HAC	LAC	HAC	LAC	HAC
AF-HS-1	45	8	-	-	-	-	-	-
TS-HS-1	156	4	-	-	-	-	31	8
TS-HS-1F	45	5	-	-	-	-	-	-
TS-HS-2	nd	nd	77	2	4	nd	24	6
TS-HS-3a	nd	nd	21	2	4	nd	-	-
TS-HS-3b	-	-	111	19	-	-	20	5
TS-HS-4	nd	nd	26	nd	10	<1	24	7
TS-HS-5a	nd	<1	85	1	nd	nd	-	-
TS-HS-5b	44	4	159	10	-	-	22	6
TS-HS-6a	nd	nd	18	<1	nd	nd	32	12
TS-HS-6b	nd	nd	19	1	nd	nd	-	-
TS-HS-7a	2	nd	420	1	4	<1	-	-
TS-HS-7b	nd	nd	520	4	5	nd	-	-
TS-HS-7c	31	4	572	37	-	-	26	4
TS-HS-8	21	6	738	11	-	-	21	4
TS-HS-9	nd	nd	170	7	<1	<1	22	5
TS-HS-10	nd	nd	150	1	nd	nd	19	5
TS-HS-11	nd	nd	98	8	nd	nd	17	4
TS-HS-12	4	<1	4	nd	nd	nd	-	-
TS-HS-13	4	<1	nd	nd	<1	nd	51	3
TS-HS-14	nd	nd	nd	nd	nd	nd	61	3
TS-HS-15	nd	nd	1	2	nd	nd	-	-
TS-HS-16	3	2	1	nd	nd	nd	30	5
TS-HS-17	5	<1	2	nd	nd	nd	21	4
TS-HS-18	nd	nd	21	2	nd	nd	58	4
TS-HS-19	nd	nd	21	3	nd	<1	53	5
TS-HS-20	nd	nd	19	2	nd	nd	23	3
TS-HS-21	nd	nd	19	2	nd	nd	22	4
TS-HS-22	15	nd	26	7	nd	<1	58	5
TS-HS-23	nd	nd	28	2	nd	nd	22	4
TS-HS-23F	nd	nd	20	4	6	1	36	3
TS-HS-24	nd	nd	51	39	nd	2	17	4
TS-HS-25	nd	nd	86	15	nd	<1	14	3
AF-HS-2	20	6	-	-	-	-	-	-
LL-HS-1	44	32	-	-	-	-	-	-
MH-HS-5	-	-	408	59	-	-	-	-
MH-HS-10	14	11	-	-	-	-	-	-
MH-HS-12	28	32	-	-	-	-	-	-
MH-HS-13	44	27	-	-	-	-	-	-

Appendix D. Repetitive counts of harbor seals and seal pups (#/#) on selected haulout sites in Prince William Sound, Alaska, June 1989. An x indicates that no survey was conducted.

Site	Date (June)								
	8	11	16	17	18	19	20	26	27
Sheep Point	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Gravina Island	2/0	0/0	0/0	0/0	0/0	3/2	19/1	0/0	0/0
Gravina Rocks	7/0	0/0	0/0	0/0	1/0	2/0	11/1	9/0	13/1
Olsen Bay	62/13	47/6	66/25	x	65/14	69/20	76/18	x	88/13
Porcupine	18/1	8/3	3/2	3/0	x	12/3	24/4	0/0	1/0
Fairmount	17/5	23/7	29/5	17/7	6/4	2/1	10/4	19/9	27/6
Payday	1/1	2/1	3/1	6/5	6/3	1/1	1/0	11/10	6/6
Olsen Island	0/0	8/1	14/1	5/1	13/3	6/2	x	17/4	13/4
Point Pellew	15/2	16/4	18/5	6/1	3/2	5/1	x	12/5	13/5
Little Axel Lind	4/0	6/0	4/0	3/1	1/0	0/0	x	0/0	2/0
Storey Island	8/1	2/1	0/0	1/1	0/0	8/1	6/0	1/0	1/0
Agnes Island	26/10	30/7	29/9	25/9	25/9	34/7	25/7	34/13	x
Little Smith I.	36/9	10/2	9/5	8/4	10/1	7/2	7/1	2/0	8/4
Big Smith Island	12/5	23/4	11/6	15/7	x	21/8	17/7	15/6	28/13
Seal Island	48/23	22/6	39/14	39/16	48/12	40/11	68/12	50/18	63/14
Applegate Rocks	199/19	x	x	133/29	126/16	x	134/23	133/56	180/44
Green Island	32/11	16/4	18/9	15/5	25/10	17/5	26/8	22/9	23/10
Channel Island	93/12	74/5	76/12	61/18	x	45/7	90/9	152/20	140/12
Little Green I.	90/6	x	85/30	83/13	64/16	82/11	93/18	118/19	88/9
Port Chalmers	104/21	67/18	62/19	61/15	65/14	86/20	91/23	83/21	54/17
Stockdale Hbr.	28/0	17/5	9/3	14/5	11/0	16/2	25/3	32/9	27/7
Montague Point	32/0	26/8	17/6	14/4	8/3	13/2	18/5	1/1	9/4
Rocky Bay	31/6	21/6	23/11	22/9	14/6	30/10	32/9	27/8	22/6
Schooner Rocks	54/5	36/4	24/8	24/6	17/3	10/4	24/5	38/10	32/6
Canoe Passage	1/0	0/0	0/0	0/0	1/0	0/0	0/0	1/0	1/0

Appendix E. Repetitive counts of harbor seals and seal pups (#/#) on selected haulout sites in Prince William Sound, Alaska, June 1990. An x indicates that no survey was conducted.

Site	Date (June)								
	6	7	8	9	10	11	12	13	15
Sheep Point	4/0	4/0	3/0	4/0	1/0	1/0	0/0	1/0	1/0
Gravina Island	1/0	13/0	18/0	15/0	17/0	14/0	0/0	7/0	14/0
Gravina Rocks	0/0	5/1	6/1	9/1	9/1	9/0	0/0	7/1	0/0
Olson Bay	67/33	41/12	x	51/18	69/17	71/27	49/21	31/12	59/24
Porcupine	1/0	2/1	3/1	2/1	2/1	1/1	2/1	3/1	0/0
Fairmount	13/5	11/4	10/0	x	x	2/2	10/6	6/1	17/9
Payday	0/0	0/0	0/0	x	x	0/0	0/0	0/0	1/1
Olsen Island	1/1	0/0	0/0	x	x	12/3	6/1	1/0	2/1
Point Pellew	5/1	7/1	7/2	x	x	8/2	8/1	9/1	10/2
Little Axel Lind	2/0	2/0	1/0	x	x	0/0	0/0	0/0	3/0
Storey Island	4/2	8/0	5/1	x	x	6/2	7/3	0/0	6/1
Agnes Island	43/18	44/12	54/14	44/12	x	54/17	30/13	35/16	36/18
Little Smith I.	18/6	24/5	25/7	18/7	x	12/5	25/11	13/3	14/6
Big Smith Island	22/11	20/4	24/5	13/4	x	16/7	15/2	15/4	21/5
Seal Island	53/22	54/20	60/21	32/17	x	53/19	40/23	37/19	45/15
Applegate Rocks	140/39	132/39	137/26	122/34	x	130/38	158/37	121/34	157/43
Green Island	23/14	20/6	26/17	29/18	x	30/15	33/18	29/17	33/22
Channel Island	x	64/2	75/2	58/3	x	86/3	96/3	53/5	76/2
Little Green I.	78/7	61/4	54/5	57/9	x	76/8	47/8	100/2	x
Port Chalmers	x	98/19	92/28	110/30	x	94/29	84/23	84/22	103/20
Stockdale Harbor	25/0	26/0	22/0	37/0	x	30/0	35/0	x	23/0
Montague Point	x	24/2	21/1	26/1	x	28/1	21/0	16/1	x
Rocky Bay	x	17/3	13/3	28/6	x	25/9	23/7	18/6	x
Schooner Rocks	35/5	21/1	17/3	31/4	x	21/4	11/2	42/4	24/4
Canoe Passage	1/0	0/0	0/0	3/2	x	3/2	0/0	0/0	0/0

Appendix F. Repetitive counts of harbor seals and seal pups (#/#) on selected haulout sites in Prince William Sound, Alaska, June 1991. An x indicates that no survey was conducted.

	Date (June)										
Site	11	12a	12b	13a	13b	14a	14b	16	18	19	20
Sheep Point	0/0	0/0	0/0	0/0	1/0	1/0	0/0	0/0	x	0/0	0/0
Gravina Island	0/0	0/0	1/0	6/0	3/0	4/0	0/0	11/1	5/1	0/0	0/0
Gravina Rocks	0/0	0/0	0/0	0/0	2/0	0/0	0/0	2/1	4/0	0/0	0/0
Olson Bay	26/12	24/14	15/10	x	13/10	8/5	21/14	24/9	33/15	20/8	46/15
Porcupine	0/0	7/4	1/0	12/3	0/0	12/3	x	9/3	10/3	10/1	2/1
Fairmount	12/2	4/2	13/3	11/4	17/3	14/4	x	x	4/1	8/5	15/6
Payday	1/1	1/1	4/1	1/0	4/2	5/1	x	x	0/0	8/1	4/1
Olsen Island	0/0	0/0	1/0	3/2	5/1	5/2	x	x	0/0	7/0	5/2
Point Pellew	6/0	0/0	3/0	8/0	5/0	1/0	x	x	1/0	3/0	4/0
Little Axel Lind	1/1	1/0	3/1	0/0	4/1	1/0	x	x	0/0	5/0	7/0
Storey Island	0/0	1/1	0/0	1/1	1/0	1/0	x	x	x	0/0	0/0
Agnes Island	26/12	20/7	31/14	39/14	46/16	42/14	37/10	47/16	x	48/15	52/17
Little Smith I.	15/3	12/5	11/8	15/6	17/8	14/6	11/6	14/6	x	12/6	19/8
Big Smith Island	29/6	22/7	27/5	19/5	32/6	28/5	15/3	21/6	x	23/5	27/5
Seal Island	56/25	70/26	63/21	76/28	87/39	74/29	70/26	69/34	x	72/34	55/26
Applegate Rocks	73/29	130/43	75/26	126/57	129/33	159/54	157/36	147/52	x	177/53	185/48
Green Island	23/10	24/7	29/14	25/11	36/10	x	19/11	19/12	x	24/15	24/10
Channel Island	29/4	52/1	45/1	60/4	46/3	61/4	53/5	53/5	x	88/3	94/4
Little Green I.	5/3	x	30/5	58/8	55/9	54/5	34/6	55/9	x	62/5	12/2
Port Chalmers	44/5	58/19	69/12	86/19	91/27	40/8	43/15	94/28	x	85/20	29/13
Stockdale Harbor	13/1	15/0	14/0	17/0	16/0	1/0	5/0	14/0	x	24/0	8/0
Montague Point	10/1	17/1	13/2	19/1	14/1	18/1	13/1	20/1	x	14/1	12/2
Rocky Bay	12/5	0/0	18/3	19/5	25/7	22/7	14/3	23/6	x	27/8	25/2
Schooner Rocks	24/1	x	20/4	9/1	28/4	25/4	24/3	39/3	x	28/4	21/4
Canoe Passage	0/0	1/1	1/1	5/1	1/1	0/0	1/1	0/0	1/1	1/0	0/0

Appendix G. Repetitive counts of harbor seals on selected haulout sites in Prince William Sound, Alaska during September, 1989. An x indicates that no count was made at a site.

Site	Date (September)									
	3	4	7	8	9	11	12	13	15	16
Sheep Point	0	0	0	x	0	0	0	x	0	0
Gravina Island	13	9	x	x	x	x	12	x	11	54
Gravina Rocks	43	50	44	x	37	23	23	x	15	28
Olsen Bay	62	66	55	x	37	19	27	x	x	33
Porcupine	12	10	x	x	4	4	13	x	2	2
Fairmount	53	47	21	39	28	48	x	x	1	23
Payday	4	1	0	0	0	4	x	x	0	3
Olsen Island	9	2	10	12	13	11	x	x	0	0
Point Pellew	32	22	24	22	25	28	x	x	32	5
Little Axel Lind	11	21	25	27	25	26	x	x	23	25
Storey Island	0	0	4	5	0	1	x	x	4	10
Agnes Island	26	60	47	54	22	29	x	x	18	26
Little Smith I.	7	24	x	40	28	9	x	x	20	17
Big Smith Island	46	44	x	52	24	x	x	x	46	34
Seal Island	41	59	x	22	26	35	x	x	30	41
Applegate Rocks	x	x	x	61	103	96	x	x	x	72
Green Island	3	29	x	28	14	17	x	x	32	2
Channel Island	x	116	x	x	x	x	x	x	x	x
Little Green I.	x	13	x	x	x	35	x	x	x	47
Port Chalmers	x	x	x	56	32	67	x	x	74	78
Stockdale Harbor	x	63	x	52	57	47	x	x	29	15
Montague Point	32	48	x	47	23	x	x	39	40	27
Rocky Bay	19	19	x	12	11	7	x	9	4	7
Schooner Rocks	63	62	x	31	58	73	x	87	67	31
Canoe Passage	0	71	8	1	34	54	x	2	2	0

Appendix H. Repetitive counts of harbor seals on selected haul-haul sites in Prince William Sound, Alaska during August- September, 1990. An x indicates that no count was made at a site.

	Date (August-September)							
Site	28	29	30	31	1	4	7	11
Sheep Point	0	0	0	0	2	0	0	0
Gravina Island	4	0	3	3	3	13	11	3
Gravina Rocks	37	x	15	31	24	24	11	8
Olsen Bay	87	79	83	104	50	62	50	39
Porcupine	1	0	0	0	0	0	4	0
Fairmount	43	19	27	36	31	4	6	6
Payday	13	0	8	2	1	2	0	4
Olsen Island	12	7	14	15	3	0	15	17
Point Pellew	33	31	20	26	24	15	17	16
Little Axel Lind	15	14	15	17	10	8	19	23
Storey Island	0	0	10	4	1	0	5	0
Agnes Island	50	41	43	45	29	19	27	37
Little Smith I.	43	33	32	20	31	21	26	29
Big Smith Island	31	27	29	32	31	18	40	x
Seal Island	39	23	41	50	46	35	x	x
Applegate Rocks	151	109	98	104	122	110	x	113
Green Island	7	28	29	47	14	13	24	24
Channel Island	x	45	36	x	x	x	x	x
Little Green I.	x	15	21	32	27	x	x	46
Port Chalmers	x	79	131	x	119	x	95	96
Stockdale Harbor	39	52	57	48	59	39	42	55
Montague Point	29	49	40	46	27	17	33	48
Rocky Bay	7	16	18	11	13	1	9	10
Schooner Rocks	25	58	48	53	51	43	6	56
Canoe Passage	41	16	12	11	61	3	0	39

Appendix I. Repetitive counts of harbor seals on selected haulout sites in Prince William Sound, Alaska during August/September, 1991. An x indicates that no count was made at a site.

	Date (August/September)									
Site	22	23	24	26	27	28	29	30	31	01
Sheep Point	0	0	0	4	0	0	0	x	0	2
Gravina Island	5	5	19	28	11	11	11	2	18	21
Gravina Rocks	13	21	38	31	28	28	29	24	32	21
Olsen Bay	119	125	75	101	85	63	58	42	60	75
Porcupine	12	13	17	2	10	17	20	17	21	12
Fairmount	22	x	x	22	1	9	26	21	22	16
Payday	3	7	x	8	11	0	2	5	2	5
Olsen Island	0	0	x	11	15	15	14	15	16	5
Point Pellew	29	41	x	13	11	20	x	24	24	24
Little Axel Lind	12	x	x	6	10	12	8	10	10	15
Storey Island	0	0	x	0	0	0	2	0	2	0
Agnes Island	61	52	x	34	32	x	48	34	27	20
Little Smith I.	26	25	18	28	23	22	22	27	28	27
Big Smith Island	42	35	x	15	34	27	34	35	40	34
Seal Island	78	x	65	50	x	x	51	52	73	70
Applegate Rocks	169	x	94	88	92	95	98	145	115	56
Green Island	10	x	40	33	29	24	29	15	x	19
Channel Island	235	x	213	211	54	x	24	36	31	35
Little Green I.	26	x	17	0	2	6	6	32	x	34
Port Chalmers	75	x	96	98	75	129	152	x	139	104
Stockdale Harbor	32	x	57	45	50	51	x	43	44	53
Montague Point	32	x	27	24	34	28	27	30	27	20
Rocky Bay	26	x	25	25	26	18	28	13	25	1
Schooner Rocks	68	x	58	56	56	81	42	47	43	49
Canoe Passage	0	27	104	75	24	45	x	x	74	55