APPENDIX L

MARINE MAMMAL OIL SPILL RESPONSE GUIDELINES

MARINE MAMMAL HEALTH AND STRANDING RESPONSE PROGRAM

Marine Mammal Oil Spill Response Guidelines



U.S. Department of Commerce National Oceanic and Atmospheric Administration National Marine Fisheries Service Office of Protected Resources Marine Mammal Health and Stranding Response Program



MARINE MAMMAL HEALTH AND STRANDING RESPONSE PROGRAM

Marine Mammal Oil Spill Response Guidelines

Shawn Johnson, Michael Ziccardi

Wildlife Health Center, School of Veterinary Medicine, University of California, One Shields Ave., Davis, CA 95616

U.S. DEPARTMENT OF COMMERCE
Carlos M. Gutierrez, Secretary
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
Conrad C. Lautenbacher, Jr., Administrator
NATIONAL MARINE FISHERIES SERVICE
William T. Hogarth, Assistant Administrator for Fisheries

NOTICE

The National Marine Fisheries Service (NMFS) does not approve, recommend or endorse any proprietary product or material mentioned in this publication. No reference shall be made to NMFS, or to this publication furnished by NMFS, in any advertising or sales promotion which would indicate or imply that NMFS approves, recommends or endorses any proprietary product or proprietary material herein or which has as its purpose any intent to cause directly or indirectly the advertised product to be used or purchased because of NMFS publication.

Correct citation of this report is:

Johnson, S., and M. Ziccardi. 2006. Marine Mammal Oil Spill Response Guidelines. NOAA Technical Memorandum..

Acknowledgements

Development and publication of the *Marine Mammal Oil Spill Guidelines* was made possible with the financial support from NOAA Fisheries, National Marine Mammal Health and Stranding Response Program and the combined effort of many different interested and willing parties and organizations. The bulk of these procedures are based on protocols developed by participant organizations within California's Oiled Wildlife Care Network (OWCN) from years of marine mammal rehabilitation and oil spill response experience. These guidelines could not have been written without the previous contribution to the field of marine mammal oil spill response by Pamela Yochem (Hubbs-SeaWorld Research Institute, San Diego, CA) and Marty Haulena (The Marine Mammal Center, Sausalito, CA). Additions and modifications to this document should be made regularly as advances in oil spill response techniques are made. Any suggestions for additional material or comments on methods included in this document are welcome.

Many individuals and organizations helped make this publication possible through the generous contributions of their time and effort and we offer our sincere thanks to everyone involved.

Table of Contents

Introdu	uctio	on	1
Organizational Structure			4
Stranding Network and Facility Requirements			8
Data C	Colle	ction	11
Safety a	and	Human Health	13
Wildlife Recovery and Transportation			16
Intake	Pro	cedures	20
Animal	1 Wa	ashing and Continued Care	24
Dispos			28
Record			32
Referen			33
Appen	aice	:s	37
1.		Search Effort Log	
2.		LIVE Marine Mammal Data Log Form	
21	b.	LIVE Marine Mammal Data Log Form, page 2	
3.		DEAD Marine Mammal Data Log Form	
31	b.	DEAD Marine Mammal Data Log Form, page 2	
4.	•	Oiled Marine Mammal Intake Form	
5.		Oiled Marine Mammal Daily Progress Form	
6.		Oiled Marine Mammal Evidence Collection Proto	col
7.		Petroleum Hydrocarbon Tissue Sampling Protoco	ıl
8.		Oil Spill Response Laboratories and Supplies	
9.		Oiled Marine Mammal Necropsy Form	
10	0.	Chain of Custody Form	
10	0 b .	Chain of Custody Form, page 2	

Internet Resources

NOAA Fisheries, Marine Mammal Health and Stranding Response Program http://www.nmfs.noaa.gov/pr/health/

NOAA, Damage Assessment and Restoration Program (DARP): http://www.darp.noaa.gov/

U.S. Coast Guard Oil Spill Response: http://www.uscg.mil/hq/g-m/nmc/response/index.htm

U.S. Coast Guard Area Contingency Plans: http://www.uscg.mil/vrp/acp/acp.shtml

U.S. National Response Team: http://www.nrt.org/

Wildlife Health Center, UC Davis: http://www.vetmed.ucdavis.edu/whc/

Oiled Wildlife Care Network: http://www.vetmed.ucdavis.edu/owcn/

Introduction

Marine Mammals and Oil: A Brief Overview

In comparison to marine birds, marine mammals are infrequently affected by oil spill incidents. The number of individuals and species affected, as well as the degree of pathological impact of such exposure, will depend on many variables, such as the location and size of the spill, the characteristics of the oil, weather and water conditions, types of habitats affected, the time of year the spill occurs, as well as the behavior and physiology of the marine mammal. Information on the effects of oil on marine mammals is sparse, and is mostly a result of the *Exon Valdez* oil spill in Alaska in 1989 and a limited number of exposure experiments on a narrow range of species exposed to relatively low doses of oil (Geraci and St. Aubin, 1990).

The sensitivity of marine mammals to spilled oil is highly variable and appears to be most directly related to the relative importance of fur and blubber to thermoregulation. In those species with relatively sparse fat stores, direct contact with oil impairs the thermal insulative value of fur thus resulting in hypothermia. External exposure can also result in dermal injury and conjunctivitis. Internal exposure of oil by ingestion (either by direct ingestion or indirect through food and water sources) can result in gastrointestinal ulcers and liver and kidney damage. Inhalation of volatile hydrocarbons can result in central nervous system and pulmonary damage and behavioral abnormalities. Depending upon the extent of external exposure, the toxicity of the petroleum product, the volume ingested or inhaled, the presenting clinical signs, and the species affected, some marine mammals exposed to oil may not need rehabilitation. Oil spill responders must consider that such procedures involving capturing, holding, treating, and releasing the wild animals places stress on the animal, and the consequences of capture and captivity may be a greater risk to its well being than contacting oil. Exceptions may include abandoned or moribund young pups of any species and species that rely on fur for thermal insulation. These animals will most likely require rehabilitation when oiled due to the physical and toxicological effects of petroleum exposure.

Pathological Effects of Petroleum Exposure

Documented clinical and histopathological effects of oil in pinnipeds and sea otters include ambulatory restrictions, thermoregulatory imbalance, central nervous system depression, interstitial pulmonary emphysema, aspiration pneumonia, anemia, conjunctivitis and corneal edema, gastrointestinal irritation, and hepatic and renal tubular necrosis/lipiosis, and adrenal gland dysfunction (Davis and Anderson, 1976; Geraci and Smith, 1976; Engelhardt et al., 1977; Engelhardt, 1985; Geraci and St. Aubin, 1988; Geraci and Williams, 1990; St. Aubin, 1990; Lipscomb et al., 1993). Small laboratory studies on the effects of oil have been conducted on ringed and harp seals (Smith and Geraci, 1975; Geraci and Smith, 1976); however most studies have been unable to correlate the degree of oiling with the type of effect and many of these lesions may be related to captivity stress or other underlying factors. Changes in acute phase proteins and cytokines (e.g. elevated IL-6, haptoglobin and creatine kinase) have been correlated with probable petroleum exposure in river otters (Duffy et al., 1993; Duffy et al., 1994). Oiled sea otters displayed evidence of hepatic and renal dysfunction as well as anemia in their blood parameters (Williams et al., 1995).

Heavy oiling did not appear to interfere with seal locomotion during the Exxon Valdez oil spill (Lowry et al., 1994), but in previous spills seal pups encased in oil have drowned due to their inability to swim (Davis and Anderson, 1976). During Exxon Valdez, harbor seals were observed exhibiting abnormally tame or lethargic behavior. These observations are most likely explained by midbrain nerve damage found in oiled harbor seals and Steller sea lions (Spraker et al., 1994). In addition to the acute mortalities associated with the loss of thermoregulation and buoyancy, many physiological and behavioral problems have been attributed to internal exposure to petroleum and polycyclic aromatic hydrocarbon (PAH) compounds in sea otters. However, many of these conditions have been difficult to differentiate from lesions attributed to, or compounded by, shock and chronic stress associated with capture and the rehabilitation process (Williams and Davis, 1995). It has become clear that animals captured during oil spill responses undergo additional stressors that may or may not be offset by the medical care they receive.

Background

The purpose of the Marine Mammal Oil Spill Response Guidelines (Guidelines) is to provide a foundation for coordination and communication between the National Marine Mammal Health and Stranding Response Program participants and other state and federal governmental agencies involved in oil spill response and marine mammal conservation and protection. The National Oceanic and Atmospheric Administration (NOAA) Fisheries, Office of Protected Resources, Marine Mammal Health and Stranding Response Program (MMHSRP) enlisted the University of California (UC) Davis, Wildlife Health Center to assist in the development of these Guidelines with input and assistance from NOAA's National Ocean Service, Office of Protected Resources, Damage Assessment and Restoration Program (DARP) and NOAA Fisheries, Office of Law Enforcement (OLE). The UC Davis, Wildlife Health Center, through its Oiled Wildlife Care Network (OWCN) program is among the world's leading experts on oiled wildlife response methods and standards. The primary purpose of the document is to: outline appropriate standardized data collection techniques for response activities and damage assessment; define chain-of-custody protocols for animal collection, necropsy and sampling; provide recommendations for protection of human health and oil spill safety training for responders; and present guidelines for best achievable care of oiled marine mammals. Standardization of this information between and among oiled marine mammal responders should allow for more accurate collection of data for analysis, which then may yield better information on the effects of oil on marine mammals and further improvements in oil spill response involving marine mammals. These Guidelines by their design do not address overall marine mammal husbandry methods in detail, but are intended to provide basic information on oil spill specific issues (such as search and collection, transport, emergency care and stabilization), and procedures specific to oil spill response. For more information on general marine mammal rescue and rehabilitation, the reader should consult references such as Marine Mammals Ashore (Geraci and Lounsbury, 1993) and the CRC Handbook of Marine Mammal Medicine (Dierauf and Gulland, 2001).

Intended Uses

These Guidelines are intended for use by the NOAA Fisheries MMHSRP, other natural resource management agencies, marine mammal stranding networks and rehabilitators, On-Site Coordinators, and Potentially Responsible Parties (PRPs) as a guide in:

- Developing appropriate sections of Area Contingency Plans (ACPs)
- Stimulating communication and documentation coordination between interested parties
- Caring for oiled marine mammals
- Evaluating marine mammal rehabilitation center capabilities for oil spill response
- Collecting evidence for assessment of impacts on marine mammals
- Making informed choices during spill responses

Responses to spills impacting marine mammal will depend upon factors including the size of the spill, species involved, type of product spilled, time of year, and location. It is important that spill responders and pre-spill planners recognize that the variability in degree of effort and complexity in marine mammal response can be significant when comparing small and large events.

This document is not intended for use as a training manual. Nor is this document an exhaustive list of techniques in this field, in which practical knowledge is being continuously refined and developed. It is to serve as guidance for acquiring the best achievable care and data collection during an oil spill response and should be periodically reviewed and updated.

Organizational Structure

Organizational Structure of Wildlife Response

Actions taken to protect wildlife resources follow an organized and agreed-upon cascade of agency notifications and activities. All activities of the oil spill response are coordinated through the Unified Command (UC) and follow an Incident Command System (ICS) structure as standardized by the National Interagency Incident Management System (NIIMS) and modified for oil and hazardous substance spill response by the National Response Team (Figure 1., NRT 2004). The UC is the governing body ultimately responsible for all decision making processes during the spill response, and is made up of a Federal On-Scene Coordinator (FOSC) (usually a Coast Guard Captain of the Port for the affected area), a State Incident Commander (IC) or On-Scene Coordinator (SOSC), and a qualified individual from the Responsible Party (RP), if known. When appropriate, local government representatives can be included in the UC. The FOSC has the ultimate responsibility for directing the oil spill response if a consensus cannot be reached among the members of the UC. Wildlife response activities usually exist within the Operations Section of the ICS, though some wildlife actions (primarily baseline assessment and planning) also occur with the Environmental Unit of the Planning Section. The Wildlife Branch within the Operations Section coordinates and initiates wildlife response activities. Guidance for dealing with oiled wildlife is not specifically provided in the National Contingency Plan, therefore the Wildlife Branch operational plan is developed uniquely within each Regional and Area Contingency Plan based on the specific resources and agency involvement.

Early but prudent initiation of a wildlife response plan and the previous development of the Wildlife Branch ensure timely mobilization of dedicated staff, equipment, and volunteers. This structure allows for effective lines of communication, making the response effort much more efficient. The degree of the wildlife response effort is designed to be flexible and scalable to the size of the oil spill - only those positions necessary and appropriate for a specific spill incident are filled.

Trustee Organizations

Under federal statutes, NOAA Fisheries, National Marine Fisheries Service (NMFS) has responsibility for managing and protecting all cetaceans and pinnipeds in U.S. waters, except walruses; U.S. Fish and Wildlife Service (FWS) has responsibility for managing and protecting manatees, walruses, sea otters, and polar bears. NOAA Fisheries is responsible for the administration of the Endangered Species Act (ESA) as it applies to certain cetaceans and pinnipeds and the FWS is responsible for the administration of the ESA as it applies to remaining marine mammals and terrestrial mammal and bird species. Following an oil spill, specific information on wildlife resources at risk and appropriate wildlife response actions are made available to the Federal On-Site Coordinator (FOSC) and other members of the Unified Command (UC) through representatives of appropriate wildlife resource managers. Therefore, the UC must immediately consult with FWS or NMFS whenever a response may affect these resources. The Marine Mammal Protection Act (MMPA) prohibits the "take" of sea otters, seals, sea lions, walruses, whales, dolphins, and porpoises, which includes harassing or disturbing these animals as well as actual harming or killing; however, Section 109(h) of the MMPA allows take by

Federal, State, or local governmental officials, during their official duties, provided the take is for the welfare and protection of the animal or public health. Accordingly, the FOSC/UC is authorized to take marine mammals during an oil-spill response if to protect the welfare of the animal. Section 12(c) of the MMPA allows NMFS to enter into cooperative agreements (e.g. Stranding Agreements) that allow stranding network participants marine mammal take in order to carry out the purposes of the MMPA. The ESA and its implementing regulations provide special provisions for consultations during emergencies (such as oil spills) with FWS and/or NMFS for making recommendations to the FOSC to avoid the taking of listed species or to otherwise reduce response-related impacts. In some State statutes, management and protection of wildlife resources are joint responsibilities between NMFS, FWS and the State. Because of these shared trust responsibilities, both federal and state agencies are required to respond to spills, or potential spills, that may impact marine mammals. To facilitate efficient and effective coordination during an oil spill response, federal and state agencies may consider developing Memorandums of Agreement (MOA's) or Memorandums of Understanding (MOU's) that pre-designate regional primary points of contact, establish lead representatives, and define roles for natural resource emergency situations.

In the wake of the Exxon Valdez spill, Congress passed the Oil Pollution Act of 1990 (OPA 90). OPA 90 sets forth an extensive liability scheme that is designed to ensure that, in the event of a spill or release of oil or other hazardous substance, the responsible parties are liable for the removal costs and damages that result from the incident. A responsible party may be liable for removal costs and damages to natural resources, real or personal property, subsistence use, revenues, profits and earning capacity, and public services. OPA 90 also set aside a significant trust fund that can be utilized quickly to implement a spill response prior to establishment of liability.

OPA 90 directs the appointed trustees to conduct natural resource damage assessments (NRDAs) and develop and implement plans to restore, rehabilitate, or replace damaged natural resources. Authority to claim damages to natural resources also stems from Clean Water Act (CWA) and Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Under the CWA, federal and state agencies with diverse jurisdictions and missions are directed to combine their response and planning efforts in the event of an oil spill or release of another hazardous substance under the aegis of a National Contingency Plan (NPC) or an Area Contingency Plan (ACP). An Area Contingency Plan must provide for efficient, coordinated, and effective action to minimize damage from oil and hazardous substance discharges. In so doing, an ACP assigns duties and responsibilities to various federal and state agencies, provides for maintenance of necessary equipment and supplies, and establishes Coast Guard strike teams with specialized training in oil and hazardous substance control. In addition, an ACP is designed to provide for surveillance and notification systems to detect oil spills as early as possible. Further, an Area Contingency Plan is to provide for a specific fish and wildlife response plan, developed with the advice of expert agencies, to minimize disruptions to fish and wildlife and their habitat. Regional and Area Contingency Plans can be located at the U.S. National Response Team website (www.nrt.org) and the USCG website: (http://www.uscg.mil/vrp/acp/acp.shtml).

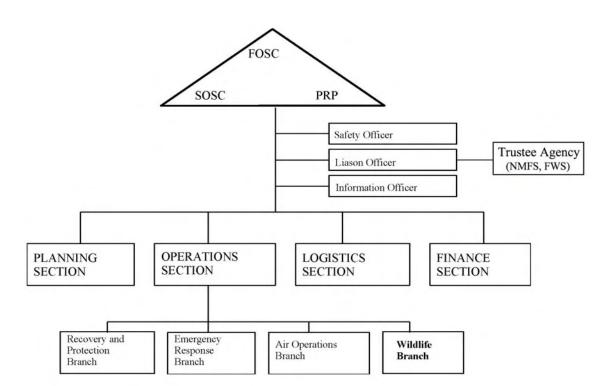


Figure 1: Incident Command Structure for Oil Spill Response (NRT 2004)

Once the FOSC activates the Wildlife Branch, several components of oiled wildlife response can be initiated, including reconnaissance to determine species and areas to focus operations, hazing of animals to prevent oiling, search and collection for live and dead animals in the spill area, treatment and rehabilitation of oiled animals, and release and monitoring of recovered animals. The agencies, organizations, and individuals responsible for these functions should be outlined in the Area Contingency Plan. An example of Wildlife Branch organization is shown in Figure 2.

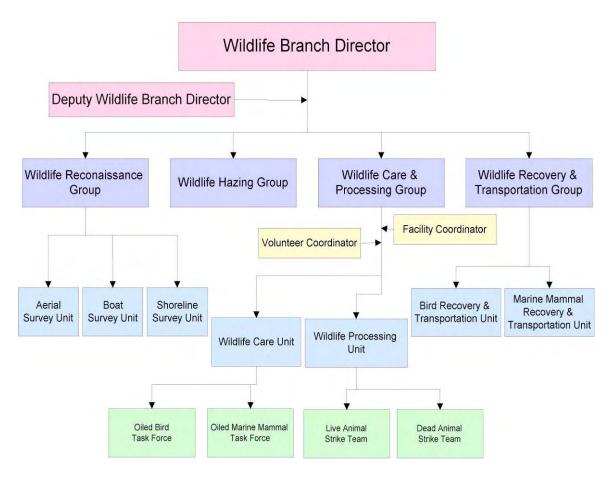


Figure 2: Wildlife Branch Organization (State of California, Wildlife Response Plan, 2004)

Under the direction of the Wildlife Branch Director (WBD), the principal objectives of Wildlife Operations during spill response and cleanup are to:

- Provide the best achievable care to impacted and/or threatened wildlife
- Document for the Unified Command the immediate impacts of the oil spill to wildlife
- Minimize injuries to wildlife
- Protect wildlife and habitats from adverse effects of wildlife recovery

To ensure these objectives are achieved with maximum efficiency, the WBD (in coordination with the Environmental Unit) manages the activities of the federal, state, and local agencies along with commercial and non-profit organizations responsible for wildlife protection and management who fall under the authority of the Unified Command during spill response

Stranding Network and Facility Requirements

Wildlife Operation plans should include (where available and appropriate) properly trained regional Stranding Network Participants because of their experience with live animal stranding response and rehabilitation for the local area. In order for Stranding Network Participants to contribute during wildlife response, they must hold a Stranding Agreement or Letter of Authorization (MMPA, Section 112(c)) with NMFS/FWS and have received specific oil spill training and meet facility requirements for oiled marine mammal rehabilitation. NOAA Fisheries, Office of Protected Resources, may include oil spill response authorization in the Stranding Agreement with the Participant when it is determined that the Stranding Network Participant meets these criteria. Authorized marine mammal rehabilitation organizations should make efforts to become engaged in the development of their Area Contingency Plans to ensure their involvement during oil spill response.

Criteria for Evaluating Marine Mammal Rehabilitation Groups

The following criteria can be used when considering and evaluating marine mammal rehabilitators for conducting oil spill response.

- Holds all necessary permits, Stranding Agreements (NMFS) and Letter of Authorizations (FWS) for marine mammal stranding and response activities.
- Experience in the capture, treatment, and care of oiled marine mammals
- Knowledge of conducting marine mammal response activities within an Incident Command System structure including appropriate communication and notification procedures
- Sufficiently trained (health/safety and animal care), equipped, and experienced supervisory staff
- Ability to train and equip personnel and volunteers for marine mammal response during an emergency oil spill response
- Ability to quickly mobilize to perform marine mammal capture, field evaluation, stabilization and transport (including to remote locations if necessary)
- Access to appropriate facilities for treating and housing oiled marine mammals (including adequate animal care, hazardous waste, and personnel infrastructure)
 - O Ability to establish and operate marine mammal intake, holding, and isolation areas within 12-24 hours of wildlife response activation.

- O Ability to establish and operate marine mammal cleaning and pre-release areas within 72 hours of wildlife response activation.
- Agreement with a licensed veterinarian experienced in the treatment of oiled marine mammals to provide necessary medical care
- Use of best practices as outlined in the remainder of this document

Facility Requirements for Marine Mammal Oil Spill Rehabilitation

General Considerations

The size of the spill, its location, and the number and species of animals oiled will help determine the type and location of a facility that can meet the required need. Not all spill responses will be in the vicinity of a permanent rehabilitation facility. Temporary facilities that can care for oiled marine mammals in the short or long-term can be established in local, fixed structures, or mobile units can be brought to a spill location to set up as a temporary facility. However, it is critical that spill responders and pre-spill planners recognize the degree of effort, the unique requirements of oiled wildlife care and the complexity required to implement and establish an adequate facility. Pre-spill planning is strongly encouraged to achieve wildlife response systems that will adequately address the needs of small as well as large rescue efforts as rapidly as possible during a spill.

There are published standards for the design of facilities housing marine mammals in captivity. In the United States, these standards are published by the Department of Agriculture, Animal and Plant Health Inspection Service (APHIS, www.aphis.usda.gov/ac/cfr/9cfr3.html) and are a requirement for facilities that wish to display animals to the public. They include such items as haul-out requirements, pool size and depth, water quality, number of animals to be kept in a particular environment, and strict standards for food preparation areas and medications. The USDA standards are useful guidelines but may not be appropriate for animals that require constant medical attention and handling, or for facilities that only keep animals for a short period of time. NMFS is in the process of developing specific marine mammal rehabilitation facility guidelines (NMFS/FWS Best Practices for Marine Mammal Stranding Response, Rehabilitation, and Release: Pinniped and Cetacean Rehabilitation Facility Guidelines).

Facility design for rehabilitation centers is an ongoing area of study and no perfect facilities exist to suit all needs for each species and age class of marine mammal. Notwithstanding, certain principles should be kept in mind when designing an oil spill response facility or when attempting to house oiled marine mammals in an existing facility (Davis and Davis, 1995). An ideal facility should include: intake/physical exam/evidence processing area; a veterinary hospital with isolation capabilities; indoor wildlife housing/caging areas; food storage and preparation facilities; animal washing and rinsing areas; drying areas; outdoor pool and pen areas; pathology facilities; volunteer training and eating areas (with restrooms); administrative offices with multiple phone/fax lines and conference space; storage; and access to a large parking area.

Minimizing stressors is an important aspect of creating a good rehabilitation environment. Specific animal needs must be taken into account when trying to provide adequate housing for animals during an oil spill. These needs may be affected by such factors as the animal's species, age, physical condition, degree of oiling, and nature of the product with which it was oiled.

Housing Requirements and Considerations

Indoor and outdoor housing should maximize safety to humans and the animals, provide an escape-proof enclosure, and minimize visual stress and human traffic. Within an oil spill response facility, housing should be set up so that there are appropriate areas for holding animals prior to intake, pre-wash assessment and stabilization, post-wash, quarantine, and longer term housing. These areas will differ in the amount of access to the animals that is required, the space that each animal requires, the degree to which the environmental temperature can be controlled, and type (if any) of water requirements (fresh versus salt). Ideally, all of these areas should have separate filtration systems. Separate systems are required for pre- and post-wash animals to prevent oil contamination of animals that have already been washed.

Environmental Control: A finer degree of environmental temperature control is required for newly admitted animals, neonates, and animals that are more compromised due to poor nutritional state, greater extent of secondary effects, or underlying disease. Animals that are compromised require easy or limited access to water, haul-out areas, and heat sources such as heating pads and lamps, but may need frequent observation to ensure that severely debilitated animals are able to move away from heat sources to prevent hyperthermia and burns. Some animals may require more frequent handling for monitoring, sample collection, feeding or medicating. Housing should minimize stress but maximize accessibility and ease of monitoring (Tuomi et al., 1995)

Ventilation: Adequate ventilation is an extremely important factor for maintaining marine mammals in captivity and is more important in oil spill situations to protect against the toxic effects of volatile agents and prevent the spread of infectious agents between animals. Ten to fifteen air changes per hour has been recommended as adequate for inside animal holding (NIH, 1985) and these standards should be adhered to if at all possible. Outdoor housing is ideal for maintaining ventilation but drawbacks include lack of environmental control, discomfort for personnel working with the animals, and more challenging access control by staff.

Quarantine: The potential for the spread of disease is an important issue to consider for marine mammals in captivity. Captured animals, staff and volunteers may carry infectious agents without showing signs of disease and could pose a threat to oiled animals. Staff should use effective quarantine protocols including foot baths containing appropriate antimicrobial solutions between housing areas, cleaning/disinfecting or changing protective clothing between animals, designating separate feeding and cleaning equipment for different areas, and minimizing movement of animals and personnel between areas. Extra care must be taken in areas where animals with infectious diseases are kept and when handling immunocompromised animals.

Water Supply: Oiled wildlife care facilities require large quantities of water to provide all areas simultaneously (e.g., wash/rinse area, pool area, laundry). The quantity should be sufficient to provide at a minimum a continuous flow of 4 gallons/minute to all indoor valves and additional supply to fill, operate filtration and ozonation equipment, and provide overflows for pools. Washing and rinsing areas require temperature-controlled hot water (98-108°F) with water hardness of 2-5 grains per gallon at pressure of 40-60 psi.

Waste Water: Facilities must dispose of all oil and animal wastewater in accordance with appropriate Federal, State, and municipal regulations. Oil contaminated water often must be contained in separate holding tanks and not released in normal sewer system.

Data Collection

Data Collection and Chain-of-Custody Procedures

Systematic search and recovery, transportation, processing, and treatment of all oil-affected wildlife are critical for guiding response actions and gaining an understanding of the short-term and long-term consequences of oil spills to wildlife populations. In addition, these data can be used after the emergency response for natural resource damage assessment activities. In order to track the samples and collect data during oiled wildlife response, the trustee agencies and response organizations must adhere to pre-established chain-of-custody and animal identification procedures. For tracking purposes, data on oiled animals are compiled on standard data log forms (Appendix 2-3). During large-scale responses, pre-identified wildlife agency personnel or their agents will complete log forms; however, field and rehabilitation responders should be familiar with the forms and their completion for smaller-scale responses and for individual oiled animals that present to participating facilities independent of a spill response. In addition to the tracking of live animal data, all samples (carcasses, samples, photos, records) that may be used in legal cases must be tracked and secured at all times.

Quality assurance (QA) procedures are necessary to ensure that data are collected in a scientifically valid manner. It is important throughout any sampling and analysis program to maintain integrity of the sample from the time of collection, through the point of data reporting, to the final sample disposition. Proper chain-of-custody procedures allow the possession and handling of samples traced from collection to final disposition. Documents needed to maintain proper chain-of-custody include:

Field Logbook: All pertinent information on field activities and sampling efforts should be recorded in a field logbook. The logbook should enable someone else to completely reconstruct the field activity without relying on the memory of the field crew. All entries should be made in indelible ink (preferably ballpoint), with each page signed and dated by the author, and a line drawn through the remainder of any page. All corrections should consist of permanent line-out deletions that are initialed. An example of a Search Effort Log is presented in Appendix 1. For tracking and chain-of-custody purposes, all live and dead animals recovered should be identified (tagged/marked) in the field and the identification noted on the Search Effort Log. Permanent tags will then be applied and logged at the processing facility.

Animal Logs: At admittance to a wildlife care and processing facility, the animal must be logged into the Live Marine Mammal Data Log or Dead Marine Mammal Data Log (Appendix 2-3) and all of the boxes on these forms must be completed. All animals collected dead or alive should be given a unique log number and identifier (e.g. tag), as well as a Level A data field number, in order to track the individual animals through the capture/collection, processing, and for live animals the rehabilitation and release process.

Sample Collection and Label: It is necessary to collect an oil sample from each individual animal. A detailed protocol for the collection of evidence is provided in Appendix 6. Each sample must be identified with a waterproof label that is securely attached to the outside of each sample container. Labels must contain the oil spill name, date, species, intake log number and Level A data field number of that animal, animal capture location, and flipper tag color and number and then sealed with evidence tape or custody seals. Custody seals are used to detect unauthorized tampering with the samples. Samples and photo must be properly stored in a secure location that has limited and controlled access.

Intake Form: For live animals, the Oiled Marine Mammal Intake Form (Appendix 4) must be completed for each animal. This form contains important questions about the extent of oiling, location and depth of oiling, as well as a place for documenting physical examination findings. For evidence documentation, a photo of the animal and oil sample must be taken during intake and admission into the wildlife care and processing centers (see Intake and Admission Procedures). During rehabilitation, each animal must have individual records documenting the treatment and care of that animal. Authorization for cleaning and later release must be documented on the Oiled Marine Mammal Intake Form and signed by the authorizing authority (i.e. attending veterinarian). For resource damage assessment purposes, a photo of the animal with identification (i.e. card with animal log number and date) must be taken prior to release.

Chain-of-Custody Forms: A chain-of-custody record must accompany every sample that is removed from the secured location in the wildlife processing and care facilities. The chain-of-custody form should be supplied by the managing agency (NMFS, USFWS) representative that is acquiring the sample. Both the person relinquishing custody of the sample(s) and the person receiving the sample(s) must sign the form and ensure that the samples and records are not left unattended unless secured properly. An example chain of custody form can be found in Appendix 10.

Tissue Sampling: Tissue samples are collected for either chemical or histological analysis. Only after authority is given by the appropriate trustee agency and the Unified Command can necropsies be performed by qualified veterinarians and pathologists to collect tissue samples and determine cause of death on collected carcasses and mortalities that occurred during rehabilitation. Each animal should be photographed prior to sampling and samples collected following the sample collection protocols described in Appendix 6.

Safety and Human Health

Worker health and safety are of primary importance in any oiled marine mammal rescue and rehabilitation effort. The earliest phases of an oil spill are generally the most hazardous to human health and safety. Thus, safe practices during field collection of marine mammals must be a priority. Rescue programs should not be initiated unless personnel can conduct activities safely.

As with all spill response activities, the marine mammal rescue and rehabilitation effort needs to be coordinated and monitored by the spill response command center operations, safety, and medical staffs. A written Site Safety Plan (SSP) must be developed and approved by the spill's Safety Officer for the rehabilitation facility. If field activities are on-going for marine mammal response, the site safety plan needs to be expanded to include these activities including any specialized equipment that will be used. All staff and volunteers working on the spill must be familiar with and sign the SSP prior to work.

Training for Marine Mammal Rescue/Rehabilitation Personnel

In addition to mastering specific marine mammal rescue and rehabilitation tasks, personnel must be trained to recognize and minimize risk of injuries from oil-related and physical hazards associated with oil spill response operations prior to being allowed to participate in on-site activities. Elements of required and recommended training will vary depending on the tasks of the individuals involved in the response. Training-hour requirements and specific courses vary with level of involvement, agency policy, and OSHA and state regulations.

Required Training

Personnel involved in oil spill response activities must comply with all applicable worker health and safety laws and regulations. The primary Federal regulations are the Occupational Safety and Health Administration (OSHA) standards for Hazardous Waste Operations and Emergency Response (HAZWOPER) published by the U.S. Department of Labor in Title 29 of the Code of Federal Regulations (CFR), section 1910.120 (<u>www.osha.gov</u>). Oiled marine mammal responders and rehabilitation centers are not specifically addressed by HAZWOPER and training to address risks associated with marine mammal stranding and oil spill response personnel may fall within the scope and application of the Hazard Communication Standard ("HAZCOM", 29 CFR 1910.1200(h)). The OSHA field compliance or Safety Officer should be contacted to ascertain the worker training requirements and develop an implementation plan to minimize the hazards of exposure to workers involved in cleanup operations. For maximum protection of the environment, OSHA has recognized the need to quickly clean-up spilled oil and has empowered the OSHA Regional Response Team representative to reduce the training requirements for responders engaged in post-emergency response operations as directed by OSHA Instructions CPL 2-2.51 (www.osha.gov). State requirements which are more restrictive will preempt Federal requirements. Marine mammal stranding network participants are responsible for training and certifying their employees and volunteers.

Recommended Training

In addition to the training required by Federal regulations, further training is highly recommended for safe and efficient operations during a spill response. This guidance is considered a minimum

essential training for marine mammal rehabilitators in accordance with the goal of establishing best practices.

Search and collection and transport personnel

- General oil spill response training
- HAZWOPER 24hr training
- Aircraft/boating/ all-terrain vehicle safety
- First aid/CPR
- Local geographical knowledge
- Marine mammal identification and capture techniques

Rehabilitation Facility Management

- Marine mammal oil spill response training
- Incident Command System
- HAZWOPER 24hr training
- Crisis management
- First aid / CPR
- Media relations

Rehabilitation/Stranding Network Facility Workers and Volunteers (Live and Dead Animal Handling)

- General oiled marine mammal training
- HAZCOM Hazardous Communication training
- First aid / CPR

Personal Protective Equipment

Personal protective equipment (PPE) must be used to protect wildlife response personnel from exposure to hazardous substances and dangers associated with animal care activities. To guard against injury from marine mammals, all workers should wear approved personal protective equipment appropriate to their task.

Recommended PPE

Full eye protection, i.e., goggles, safety glasses, or face shield

Oil resistant rain gear or oil protective clothing (coated Tyvek, Saranex, etc.)

Gloves (neoprene or nitrile) that are oil resistant and waterproof

Non-skid shoes/boots that are oil resistant and waterproof

Ear protection (muff or ear plug type) when using pyrotechnic devices or operating machinery Personal flotation device when working on or near water

Respiratory protection from organic vapor hazards may also be required for some operations. If respirators are used, training and fit testing are required. All workers must be trained on the proper use and limitations of all personal protective equipment prior to using the equipment.

Hazardous Substances

Rescue and rehabilitation workers may be exposed to spilled oil, and must be so informed. Prior to handling a contaminated marine mammal, the Material Safety Data Sheet (MSDS) for the

spilled material should be reviewed and all recommended precautions followed. Workers and the rehabilitation facility shall be periodically monitored, using calibrated instruments and devices to determine exposure. Ventilation in all work areas should prevent the buildup of airborne contaminants.

A portion of the rehabilitation facility should be designated for the storage of contaminated clothing, equipment, and medical waste until the items can be decontaminated or disposed of properly in accordance with the site safety plan.

Volunteers

Wildlife response programs regularly use volunteers, particularly at the rehabilitation facility. Wildlife response managers need to ensure that volunteers are appropriately trained, supervised, and informed of all hazards. A comprehensive volunteer management program is an essential component of an efficient wildlife response. This management program needs to address, at a minimum, volunteer safety, training, supervision, scheduling, and liability.

Wildlife Recovery and Transportation

Agency Oversight

Wildlife Recovery and Transportation involves the collection/capture of dead and live oiled wildlife and their transport to processing centers. Under the proposed ICS Wildlife Operation structure presented in Figure 2, these activities are performed by the Wildlife Recovery and Transportation Group, in close coordination with the UC and the state and federal trustee agencies. Marine mammal collection by any agency or organization must be done under the direction of the UC and under the agreements/permits from the appropriate management agencies (i.e., NMFS, FWS). Recovery and Transportation usually include personnel from state and federal trustee agencies, approved contractors, and marine mammal stranding network and rehabilitation organizations. Trained, qualified volunteers can be used utilized as long as OSHA and other training requirements are met and adhered to.

Search and Collection Guidelines

Rescue Team: Teamwork is essential to safe, efficient collection of oiled marine mammals. Each team should consist of at least two people, and should be outfitted with the resources and equipment necessary to complete its assignment. A plan of action should be developed and discussed among all search and collection personnel and approved by the Wildlife Branch Director prior to entering the search area. Each capture site should be evaluated and strategies developed to suit the terrain and species involved. Capture of affected animals should not be attempted if adverse weather, sea conditions, cliffs, or other physical and chemical hazards in the "hot zone" are present. Communication between the Rescue and Transportation Group and the reconnaissance personnel (within the Operation Section or the Environmental Unit) is important to maximize the success of search effort.

Equipment: Prior to a response, ensure that all equipment is ready and in working condition. Capture materials should include communication equipment (portable phone or radio), specialized vehicles (4-wheel drive with lifting tailgate or crane, adequate floor space, easily cleaned, and good ventilation), boats (capture vessel and support vessel), aircraft (fixed wing or helicopter), SCUBA gear, nets (type varies by species and location of capture), cages and transport boxes (type varies by species), herding boards, personal protection equipment (PPE) and a first aid kit for humans. Any injuries to staff or volunteers should be treated immediately and reported to the site safety officer. In addition to PPE required by the Safety Officer to protect personnel from oil exposure, appropriate attire for capture teams includes closed-toed shoes or boots, long-sleeve shirts, long pants, rain gear, coveralls, and organizational identification (e.g., clothing labeled with insignia or logo).

Procedures: Record the details of the beach search effort on the appropriate Form (Search Effort Log, Appendix 1) and include data on the start and end of a search segment, observations of oiled animals, and detailed info on the stranding and/or collection (location of capture, GPS decimal degree coordinates, reason for capture). If oil or medical samples are collected from the animal prior to reaching the intake facility, make sure they are labeled properly with a unique field

identification number for each animal. For further details on oil sample collection consult Appendix 6, Evidence Collection Protocol.

Domestic animals should not be permitted near the capture location nor should they come into contact with marine mammals. Domestic animals should not be allowed in the transport vehicle, and if the vehicle has previously been used to transport domestic animals, it should be disinfected and cleaned prior to transporting marine mammals.

Capture: The potential benefits of capture must outweigh potential negative consequences. In general, no rescue should be initiated on free-swimming or beached pinnipeds in the vicinity of an oil spill unless the animal in question is in obvious distress. Also, no rescue should ever be initiated on free-swimming cetaceans in the vicinity of an oil spill, but a rescue should be attempted on a beached cetacean. A decision to capture should consider such factors as sex, age, reproductive state, and size of individual animal, and their location with respect to other marine mammals. Additionally, all captures must be approved by the appropriate trustee agency (NMFS, FWS) prior to initiation.

Capture and transportation of oiled mammals should be performed only by qualified personnel who have received the appropriate safety training as well as marine mammal handling and restraint training. Because recovery and transportation duties vary with each response and may involve more risk than other duties, the Safety Officer will communicate to the Wildlife Branch Director what level of training is appropriate for field response personnel; this training may include a 24-hour HAZWOPER training (Hazardous Waste Operations and Emergency Response), first aid/CPR, water safety, or boat safety courses (see Safety and Human Health).

The method of capture may vary according to species and situation. Captures should generally be considered for isolated individuals on beaches, spits, tide flats or other relatively flat surfaces, using herding boards and nets (brail, breakaway or steel frame pole). Less often, captures may be attempted from rock jetties, piers, docks or even in the water for severely debilitated animals. Long-handled dip nets, floating bag nets, and a net gun have all been used with some success. Depending on the species involved, aquatic captures may use tangle nets, float nets, or Wilson traps.

Unless specifically authorized by appropriate trustee agencies, no non-oiled animals will be collected during spill incidents. Preemptive captures to prevent the oiling of sensitive species may be considered only under dire circumstances at the direction of the UC and trustee agencies and when adequate transport and holding facilities exist. Beached cetaceans should not be pushed back out to sea without first being examined by a NMFS-approved marine mammal veterinarian and the action approved by the NMFS. Prior to being returned to the open ocean, cetaceans should be affixed with a NMFS approved tag or brand.

All wildlife captured during spill responses should if at all possible be retrieved and transported to the wildlife processing and care center(s), regardless of the status and condition (i.e. degree of decomposition, degree of oiling). In addition, all capture-related information (i.e. location, name of captor, GPS decimal degree coordinates, date, and time) must accompany the animal to the facility. The presence of such documentation must be verified when processing centers receive wildlife from the Wildlife Recovery and Transportation Group. All information necessary to

complete either the live or dead mammal log should be collected prior to the animal entering the rehabilitation process or storage respectively.

Transport Procedures

Prior to transport, field stabilization techniques may be used if it will be more than one or two hours until the animal reaches the rehabilitation facility. These techniques may involve assessing the animal for hypo- or hyperthermia and treating accordingly; administering oral electrolyte solution and subcutaneous fluids; removing large amounts of oil from the eyes and nares; and administering emergency medications (under the guidance of a veterinarian).

After capture and field stabilization, the oiled animal should be placed in a well-ventilated area on a stretcher or foam (for small cetaceans) or in a transport box, airline kennel, or cage (depending on pinniped species) for transport. Animals should be staged in a quiet, sheltered area or moved directly into the transport vehicle. The cage should be large enough to allow the animal to lie down in a comfortable position. Only one animal per transport cage is recommended for the safety of the animals and to prevent cross-contamination of oil. Females and their pups are most safely transported in separate cages, although they should be positioned so that they can hear, see, and smell each other. Pinnipeds less than 70 kg (145 lbs) can be transported in large airline sky kennels. Aluminum or other lightweight material is recommended to minimize weight of cages designed for larger animals. Each cage must be firmly tied or otherwise secured in the vehicle.

Sea otter transport kennels should be fitted with a raised bottom grate to avoid additional fur fouling. Shaved ice or any other form of fresh water ice (to combat dehydration) and chew toys (to combat tooth damage, e.g. plastic/rubber dental chews manufactured for large breed dogs) are usually provided for sea otters in transport kennels, but food should be offered if transport time is greater than four or five hours.

Animals must be monitored periodically on transports greater than one hour, as directed by a response veterinarian. In most cases, sedation during transport is not recommended. Critical cases (e.g., unstable, hypo- or hyperthermic animals) may require more frequent monitoring. Personnel transporting animals between the field and the rehabilitation center must maintain contact with their supervisor at all times so that departure and arrival times may be anticipated.

Hyperthermic animals may be sprayed gently with water, or ice cubes may be added to the top of the cage and allowed to drip onto the animal as it melts. In order to prevent inhalation and subsequent drowning by unconscious animals, do not allow water to accumulate in the bottom of transport cages. Hypothermic animals should be placed in a sheltered location out of the wind, although good ventilation must be maintained to prevent animals and humans from inhaling petroleum fumes. Keep in mind that oiled, stressed, or injured seals are not able to regulate their body temperature effectively, and their conditions can change within minutes. Animals are generally transported in either a pick-up truck or an enclosed van-type vehicle. Adequate ventilation must be maintained to protect both humans and animals from inhaling fumes emitted by freshly oiled animals. Unless hypothermia is observed or suspected, keep animals damp and cool. The preferred air temperature for pinniped transport is 50-68°F (10-20°C) but should not exceed 59°F (15°C) for sea otters (Geraci and Lounsbury, 1993; Benz and Britton, 1995). Fur seals or sea otters whose coats are oiled or saturated, neonates of all species, and animals with extensive wounds or severe emaciation may require higher temperatures compared to minimally oiled animals or non-oiled, stranded animals. Keep in mind that human comfort during transport

may not be synonymous with or sufficient for the temperature and ventilation needs of the transported marine mammals.

Beached Carcass Removal

Measures must be taken to ensure that dead animals are appropriately collected, identified, documented, and not disposed of until approved by the trustees. In addition, the prompt removal of disabled and dead oiled and unoiled animals from the environment can be critical to minimize the occurrence of secondary oiling, poisoning of predators and scavengers, and decreasing reidentification of carcasses on subsequent days. Since it is not feasible, reliable, or practical to attempt to discriminate between spill-related and non-spill-related casualties while conducting beach surveys, all carcasses must be collected. For example, scavenged carcasses, animals with dark plumage, wet carcasses, or carcasses with oil sheen or small amounts of oil that may be spill related are not always identifiable in the field as such. Because all carcasses found within a spill area are evidence, they must be handled according to established chain of custody protocols in accordance with spill incident-specific instructions (refer to the Data Collection section of this document). Each carcass must be labeled with the date, time, location, species (if known), and collector's name; taken to a designated morgue location; logged into the Dead Marine Mammal Log form and placed in a refrigerated unit until further processing can be accomplished. If a necropsy cannot be performed within 24hrs the carcass should be frozen (see Disposition Section for necropsy details).

Carcass removal, storage, and disposal expenses are considered a response activity cost that should be reimbursed to the Stranding Network Participant. It is the responsibility of the Participant to notify the Unified Command of current and future carcass storage and disposal expenses during the initial cost assessment of the response activity.

Intake Procedures

Initial Intake Procedures

While completing intake procedures, it is important to perform a thorough evaluation, collect all samples and data, be safe, and minimize the animal handling time. All personnel performing intake procedures should wear appropriate PPE including safety goggles, protective clothing, and nitrile gloves (or nitrile gloves inside leather gloves). It is best to work in teams of at least two (handler, examiner) or three (handler, examiner, recorder) in order to perform the intake in an efficient manner. For larger animals, more than one handler may be required. Physical restraint devices such as squeeze cages, otter restraint boxes, and stuff bags may be needed for larger pinnipeds and sea otters (Geraci and Lounsbury, 1993; Williams and Sawyer, 1995). Some animals (e.g., sea otters, adult sea lions) may require chemical restraint for safe handling and examination (Williams and Sawyer, 1995; Haulena and Heath, 2001).

Several different forms must be completed for every animal captured for rehabilitation during an oil spill. The animal must first be logged into a **Live Marine Mammal Data Log** (example in Appendix 2) and all of the boxes on that form must be completed. In addition, an **Oiled Marine Mammal Intake Form** (example in Appendix 4) must be completed for each animal. This form contains important questions about the extent of oiling, location and depth of oiling, as well as a place for documenting physical examination findings. In addition to the intake form, the rehabilitation facility's standard forms for stranded marine mammals can be used to record physical exam findings, laboratory values, treatments, and feedings, provided that all information is clearly documented and assigned to the specific animal.

A brief physical examination is performed upon admission of each individual oiled animal (see below). A veterinarian or animal care specialist should conduct the examination and treat any conditions that are considered to be life threatening. The capture, transport, and intake process is extremely stressful and an oiled animal's condition may be very unstable. The intake area should be as dark and quiet as is practical and animals must be monitored closely during the examination and intake process. If an animal's condition deteriorates and a veterinarian is not participating in the examination, seek veterinary advice immediately.

General Intake Procedure for Oiled Marine Mammals

- 1. Obtain and Complete Intake Forms
 - Live Mammal Data Log
 - Oiled Marine Mammal Intake Form
- 2. Physical Examination
- 3. Flipper tag application
- 4. Oil sample collection
- 5. Photograph

Animals need to be identified to species and, when possible, age class (pup, yearling, subadult, adult) and sex should be determined. Consult charts on age estimation for pinnipeds and sea

otters from marine mammal guides such as Geraci and Lounsbury (1993), Reeves et al., (1992) and Ainley et al., (1980) for species and sex identification. All animals should be tagged or marked for individual identification. This can be done with plastic livestock ear tags (e.g., Rototag, Temptag), by applying hair dye, colored livestock markers, and bleach marks to the pelage, or by clipping a small patch of pelage on the flank in a recognizable pattern (phocids and sea lions only). Dye marking and clipping is not advisable for fur seals or sea otters and may be difficult in other species depending on the location and extent of oiling. Sea otters and possibly other species may be identified using a commercially available pet microchip inserted subcutaneously at the inguinal region.

For legal purposes, it is necessary to collect an oil sample from each individual animal. A detailed protocol for the collection of evidence is provided in Appendix 6. Briefly, visible oil should be scraped from the fur with a clean wooden spatula and placed into a chemically cleaned glass jar. For animals with no visible gross oiling, an affected area is rubbed with a 4x4 piece of fiberglass cloth or cotton gauze with forceps or hemostats that have been cleaned with isopropyl alcohol. Precautions must be taken to collect the sample without allowing nitrile gloves to touch the oil sample or the cloth it is collected on. The oil sample should be placed in a glass container and labeled appropriately with the following information: the oil spill name, date, species, intake log number of that animal, animal capture location, and flipper tag color and number and then sealed with evidence tape and placed in secure freezer. Sampling supplies (glass jars and cloth) can be obtained through the trustee agencies.

It is also necessary to take a Polaroid photograph of the oiled animal. The photograph should include the entire animal, the oiled region, and if possible, show the flipper tag numbers. After the photograph develops, it should be labeled with the same information as the oil sample; the oil spill name, date, species, intake log number of that animal, animal capture location, and flipper tag color and number. The photograph and oil sample are both pieces of evidence and should be securely stored. If samples are to be sent for analysis, a completed Chain of Custody form is required and will be provided by the lead trustee agency.

Physical Examination

Animals are to be weighed and measured (standard length and axillary girth, xiphoid girth in sea otters) and their temperature measured with an electronic thermometer with a flexible thermister probe (e.g., Physitemp Model BAT-12 Digital Laboratory Thermometer) inserted 15 cm into the rectum. Standard thermometers can be used in sea otters, but do not accurately measure core temperatures in pinnipeds. Normal core temperature for sea otters is 99.5-100.6 °F (37.5-38.1 °C) and most pinnipeds range from 98-102 °F (Dierauf and Gulland, 2001). If the use of a thermometer is not possible, feel the flippers (e.g., icy cold or dry and hot) and observe the animal's behavior (e.g., shivering, agitation) in order to evaluate abnormally high or low body temperature. If an animal is dry and alert/active prior to the exam, assume it will overheat with handling.

A complete whole body examination should be conducted, making note of the degree and nature of oil contamination. Assess behavior, activity level and alertness; if possible, observe the animal in the transport cage prior to handling to evaluate locomotion and central nervous system status. Evaluate overall body condition and estimate the percent dehydration. Most stranded animals are at least slightly dehydrated (<5%, demonstrated by decreased tear production and subdued behavior). More severely dehydrated animals (5-10%, demonstrated by lack of tear production,

thick ocular mucus, "sunken" or crusty eyes, dry mucous membranes, skin tenting in otariids, curling of the vibrissae in harbor seals, and lethargic or depressed behavior) may need to be treated with fluids prior to continuing the examination and intake procedures; however, it is preferable to obtain blood samples prior to hydration treatments.

Due to the risk of being bitten, a thorough oral exam is possible only in anesthetized, dead, comatose, and young animals, but a visual inspection of the oral cavity is often possible during vocalization in alert animals. Palpate the neck and thorax for evidence of subcutaneous emphysema and the musculoskeletal system for fractures, wounds, or swellings. Subcutaneous emphysema is often found in the neck and axillary area in oiled sea otters and is an indicator of severe pulmonary damage. Palpate the abdomen gently to detect masses, pregnancy, or fluid accumulation and observe the urogenital area for urine, feces, or abnormal discharges.

Routine Blood Sampling

Following the general examination, blood samples should be drawn for hematology (collected in an EDTA anticoagulant, lavender-top tube, LTT) and chemistry panels (collected in a serum separator tube, SST, or red-top tube, RTT) and serum banking. In phocids, blood is generally drawn from the epidural sinus or ventral (plantar) interdigital veins (at the apex of the web between the inner digits) of the hind flippers (e.g., harbor seals, elephant seals). In otariids, the caudal gluteal vein and plantar network (dorsal or ventral surface of the hind flipper just medial to the lateral digit or just lateral to the medial digit) are used for blood collection (sea lions and fur seals). In sea otters, blood may be drawn from the popliteal (saphenous) or femoral vein on a non-anesthetized animal using a restraint box and/or stuff bag. Alternatively, the jugular vein can be used on an anesthetized otariid or sea otter.

Blood samples should be collected at least three times during the rehabilitation process: on admission/intake, immediately prior to washing, and prior to release. Repeat sampling may not be necessary for wash or release procedures, if preformed within 48hrs of previous blood sampling or at the discretion of the response veterinarian. At these times, baseline blood work should include a complete blood count and standard serum chemistry tests. Normal blood values for marine mammal species can be found in Bossart et al. (2001).

Standard Blood Tests

Complete Blood Cell counts (CBC): White cell blood count, red cell blood count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), a differential cell count, platelet and reticulocyte counts. One full lavender-top tube (EDTA) (1 or 3 ml) should be taken and refrigerated until analysis.

Chemistry Profile: Albumin, alkaline phosphatase, bicarbonate, bilirubin (total and direct), BUN, calcium, chloride, cholesterol, CK, creatinine, globulin, glucose, phosphorus, potassium, total protein, sodium, AST (SGOT), ALT (SGPT), GGT, and ratios of albumin:globulin, BUN:creatinine, and sodium:potassium. Blood should be placed in a serum separator tube or red top tube, allowed to clot, centrifuged, and refrigerated prior to analysis. Excess serum should be saved and banked (frozen) at the rehabilitation facility.

Special Biomedical Sampling Protocols

At times, additional protocols may be used that require additional blood samples for other tests (e.g., PAH estimation, immune function assays, serum protein electrophoresis, plasma chemistries, serological tests for infectious diseases). Other biomedical samples (e.g., urine sample, fecal sample, microbiological swab, blubber biopsy) may also be collected at the discretion of the response veterinarian.

Post-examination Intake Procedures

Initial Treatment

- Fluid therapy: oral, subcutaneous, intravenous
- Activated charcoal (ToxiBan) tubing if oil ingestion suspected

All animals are assumed to be at least 5% dehydrated. Administer isotonic fluids to animals that appear to have not ingested oil orally at a rate of 10-20 ml/kg once either orally (e.g., Pedialyte) or subcutaneously (lactated Ringer's solution, LRS). If the animal is alert and is likely to have ingested oil (e.g., fur seals during grooming, neonates during nursing), administer activated charcoal slurry (ToxiBan, 6 ml/kg) orally.

Animals that are chemically immobilized for intake procedures or are weak and obtunded should not be given oral fluids. Subcutaneous fluids (e.g., lactated Ringer's solution), may be administered instead at 20-40 ml/kg. If ingestion of oil is suspected, ToxiBan slurry (6ml/kg) can be administered via a stomach tube just prior to anesthetic reversal (Williams and Sawyer, 1995). Extreme care must be taken to prevent gastric reflux and aspiration during this procedure. The risks associated with passing a stomach tube must be weighed against the risks associated with continued exposure to ingested petroleum.

Severely depressed animals may require intravenous fluid administration and other medication in addition to isotonic fluids. Additional fluid therapy (maintenance fluids plus correction of fluid deficits) should be determined by the attending veterinarian, based on an evaluation of blood work, concurrent fluid losses, and continuing assessment of the animal's condition. The fluid deficit is calculated by multiplying an animal's mass in kg x 1000 ml fluid/kg x the percent dehydration (e.g., 5% = .05). This should be added to the animal's daily maintenance fluid requirement (at least 40 ml/kg/day) and administered within the first 24 hr if possible.

Monitoring

Animals should be regularly monitored during the rehabilitation process. Clinical observations, feeding observations (food consumption and/or preferences), and behavior should be written on the medical records. Body weight should also be monitored repeatedly during rehabilitation and recorded, at a minimum, upon admission, pre-washing, and prior to release. More extensive body weight monitoring may be required in critical cases. Physical examinations should be performed upon admission, prior to washing, and prior to release with all information recorded on individual medical records. Whenever medications are administered, the name of the drug, dose and route (oral, SQ, IM, IV) should be recorded as well as the initials of the person who administered the medication. Medical records are viewed as potential evidence by the law and should be carefully and completely filled out by animal caretakers.

Animal Washing and Continued Care

General Topics Associated With Cleaning

The facility where oiled animals will be cleaned should be designed to accommodate the variety of species that might be cared for at that facility. Each wash station must have adequate space for the animals, animal handlers, and restraint equipment that might be necessary. Water hardness should be tested before washing animals and adjusted to 3-5 grains of hardness (Clumpner, 1991). Dawn dishwashing liquid is the preferred washing product and has been shown to be safe and effective for removing oil from the coats of sea otters and harbor seals (Rash et al., 1990). Wastewater storage, containment, and removal must meet the requirements of the municipality, city, and county. A minimum team of two or three persons usually wash animals. Fur seals and sea otters may require teams of four or five persons because the density of their fur requires much greater effort. Large animals such as elephant seals may require a washing team with three or four persons to properly restrain the animal. Large animals, aggressive animals, fur seals and sea otters may require sedation and veterinary assistance for washing and cleaning.

General Washing Needs

- Softened water (3-5 gr)
- Temperature controlled warm water (80-98°F, 27-37°C)
- Pressured spray nozzles (30-40 psi)
- Dawn detergent
- Wastewater storage and removal

Pre-Wash Evaluation

Oiled marine mammals will require at least 24 hours of supportive care prior to being washed. Initial care is focused on addressing thermoregulatory problems, rehydration, and providing nutritional sustenance so animals are no longer in a negative metabolic balance. The washing procedure is very stressful; therefore, prior to the procedure, the animal needs to have regained strength. In the case of sea otters, they also need to be able to tolerate anesthesia and start to groom once recovered. A veterinarian should conduct a pre-wash evaluation that includes a physical examination, evaluation of alertness, strength and body condition, and blood parameters. If the animal passes the pre-wash evaluation, it is referred to the washing team.

Removing Tar Patches from Animals

If the oil present on an animal is a tar patch or very weathered, pretreatment may be necessary. This is accomplished by applying warmed (95-98°F or 35°C) olive oil, canola oil, or methyl oleate to the affected region. The pretreatment solution should be manually worked into the tarred areas for up to 30 minutes or until the tar loosens and can be wiped off using an absorptive pad or towel. While pretreating the animal, it is important to monitor the animal's body temperature and be prepared to treat the animal for hyperthermia or hypothermia. Tar removal is necessary for furred marine mammals and non-furred marine mammals if the patch(es) are large, potentially interfering with thermoregulation, or contribute to toxicity and result in clinical symptoms. Clipping away tar patches (with accompanying fur) is recommended unless molt is imminent

because the animal will have a bald patch that could cause reduction of heat retention. This procedure could have serious or life-threatening implications for fur seals, sea otters, or debilitated animals.

Washing Harbor Seals, Elephant Seals, Sea Lions

Sea lions, harbor seals and elephant seals rely on their thick blubber layer for insulation, making them less susceptible to hypothermia when they become externally oiled. These species are washed with Dawn detergent in thermal-neutral (~ 98°F or 37°C) water. Soap is applied and rubbed on the fur until the oil is visibly removed. The detergent can be made into a uniform solution by mixing it with water at a 1:1 ratio prior to applying thus making it easier to work into the hair and oil. Washing pinnipeds takes between 10-30 minutes depending on the extent and type of oil, species and health of the animal, and the proficiency of the staff. An initial quick rinse can be done at the wash station and then completed with the animal unrestrained in its pen using a pressure nozzle. This modified rinse procedure decreases the duration of manual restraint. In general, rinsing should be continued until there is no evidence of oil or detergent in the rinse water. Most pinnipeds are placed directly into their outdoor pens to dry.

General Guidelines for Washing Pinnipeds

- 1. Thermal neutral water (~ 98°F or 37°C)
- 2. Dawn detergent rubbed onto fur until oil is removed
- 3. Pressurized rinse in pen until oil and detergent removed
- 4. Air dry in pen

Washing Fur Seals

In contrast, fur seals possess a thin subcutaneous fat layer and a thick pelage that thermally insulates these animals (Reidman, 1990). Since they rely more heavily on their fur, fur seals are washed in a similar fashion to otters. Oiling 30% of a fur seal's coat will result in a 50% increase in heat loss (Geraci and St. Aubin, 1990), emphasizing the need for these animals to be closely monitored during the washing procedure. Fur seals are washed using a thermal-neutral (~98°F or 37°C), 5% diluted Dawn dish washing detergent solution. The diluted detergent solution is gently massaged into the fur and, as with other species, the washing duration depends on the extent and type of oil, the strength of the animal, and the proficiency of the staff. Fur seals are rinsed with fresh, soft (3-5 gr) water under moderate pressure (30-40 psi) with a spray nozzle. This process can require up to 40-60 minutes and animals are rinsed until no oil is visible in the rinse water and no petroleum odor is detectable on the fur (Davis and Hunter, 1995). For all pinnipeds, animals may become hyperthermic during washing in which case they may need to be washed and rinsed in cold water.

Fur seals, which depend on their coat for thermoregulation, may need to be placed in a drying enclosure that is warmed with an industrial pet dryer that blows room temperature air (68°F or 20°C). Animals in drying pens must be monitored for dehydration, hyperthermia, hypothermia, and alertness. Once dry and alert, fur seals can be returned to their outdoor pens.

Washing Sea Otters

Sea otters have the densest fur of any mammal, and, unlike most other marine mammals, replace their fur throughout the year instead of undergoing a seasonal molt (Tarasoff, 1974; Williams et al., 1992). Otters have guard hairs and many fine under-hairs that are microscopically interlocked to trap air, thus providing waterproofing, thermal insulation, and buoyancy. Oil contamination

causes fur clumping which leads to a loss of insulation and predisposes otters to hypothermia from the cold ocean water.

General Guidelines for Washing Sea Otters

- 1. Anesthesia/sedation
- 2. Diluted Dawn solution
- 3. Temperature controlled warm water
- 4. Pressurized rinse (40-60 minutes)
- 5. Dry with towels and blow dryers
- 6. Anesthesia reversal

Anesthesia

Due to their aggressive temperament, sea otters generally require sedation or anesthesia to be washed. A variety of anesthetics have been used, however, the current preferred drug combination in adult sea otters for nonsurgical procedures is fentanyl (0.22 mg/kg) and diazepam (0.07 mg/kg) used together intramuscularly. The opioid antagonist naltrexone at 0.44 mg/kg is recommended for reversal, but often 3 - 4 times the total dose of fentanyl administered is needed for complete reversal (Monson et al., 2001). While sedated, supplemental oxygen is routinely provided either via facemask, or, if the sea otter is immobilized enough to tolerate it, via endotracheal tube. During sedation and cleaning, the core temperature of the sea otter must be monitored continuously because otters can become hypothermic or hyperthermic very quickly. Whenever a sea otter is sedated, bags of crushed ice should be readily available and placed under the animal's neck and flippers if hyperthermia occurs.

Washing and Rinsing

Sea otters are washed with multiple applications of diluted (5%) Dawn dishwashing detergent. Ideally, washing tables are equipped with three or four well aerated nozzles dispensing temperature controlled (28-37 °C, 80-98 °F), softened (3-5 gr.) fresh water. The water temperature affects the body temperature and needs to be adjusted according to the otter's body temperature to prevent hyper or hypothermia (Davis and Hunter, 1995; Stoskopf et al., 1997). Four to six people are required per washing table, one (with heavy gloves) specifically to hold the head and forearms. The detergent is gently massaged into the oiled fur and then rinsed off under moderate pressure (30-40 psi) with a spray nozzle. Washing should consist of a wash, rinse, wash, rinse cycle until there is no indication of oil in the rinse water and no petroleum odor on the fur. Depending on the degree of oiling, washing will usually take from 40-60 minutes. A final rinse with a spray nozzle lasting an additional 40 minutes to one hour is essential to thoroughly remove the detergent and restore the furs' water repellency. Otters are initially hand dried with dry, clean, cotton terry cloth towels. Once the bulk of the water has been absorbed, the fur is dried with commercial pet dryers that deliver a high volume of temperature controlled air (Davis and Hunter, 1995). Sea otters become increasingly prone to hyperthermia as their hair is drying and cool (room temperature) air may be necessary for drying as the sea otter's body temperature increases.

Drying

Following drying, each animal is reversed from the anesthetic and placed in a large, slat-floor kennel with a sliding top or other easily accessible dry pen for intensive care monitoring. Animals in dry holding should be closely monitored for hyperthermia and fecal, urine, or food debris must be rinsed away immediately. When fully recovered from anesthesia, otters should be offered small blocks of ice to chew on and food (Davis and Hunter, 1995). Once the animal is stable and medical conditions allow, each otter should be moved to a pool with haulout(s) serviced by

abundant, clean, chlorine-free salt water (if available). Pools must have high seawater flow rates (e.g. 5 gallons per minute for 150 gallon pool) and drain skimmers at water level to collect debris from the pool. Fecal and food contamination of the pool water can cause fur fouling and prevent restoration of water repellency. Sea otters are not waterproof after washing and drying and must reintroduce trapped air into their fur by grooming.

Post-wash monitoring and care

During rehabilitation, sea otters need to be monitored around-the-clock by qualified personnel familiar with normal sea otter behavior and who are able to recognize clinical signs of distress. Sea otters often develop hypothermia post-wash due to lack of air insulation in washed fur and inadequate grooming. Otters that appear hypothermic, having difficulty hauling out, or experiencing seizures should be immediately removed from the water and evaluated by a veterinarian. As health and fur condition improve, otters may be moved to larger pools and/or floating holding pens. All pools should have abundant haul-out space. It will generally take a minimum of seven to ten days for the fur to recover its water repellency (Tuomi et al., 1995).

Common Problems Encountered While Washing Animals

- 1. Oil is not coming off with Dawn
 - Pretreatment with canola oil, olive oil, or methyl oleate is required.
- 2. The animal's coat is not clean
 - The animal may not have been washed or rinsed adequately. In either case, the animal may need to be re-washed or re-rinsed.
 - The wash or rinse water is too hard and mineral deposits are forming on the fur. Water hardness should be rechecked to make sure it is 3-5 grains.
 - The holding pool is not clean. Check whether the water is turbid or if there is fish oil or debris floating on the pool surface. Water flow may need to be increased or pool cleaned.

Nutritional Guidelines

The dietary requirements of stranded marine mammals are generally grouped into two categories according to age and nutritional needs: unweaned pups and weaned animals. Pups need special dietary formulas and feeding regimes based on species and age while free-feeding animals are generally fed a diet of good quality fish such as herring. Adult sea otters are usually fed a variety of fish and shellfish depending on their preference. Marine mammals also usually need to receive a supplemental multivitamin, vitamin E, and salt tablets (if housed in fresh water) with amounts based on species and weight. Monitoring fecal production and hydration status is especially important when beginning any formula, switching diets, or weaning animals. Recommended diets change with continued research and experience and stranding network participants should play an important role in the development of dietary protocols for each species and facility. More information can be obtained on marine mammal nutrition and energetics from Worthy (2001), and hand-rearing and artificial milk formulas from Williams and Davis (1995) for sea otters, and Townsend and Gage (2001) and Gage (2002) for pinnipeds.

Disposition

Release

The goal in rehabilitating oiled marine mammals is to release healthy animals back into their natural environment. Rehabilitators, in consultation with designated trustee representatives (NMFS/FWS) must prepare a release plan that is communicated to and authorized by the Unified Command through the Liaison Officer. Certain criteria must be met prior to releasing marine mammals back into wild populations. For those animals that do not meet release criteria, several options are available including additional rehabilitation, euthanasia, or placement in a long-term holding facility.

While little is known about optimal oiled marine mammal release criteria, current recommendations are based on information derived from the Exxon Valdez spill and husbandry practices at aquaria and rehabilitation centers in the United States. NMFS and FWS have developed guidance and criteria for release based on optimizing the chances for survival and minimizing the risk to wild populations (Best Practices for Marine Mammal Stranding Response, Rehabilitation, and Release – Standards for Release, http://www.nmfs.noaa.gov/pr/health). The Standards for Release document describes how to characterize and assess animals using several parameters.

Standards for Release

- 1. Historical Assessment
- 2. Developmental and Life History Assessment
- 3. Behavior Assessment and Clearance
- 4. Medical Assessment and Clearance
- 5. Release Logistics
- 6. Post Release Monitoring

Current criteria require that animals show normal species-specific behavior (feeding, swimming, and diving), adequate body weight for age class and species, pelage proven to be in good condition, hematological and serum chemistry values within the normal range, no evidence of infectious diseases, and physical exam findings should be unremarkable. Other ancillary tests (e.g. *Leptospira* titer, morbillivirus titer, microbiological cultures, urinalysis, fecal examinations, etc.) may also be performed on a case-by-case basis depending on individual animal and population level concerns. The Unified Command will decide upon the location of the release with guidance from the trustee agencies

Upon approval for release by UC, an exit photo of each marine mammal must be taken and specifics of the release (location, time, personnel) recorded for Natural Resource Damage Assessment purposes.

Post-release monitoring, if at all possible, should be undertaken during marine mammal releases following oil exposure using radio or satellite telemetry. This effort should focus on survival rates, behavior, and reproductive success following oil contamination and rehabilitation, thus enabling

oiled marine mammal responders to evaluate the efficacy of oiled marine mammal care. Post-release monitoring is not usually considered a response activity expense and must be funded by the stranding network participate, trustee agency or NRDA.

Mortalities

All oiled dead marine mammals should be collected from beaches and taken to a designated morgue. Dead animals should be logged in at the morgue using a Dead Marine Mammal Data Log (example in Appendix 3). Under certain circumstances, an oiled animal may need to be humanely euthanized in order to alleviate suffering. Animals that die during an oil spill response must have this disposition information recorded on their individual animal record as well as on the Live Marine Mammal Data Log (Appendix 2). The carcass should be identified with a written tag including the species name, date of stranding and/or admission, date of death, and the flipper tag (if a tag was affixed prior to death). If a flipper tag is present, it should remain with the carcass until final disposition of the carcass. The carcass should be refrigerated or kept on ice until a necropsy is performed. If a necropsy cannot be performed within 24 hours of death, the carcass needs to be frozen.

Euthanasia

During an oil spill response, there are circumstances under which it may be necessary to humanely euthanize animals. For each spill where marine mammal rehabilitation is undertaken, the rehabilitator must prepare a written euthanasia plan in consultation with the trustee representative. Euthanasia is appropriate for oiled animals with injuries that will render it unable to survive in the wild or unsuitable for use in captivity. If animals are euthanized in the field, they are collected following the procedures outlined in the Recovery and Transportation section of this document. To prevent secondary contamination or poisoning, euthanized carcasses are never left in the field.

Necropsy

Necropsies may be performed concurrent with response activities to identify cause of death in order to differentiate between a natural versus pollution related mortality. Fatalities to apparently un-oiled wildlife may necessitate necropsies to determine if death was caused by human interactions or if sub-apparent oil exposure or ingested petroleum contributed to the mortality. Additionally, captivity-related diseases may necessitate necropsies be performed on animals that die during rehabilitation to identify potential pathogens or husbandry techniques that are detrimental to recovery.

Prior to performing a necropsy on an oiled marine mammal, specific permission must be obtained from Unified Command and the appropriate NMFS/FWS enforcement officer. The spill response veterinarian-of-record should conduct or supervise all necropsies, in consultation with the designated representative FWS or NMFS enforcement officer. In most cases, a veterinary pathologist with specialized training on marine mammals will be asked to perform the necropsy. Necropsy methods and techniques are diverse, but general procedures for marine mammal necropsies can be found in Rowles et al. (2001), Galloway and Ahlquist (1997), and Geraci and Loundsbury (1993). Specific protocols have also been developed for some marine mammals including phocids (Winchell, 1990), Killer whales (Raverty and Gaydos, 2004), Right whales (McLellan et al., 2004), and Hawaiian Monk seals (Yochem et al., 2004). These species specific procedures should be followed whenever possible in order to maintain consistency with previous data. Prior to conducting a necropsy, the trustee agency and veterinarian should agree on which forms to use; which samples to collect; how those samples will be prepared (e.g., formalin or

frozen), stored, and shipped; and where samples will be analyzed. Specific oil spill necropsy information and forms are detailed in Appendix 7-9. Tissue samples for standard histopathology, disease profiling, and petroleum hydrocarbon analysis should be collected. Sampling for oil exposure, must be performed under specific conditions detailed in Appendix 7, in order to prevent contamination of the sample. Necropsy reports are filed and all samples handled and stored using appropriate chain-of-custody protocols, as discussed previously (Data Collection) and provided by the trustee representative.

Laboratories performing the petroleum analysis must be contacted as soon as possible in order to verify that sampling protocols and sample sizes are consistent with that specific laboratory requirement. Considerations in choosing the lab should include details of forensic capabilities (ability to produce legally defensible results), quality assurance and quality control (QA/QC), and consistency with the analysis of other materials from the spill. Results can vary between labs and data should be comparable between the environmental and tissues of the different species sampled. Appendix 8 lists laboratories (not an exhaustive list), with expertise in petroleum hydrocarbon chemistry that can be contacted for oil spill sample collection and analysis information. Petroleum hydrocarbon analysis is a reimbursable response expenses if pre-approved by the UC. However, often the RP (responsible party) assumes ownership of the oil and analysis may not be preformed.

Petroleum and Polycyclic Aromatic Hydrocarbons (PAH) Analysis

In general, all crude oils are mixtures of the same hydrocarbon and non-hydrocarbon compounds, but vary in the percent composition of these compounds. Natural weathering of oil in the environment also results in highly variable compositions. Because of the continual dynamic changes in spilled oil, it can be difficult to identify and quantify all PAHs potentially present in or on an animal in the aftermath of an oil spill. Oil and tissue samples collected from marine mammals can be analyzed to determine the total amount of PAHs in tissues and identify and quantify dangerous PAHs that may have caused clinical and pathological effects. Samples can also be tested to characterize and fingerprint petroleum hydrocarbons to determine their source.

Determining source-dependent petroleum exposure during an oil spill using GC/MS or HPLC techniques on marine mammal tissues requires baseline knowledge of petroleum hydrocarbon levels and composition in the spill area and of the spilled oil. At present there are few data available on PAH levels in marine mammals inhabiting North American coastal waters. Studies have only measured PAH levels in seals and whales from the Eastern Canada (Hellou et al., 1990, Zitko et al., 1998) and Northeastern United States (Lake et al., 1995). Overall, the low concentrations of bioaccumulated PAHs in tissues from these marine mammals are fairly similar to those reported in atmospheric fallout PAHs from combustion sources (Zitko et al., 1998). Alkylated and heterocyclic PAHs are the predominant forms of PAHs in oil and coal products, and can be missed if tissues are tested only for the 16 traditionally-studied, parent PAHs listed as priority pollutants by the Environmental Protection Agency (EPA) and World Health Organization (WHO) (Means 1998). Different members of the isomeric alkylated PAHs exhibit differential toxicity, diffusion, and degradation rates, further emphasizing the importance of compound-specific analysis. With the lack of baseline PAH levels from marine mammals, control samples for comparisons were harvested at the time of Exxon Valdez oil spill from animals inhabiting nearby non-oiled areas (Mulcahy and Ballachey, 1994; Frost et al., 1994).

In experimental exposure studies (both immersion and ingestion) involving ringed seals (*Phoca hispida*), differences in detectability of PAHs in various tissues were noted (Engelhardt et al., 1977). In the immersion experiment, PAHs were highest in urine and bile, less elevated in blood and plasma, and lower in tissues (lowest in lung) at 2 days post-immersion. Tissue sampling in the ingestion study was limited with PAHs highest in blood, and higher in liver and blubber compared to muscle. These studies illuminate the importance of selecting appropriate tissues for PAH analysis. Specific tissue collection techniques are provided in Appendix 7.

Records

The importance of recording information cannot be over-emphasized. Record collection enhances individual animal care, response evaluations, and the ability to accurately characterize the best practices for appropriate care. In-house records are maintained at the rehabilitation facility and copies provided to the trustee agency. Final reports, including chain-of-custody and sample collection records, must be delivered to the trustee agency within 30 days of the date the Federal OSC declares the response closed.

Scientific Records

The following types of records are necessary to preserve vital information for scientific study, natural resource damage assessment, and improved rehabilitation practices and techniques:

- Oiled mammal sighting: records and maps for all reports of oiled mammals
- Search Effort Log
- Live Mammal Log
- Dead Mammal Log
- Marine Mammal Intake Form
- Rehabilitation Records: documents care for each animal, including feedings, treatments, medications, normal/abnormal activities.
- Lab Analyses Report: identifies all samples sent to labs, requested analyses, lab results.
- Marine Mammal Stranding Report Level A Data (NOAA 89-864, OMB #0648-0178)
- Marine Mammal Rehabilitation Disposition Report (NOAA 89-878, OMB #0648-0178)
- Human Interactions Form
- Necropsy Report

References

- Ainley, D.G., G.W. Page, L.T. Jones, L.E. Stenzel, and R.L. LeValley. 1980. Beached marine birds and mammals of the North American West Coast: a manual for their census and identification. U.S. Department of the Interior, U.S. Fish and Wildlife Service, Biological Services Program, FWS/OBS-80/03. 207p.
- Benz, C.T. and R.L. Britton. 1995. Sea otter capture. In *Emergency Care and Rehabilitation of Oiled Sea Otters: A Guide for Oil Spills Involving Fur-bearing Marine Mammals*, T.M. Williams and R.W. Davis, eds., Fairbanks, AK: University of Alaska Press. pp. 23-37.
- Bossart, B.D., T.H. Reidarson, L.A. Dierauf, B.A. Duffield. 2001. Clinical Pathology. In *CRC Handbook of Marine Mammal Medicine*. L.A. Dierauf and F.M.D Gulland, eds., Boca Raton, FL: CRC Press, Inc. pp. 383-436.
- Clumpner, C. 1991. Water hardness and waterproofing of oiled birds: lessons from the Nestucca, Exxon Valdez, and the American Trader spills. In *The Effects of Oil on Wildlife: Research, Rehabilitation & General Concerns*, J. White, ed., Hanover, PA: The Sheridon Press. pp. 101-102.
- Davis, J.E., and S.S. Anderson. 1976. Effects of oil pollution on breeding grey seals. *Marine Pollution Bulletin* 7, 2761-2767.
- Davis R.W. and C.W. Davis. 1995. Facilities for Oiled Sea Otters. In Emergency Care and Rehabilitation of Oiled Sea Otters: A Guide for Oil Spills Involving Fur-bearing Marine Mammals, T.M. Williams and R.W. Davis, eds. Fairbanks, AK: University of Alaska Press. pp. 159-175.
- Davis, R.W. and L. Hunter. 1995. Cleaning and Restoring the Fur. In *Emergency Care and Rehabilitation of Oiled Sea Otters: A Guide for Oil Spills Involving Fur-bearing Marine Mammals*, T.M. Williams and R.W. Davis, eds. Fairbanks, AK: University of Alaska Press. pp. 95-102.
- Dierauf, L.A. and F.M.D. Gulland, eds. 2001. CRC Handbook of Marine Mammal Medicine. Boca Raton, FL: CRC Press, Inc. 1063p.
- Duffy, L.K., R.T. Bowyer, J.W. Testa, and J.B. Faro. 1993. Differences in blood haptoglobin and length-mass relationships in river otters (*Lutra canadensis*) from oiled and nonoiled areas of Prince William Sound, Alaska. *Journal of Wildlife Diseases* 29, 353-359.
- Duffy, L.K., R.T. Bowyer, J.W. Testa, and J.B. Faro 1994. Chronic effects of the *Exxon Valdez* oil spill on blood and enzyme chemistry of river otters. *Environmental Toxicology and Chemistry* 13, 643-6470.
- Engelhardt, F.R. 1982. Hydrocarbon metabolism and cortisol balance in oil-exposed ringed seals, *Phoca hispida. Comparative Biochemistry and Physiology. C: Comparative Pharmacology* 72 (1), 133-136.

- Engelhardt, F.R., J.R. Geraci, and T.G. Smith. 1977. Uptake and clearance of petroleum hydrocarbons in the ringed seal, *Phoca hispida. Journal of the Fisheries Research Board of Canada* 34, 1143-1147.
- Frost, K.J., C.A. Manen, and T.L. Wade. 1995. Petroleum hydrocarbons in tissues of harbor seals from Prince William Sound and the Gulf of Alaska. In *Marine Mammals and the Exxon Valdez*, T. R. Loughlin, ed., San Diego: Academic Press. pp. 331-358.
- Gage, L.J., ed. 2002. Hand-Rearing Wild and Domestic Mammals. Ames, IA: Iowa State Press. 279p.
- Galloway, S.B., and J.E. Ahlquist, eds. 1997. *Marine Forensics Manual, Part 1 Marine Mammals*. National Marine Fisheries Service, Southeast Fisheries Science Center, Charleston laboratory, Charleston, SC, 90p.
- Geraci, J. R., and T. G. Smith. 1976. Direct and indirect effects of oil on ringed seals (*Phoca hispida*) of the Beaufort Sea. *Journal of the Fisheries Research Board of Canada* 33, 1976-1984.
- Geraci, J.R., and D.J. St. Aubin. 1988. *Synthesis of effects of oil on marine mammals*. Ventura, CA: Battelle Memorial Institute. Final report. MMS 88-0049
- Geraci, J.R., and D.J. St. Aubin. 1990. Sea Mammals and Oil: Confronting the Risks. San Diego: Academic Press. 221p.
- Geraci, J. R., and T. D. Williams. 1990. Physiologic and toxic effects on sea otters. In *Sea mammals and Oil: Confronting the Risks*. D.J. St. Aubin and J.R. Geraci, eds., San Diego: Academic Press. pp. 211-221.
- Geraci, J.R. and V.J. Lounsbury. 1993. *Marine Mammals Ashore: A Field Guide for Strandings*. Galveston, TX: Texas A&M Sea University Sea Grant Publication. 305p.
- Haulena, M. and R.B. Heath. 2001. Anesthesia. In *CRC Handbook of Marine Mammal Medicine*. L.A. Dierauf and F.M.D Gulland, eds., Boca Raton, FL: CRC Press, Inc. pp. 655-684.
- Hellou, J., G. Stenson, I. H. Ni, and J.F. Payne. 1990. Polycyclic aromatic hydrocarbons in muscle tissue of marine mammals from the Northwest Atlantic. *Marine Pollution Bulletin* 21 (10), 469-473.
- Lake, C.A., J.L. Lake, R. Haebler, R. McKinney, W.S. Boothman, and S.S. Sadove. 1995. Contaminant levels in harbor seals from the northeastern United States. Archives of Environmental Contamination and Toxicology 29, 128-134.
- Lipscomb, T.P., R.K. Harris, R.B. Moeller, J.M. Pletcher, R.J. Haebler, and B.E. Ballachey. 1993. Histopathologic lesions in sea otters exposed to crude oil. *Veterinary Pathology* 30 (1), 1-11.
- Loughlin, T. R., ed. 1994. *Marine mammals and the Exxon Valdez*. Orlando, Florida: Academic Press, Inc. 395p.
- Lowry, L.F., K.J. Frost, and K.W. Pitcher. 1994. Observations of oiling of harbor seals in Prince William Sound. In *Marine mammals and the Exxon Valdez*. T. R. Loughlin, ed., San Diego, New York: Academic Press. pp. 209-225.

- NRT, National Response Team. 2004. ICS/UC Technical Assistance Document. website: http://www.nrt.org/
- Means, J.C. 1998. Compound-specific gas chromatographic-mass spectrometric analysis of alkylated and parent polycyclic aromatic hydrocarbons in waters, sediments, and aquatic organisms. *Journal of AoAC International* 81, 657-672.
- McLellan, W.A., Rommel, S.A., Moore, M., Pabst, D.A. 2004. Right Whale Necropsy Protocol. Final report to NOAA Fisheries for contract #40AANF112525. 51p.
- Monson D.H., C. McCormick, and B.E. Ballachey. 2001. Chemical anesthesia of northern sea otters (Enhydra lutris): results of past field studies. *Journal of Zoo and Wildlife Medicine* 32(2),181-189.
- Mulcahy, D.M., and B.E. Ballachey. 1995. Hydrocarbon residues in sea otter tissues. In *Marine Mammals and the Exxon Valdez*. T. R. Loughlin, ed., San Diego: Academic Press. pp. 313-330.
- Raverty, S.A., and J.K. Gaydos. 2005. Killer Whale Necropsy and Disease Testing Protocol. UC Davis Wildlife Health Center, Sea Doc Society. 61p. http://mehp.vetmed.ucdavis.edu/pdfs/orcanecropsyprotocol.pdf.
- Rash, J.A., C.R. McCormick, R. Alexander, S.J. Nichol, and D.C. Perrollaz. 1990. Coat Gradation and Conditioning of Sea Otters at the Seward Rehabilitation Center. In Sea Otter Symposium: Proceedings of a Symposium to Evaluate the Response Effort on Behalf of Sea Otters after the T/V Exxon Valdez Oil Spill into Prince William Sound. Anchorage, AK: US Fish and Wildlife Service. Biological Report 90 (12).
- Rowles, T.K., R.M. Van Dolah, and A.A. Hohn. 2001. Gross Necropsy and Specimen Collection Protocols. In *CRC Handbook of Marine Mammal Medicine*. L.A. Dierauf and F.M.D Gulland, eds., Boca Raton, FL: CRC Press, Inc. pp. 449-470.
- Reeves, R.R., B.S. Stewart, and S. Leatherwood. 1992. *The Sierra Club Handbook of Seals and Sirenians*. Sierra Club Books, San Francisco. 359p.
- Reidman, M. 1990. *The Pinnipeds: Seals, Sea Lions and Walruses*. Berkeley, CA: University of California Press, pp. 16-17.
- Ridgeway, S.H. 1972. Homeostatis in the aquatic environment. In *Mammals at Sea: Biology and Medicine*. S.H. Ridgeway, ed., Springfield, II: Charles C. Thomas
- Smith, T.G., and J. R. Geraci. 1975. The effect of contact and ingestion of crude oil on ringed seals of the Bearfort Sea. *Bearfort Sea Project*, Tech. Rep. No. 5. Inst. Ocean Sci., Sidney, British Columbia.
- Sparker, T.R., L.F. Lowry, and K.J. Frost. 1994. Gross and histopathological lesions found in harbor seals. In *Marine mammals and the Exxon Valdez*. T. R. Loughlin, ed., San Diego: Academic Press. pp. 281-312.

- St. Aubin, D.J. 1990. Physiologic and Toxic Effects on Pinnipeds. In *Sea Mammals and Oil: Confronting the Risks*. J.R. Geraci and D.J. St. Aubin, eds., San Diego: Academic Press. pp. 103-125.
- Stoskopf M.K., L.H. Spelman, P.W. Sumner, D.P. Redmond, W.J. Jochem, and J.F. Levine. 1997. The impact of water temperature on core body temperature of North American river otters (Lutra canadensis) during simulated oil spill recovery washing protocols. *Journal of Zoo and Wildlife Medicine* 28(4), 407-12.
- Tarasoff, F.J. 1974. Anatomical adaptions in the river otter, sea otter, and harp seal with reference to thermal regulation. In *Functional Anatomy of Marine Mammals*. R.J. Harrison, ed., London: Academic Press, pp. 111-142.
- Townsend, F.I. and L. J. Gage. 2001. Hand-rearing and artificial milk formulas. In *CRC Handbook of Marine Mammal Medicine*. L.A. Dierauf and F.M.D Gulland, eds., Boca Raton, FL: CRC Press, Inc. pp. 829-849.
- Tuomi, P.A., S. Donoghue, and J.M. Otten-Stanger. 1995. Husbandry and Nutrition. In *Emergency Care and Rehabilitation of Oiled Sea Otters: A Guide for Oil Spills Involving Fur-bearing Marine Mammals*. T.M. Williams and R.W. Davis, eds., Fairbanks, AK: University of Alaska Press. pp. 103-119.
- Williams, T.D., D.D. Allen, J.M. Groff, and R.L. Glass. 1992. An analysis of California sea otter (*Enhydra lutris*) pelage and integument. *Marine Mammal Science* 8(1), 1-18.
- Williams, T.M. and R.W. Davis, eds. 1995. *Emergency Care and Rehabilitation of Oiled Sea Otters: A Guide for Oil Spills Involving Fur-bearing Marine Mammals*. Fairbanks, AK: University of Alaska Press Fairbanks. 279p.
- Williams, T.D. and D.C. Sawyer. 1995. Physical and chemical restraint. In *Emergency Care and Rehabilitation of Oiled Sea Otters: A Guide for Oil Spills Involving Fur-bearing Marine Mammals*, T.M. Williams and R.W. Davis, eds., Fairbanks, AK: University of Alaska Press. pp. 39-43
- Williams, T.D., J.F. McBain, P.A. Tuomi, and R.K. Wilson. 1995. Initial clinical evaluation, emergency treatments, and assessment of oil exposure. In *Emergency Care and Rehabilitation of Oiled Sea Otters: A Guide for Oil Spills Involving Fur-bearing Marine Mammals.* T.M. Williams and R.W. Davis, eds., Fairbanks, AK: University of Alaska Press. pp. 45-57.
- Winchell, J.M. 1990. Field Manual for Phocid Mecropsies (specifically *Monachus schauinslandi*). U.S. Dep. Commer., NOAA Tech. Memo., NOAA-TM-NMFS-SWFSC-146, 55p.
- Worthy, G.A. 2001. Nutrition and energetics. In *CRC Handbook of Marine Mammal Medicine*. L.A. Dierauf and F.M.D Gulland, eds., Boca Raton, FL: CRC Press, Inc. pp. 791-798
- Yochem, P.K., R.C. Braun, B. Ryon, J.D. Baker, and G.A. Antonelis. 2004. Contingency Plan for Hawaiian Monk Seal Unusual Mortality Events. U.S. Dep. Commer., NOAA Tech. Memo., NOAA-TMNMFS-PIFSC-2, 297p.
- Zitko, V., G. Stenson, and J. Hellou. 1998. Levels of organochlorine and polycyclic aromatic compounds in harp seal beaters (Phoca groenlandica). *Science of the Total Environment* 221, 11-29.

Appendices

- 1. Search Effort Log
- 2. LIVE Marine Mammal Data Log Form
- 2b. LIVE Marine Mammal Data Log Form, page 2
- 3. DEAD Marine Mammal Data Log Form
- 3b. DEAD Marine Mammal Data Log Form, page 2
- 4. Oiled Marine Mammal Intake Form
- 5. Oiled Marine Mammal Daily Progress Form
- 6. Oiled Marine Mammal Evidence Collection Protocol
- 7. Petroleum Hydrocarbon Tissue Sampling Protocol
- 8. Oil Spill Response Laboratories and Supplies
- 9. Oiled Marine Mammal Necropsy Form
- 10. Chain of Custody Form
- 10b. Chain of Custody Form, page 2

Appendix 1. Search Effort Log

Search Effort Log

Please record all beaches searched even if no animals are found.

Spill	Name:	Date	2:
Search	ners:		

Note: Time should include all time spent on the beach, even when backtracking. North and south endpoints should be GPS pts. If not, please provide a good description of the area covered. For collected animals, put GPS location here.

	Beach Name	Start Time	End Time	North/West Extreme (Lat/Long)	South/East Extreme (Lat/Long)	Total Distance Searched	Method (foot, ATV, scan)	Mammals Collected Note: (live/ dead, GPS, ID #)
A								
В								
C								
D								
E								
F								
G								
Н								
Ι								
J								
K								

Oiled Marine Mammal Data Log: LIVE Animals

Oil Spill Name:	.e:			Facility:					
Intake Log Number (L-xxxx)	Date Collected (m/d/y)	Time Coll'ted (24 hr)	First Initial & Last Name of Collector	Beach Search Number	Collection Location (Beach Name)	GPS Coordinates (N) GPS Field Tag Coordinates (W) #	Field Tag #	Date Arrived (m/d/y)	Time Arrived (24 hr)
						111			
							8 s 1		71

Front Side of Page of

Oiled Marine Mammal Data Log: LIVE Animals (continued from front side)

Oil Spill Name:				Facility:								
Intake Log Number	Date Processed (m/d/y)	Time Processed (24 hr)	First Initial & Last Name of Examiner	Species	Level A Field #	Tag Color/#	% Oiled	Sample/ Photo Taken? (Y/N)	Disposition Date (m/d/y)	Disposition Status (R,D,E,T)	Release Tag#	Morgue ID
					-							
0					. 6	1 2 3				0 1		
					-							

ਰ

Back Side of Page_

Oiled Marine Mammal Data Log: DEAD Animals

Oiled Marine Mammal Data Log: DEAD Animals (continued from front side)

	Notes (any other observations, contamination by petroleum products such as plastic or another specimen)						
	(any other						
	Necropsy (Y/N)			- 1			
	Morgue						
	AG (cm)						
	SL (cm)						
	Sex						of
	Age			- 4			
	Tag #						of Page
Facility:	Level A Field#						Back Side of Page
	Sample/ Photo Taken? (Y/N)						
	Depth Oiled						
	bəliO %						
	angia gnilio						
	Scavenging						
He:	Condition						
Oil Spill Name:	Intake Log Number (D-xxxx)						

		Oiled Marine Ma	mmal Inta	ake Form
Spill N	lame:		Level A Field #:	Log Number:
URE	Capture Date/Time:		Capture Location	n:
CAPTURE	Field Band:		Collector:	
SSING	Intake Date/Time:		Species:	
PROCESSING	Tag Color/#:		Examiner's Signo	ature:
D	Signs of Oiling	Oil Visible Skin Burns Smell	Area Oiled	Head Body Multiple Entire
EXT. OIL ID	Oil Color	Black Brown Clear Other	Depth of Oiling	Deep Moderate Surface
(i)	% Oiled	<2% 2-25% 26-50% 51-75% 76-100%	Samples	Hair Swab Photo
	Weight/Temp.	grams °F	Age	Pup Sub-adult Adult Unknown
	Std Length/Girth	cm cm	Sex	Male Female
	Heart Rate	WNL beats/min.	Body Condition	Normal Thin Emaciated
	Resp. Rate	WNL breaths/min.	Attitude	BAR QAR Nonresponsive Seizing
	Dehydration	None Mild Moderate Severe	CRT/mm color	Sec. / Pink Pale White Purple
W	Human Interaction	☐ Yes ☐ No Type: Boat Collision, Shot, Fisl	neries, Other:	
PHYSICAL EXAM	Neurologic	NSF Other:		
HYSIC	Head/Mouth	NSF Other:		
_	Eyes/Ears	NSF Other:		
	Heart/Lungs	NSF Other:		
	Gastrointestinal	NSF Other:		
	Musculo-skeletal	NSF Other:		
	Integument	NSF Other:		
	Comments			
TX-DX	Blood taken? HCT	LTT RTT GTT Toxiban: yes	no time:	
<u>~</u>	Pre-wash Exam:	Veterinarian Signature	e Washed :	Weight: Bloodwork Attached □
Z		Veterinarian Signature	n Date:	Weight: Bloodwork Attached □
DISPOSITION	Disposition Date:	Disposition Location:		
DISP	Disposition Status: R			AINED Necropsied by:
	Flipper Tag No.:	Location: RF	LF	RH LH

Appendix 5. Oiled Marine Mammal Daily Progress Form

OILED MARINE MAMMAL DAILY PROGRESS FORM SpillName_ _Log# ___ __ Tag Color/#_ Species_ Treatment and Progress Notes Date Init.

Appendix 6. Oiled Marine Mammal Evidence Collection Protocol

The photograph and oil sample are both considered to be legal evidence therefore it is important that the following procedures are followed closely.

Photograph Evidence

- 1. Use a Polaroid camera (if possible).
- 2. Photograph should include the entire animal, highlighting the oiled region, and if possible, the tag number.
- 3. Label the photograph with Spill Name, Date, Species, Log #, Capture Location, and Tag # and Color.

Sample Collection Techniques for Visible Oiling

- 1. Scrape visible oil from fur/skin with wooden spatula (tongue depressor).
- 2. Place oil covered spatula in solvent-rinsed glass jar with a Teflon-lined lid (e.g. I-Chem) and break off the remaining un-oiled portion, allowing the lid to close. If jar is not available, wrap sample in aluminum foil (dull side to sample).

Note: Avoid touching /contaminating oil sample with your nitrile gloves.

- 3. Label the glass jar (use waterproof labels).
 - Label must include: Spill Name, Log #, Species, Tag #, Arrival Date, Sampling Date, and Capture Location.
- 4. Fill out Custody Seal and apply it across the lid of the jar and onto the sides of the glass.
- 5. Keep sample refrigerated or on ice until it can be stored.
- 6. Lock sample in a -20°C (or colder) freezer.

Sample Collection Techniques for No Visible Oiling

- 1. Rub an affected area with a 4x4 fiberglass or cotton cloth (or gauze) with sterile forceps or hemostats that have been cleaned with isopropyl alcohol.
 - **Note:** Do not allow the nitrile gloves to touch the oiled area or the cloth.
- 2. Place the oiled covered cloth into a solvent-rinsed glass jar with a Teflon-lined lid.
- 3. Seal and fill out the information on the waterproof label (as above).
- 4. Fill out the Custody Seal and apply it across the lid of the jar and onto the sides of the glass.
- 5. Keep sample refrigerated or on ice until it can be stored
- 6. Lock sample in a -20°C (or colder) freezer.

All evidence should be securely stored and refrigerated/frozen until the Wildlife Branch Director provides further instructions. If samples are to be sent for analysis, a Chain of Custody Form is required.

Appendix 7. Petroleum Hydrocarbon Tissue Sampling Protocol

Supplies for sampling

All instruments used in handling (e.g. scalpels and forceps, cutting boards) or storing (e.g. jars, foil, sheets) samples must be made of a non-contaminating material consisting of stainless steel, glass, Teflon, or aluminum.

- Solvent-rinsed glass containers with Teflon-lined lids for tissues
- Solvent-rinsed Teflon sheets for tissues
- Aluminum foil (if Teflon sheets are not available) sample to the dull side
- Sterile syringes and needles
- Amber glass vials or glass vials covered with foil with Teflon lids (for bile, urine)
- Teflon screw top vials (for blood storage and urine)
- Stainless steel scalpels, knifes, forceps
- Isopropyl alcohol (99.9% pesticide free IPA) to rinse instrument
- Wooden tongue depressors (can be used to handle tissues if necessary)
- Whirl-pak bags or Zip-lock freezer bags
- 10% buffered formalin and appropriate containers for histopathology samples
- Permanent marker or pen
- Evidence/Custody tape and labels
- Sample Log/Chain of Custody forms

Sampling Protocol

Tissues to collect for petroleum hydrocarbon analysis in order of preference:

- a. bile
- b. urine
- c. whole blood
- d. stomach and intestinal contents
- e. blubber/fat
- f. liver
- g. kidney
- h. lung
- i. intestine
- j. brain
- k. muscle
- i. Samples taken for analysis should only be collected from **alive** or **freshly dead animals**. If a necropsy cannot be performed within 24 hrs after death, the carcass should be frozen for later examination.
- ii. Recommended minimum sample size is 10-20 g of tissues (approx. 1-2 tablespoons) and 5 ml for fluids (blood, urine, bile, feces, stomach contents). However, analysis can be performed on as little as 100 μL of bile; therefore collect whatever amount is present.

Appendix 7. Petroleum Hydrocarbon Tissue Sampling Protocol, page 2

- Fluids such as blood, urine, and bile should be collected using sterile syringes or pipettes and transferred to Teflon vials (blood) or amber glass vials (bile, urine).
- iv. Use powder-free nitrile gloves. Vinyl gloves are an acceptable alternative. Avoid contact of gloves with samples.
- v. Scalpels, knifes, and cutting tools used for tissue collection should be cleaned and rinsed with isopropyl alcohol between tissues. If heavily contaminated with oil, instruments can be cleaned with detergent (e.g. Dawn), rinsed with water, and then rinsed with alcohol.
- vi. Samples are stored preferably in solvent-rinsed Teflon-lined glass jars, labeled, and secured with evidence tape/custody seal. If glass jars are not available, samples can be placed in Teflon sheets or aluminum foil (dull side to sample) and stored in whirl-paks/freezer bags.
- vii. If samples/tissues have come in contact with a contaminating material (e.g. plastic bag), collect and store a representative example of that material (e.g. plastic bag) using the same method as for collecting tissues.
- viii. Collect a representative sample of each tissue (< 1 cm thick) preserved in 10% buffered formalin for histopathology. Duplicate hydrocarbon and histology samples whenever possible.
- ix. Each sample must be labeled with **Spill Name**, **Log #**, **Level A Field #**, **Species**, **Tag#**, **Arrival Date**, **Sampling Date**, and **Capture Location** and securely stored.
- x. Samples for PAH analysis should be chilled immediately on ice/refrigeration and then frozen as soon as possible to -20°C or colder in a locked freezer. Histopathology samples are stored at room temperature.

All evidence should be securely stored and refrigerated/frozen until the Wildlife Branch Director provides further instructions. If samples are transferred to a different location or sent for analysis, a Chain of Custody form is required. A Chain of Custody form can be found in this document, but are often provided by the laboratory.

Shipping:

Ship samples frozen on blue ice or with ~5 lbs dry ice according to laboratory specification using Federal Express (FedEx). FedEx follows IATA regulations for shipping hazardous materials and maintains chain of custody record by tracking packages.

Sampling supplies such as jars, label, and custody seals are often supplied by the analytical laboratory and are produced by:

I-Chem[™] Brand, Certified 300 Series jars Order: 1-800-451-4351, www.ichembrand.com

Appendix 8. Oil Spill Response Laboratories

Laboratories with tissue petroleum hydrocarbon analysis expertise

Northwest Fisheries Science Center 2725 Montlake Boulevard East	Alaska Fisheries Science Center Auke Bay Laboratory
Seattle, WA 98112-2097	11305 Glacier Highway
Jon Buzitis, (206) 860-3309	Juneau, Alaska 99801-8626
Gina Ylitalo, (206) 860-3325	Jeep Rice, (907) 789-6020
Petroleum Chemistry Laboratory	TDI-Brooks International
Office of Spill Prevention and Response	1902 Pinon
California Department of Fish and Game	College Station, TX 77845
1995 Nimbus Rd	(979) 693-3446
Rancho Cordova, CA 95670	Thomas McDonald, (979) 220-3821
(916) 358-2803	, ,
Alpha Woods Hole Laboratories	Zymax Forensics
375 Paramount Drive	71 Zaca Lane
Raynham, MA 02767	San Luis Obispo, CA 93401
Peter Kane, (508) 822-9300	(805) 544-4696
	Alan Jeffrey, (805) 546-4693
Mote Marine Laboratory	Geochemical & Environmental Research
1600 Ken Thompson Parkway	Group (GERG)
Sarasota, Florida 34236	Texas A&M University
(941) 388-4312	833 Graham Road
Dana Wetzel, (941) 388-4441	College Station, Texas 77845
	(979) 862-2323

The laboratory should be able to perform analysis of the 16 traditionally-studied, parent PAHs listed as priority pollutants by the Environmental Protection Agency (EPA) in addition to the 44 alkylated and heterocyclic PAHs.

Unified Command and Trustee Agencies will make final decision on laboratory use.

O	iled Marir	ne Mamn	nal	Form completed	by:	Dat	te:
	Gross Necr	opsy Report		Enforcemnent O	fficer:		
Spill Name:				Strand/Capture I	ocation:		
Animal Log#	Į –			Collecters Name	\$		
Level A Field	#	Tag#		Intake date:			Euthanasia
Species:		777		Death date:		Time:	□yes
Sex:		Age:		Post mortem dat	e:	Time:	□no
Weight:	ka	. (estimate/ad	etual)	Carcass Classi		Frozen: 🗆 y	
						The state of the s	
Measurement	ciated 1 2 3 4	Blubber der		2-πesn, Photographs: [intact), 4-poor, 5-	- mummmed
	- 18		dispersion of the control of the con				
SL cm	AG cm	UG cm	XG cm	Radiographs:			
Clinical signs/o							
Antibiotics give							
Pertinent lab re	esuits						
GROSS NECF	ROPSY ABNOR	RMALITIES:					
-							
é							
HYDROCARE	SON ANALYSIS	SAMPLES		MICROBIOLOG	Ŷ	SWABS: oth	ier
bile	kidney	blubber/fat		Lung		a Carolina	
blood	muscle	other:		Liver			
urine liver	lung intestine			other 🗆		4	
liver	A DATE OF THE CONTRACT			11			
HISTOLOGY S	of the second second	iloum	kidnov	mamman/ sland	mucala	LYMPH NODES	
lung fraches	thyroid	ileum	kidney	mammary gland	muscle	Me that a draw way	
trachea heart	tonsil	colon	ureter	adrenal	gonad	colonic	gastric
heart	tongue	pancreas	urinary bladder	skin	prostate	sublumbar	hepatic
aorta	esophagus	spleen liver	urethra blubber	eye (L/R)	uterus	inguinal	mediastinal submandibular
pulmonary artery	stomach duodenum	gall bladder	bone marrow	fatsite:	∨agina cervix	axillary mesenteric	tracheobronchial
thymus salivary aland	A STATE OF STATE OF	300000000000000000000000000000000000000		whole repro		mesentenc	nacieonionenial
salivary gland other:	jejunum	brain	spinal cord	whole repro	penis		
	th (proliminant	diagnosis).				-	
A-5-2-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1	th (preliminary	ulagilosis):	Figure 100	Cross States		Baire	
Examiner:		4 4	Examiner's	signature:		Date:	

NOAA (F	CHA	AIN OF CU	JSTODY REC	ORD (1997)	Case Number:
DATE AND		DUTY STATIC	ON:	EVIDENCE/PR	OPERTY SEIZED BY:
or location) TA RE			(person and/ DEFEN	DANT/COMPANY NAI	ME AND REMARKS:
ITEM NO:	DESCRI	TION OF EVID	ENCE/PROPERTY (inc	lude Seizure Tag numbers a	nd any serial numbers):
ITEM NO:	FROM (PRI	NT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: FEDEX
	TO (PRINT	NAME, AGENCY)	RECEIPT SIGNATURE	RECEIPT DATE:	U.S. MAIL IN PERSON OTHER:
ITEM NO:	FROM: (PRI	NT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: FEDEX
	TO: (PRINT	NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE:	U.S. MAIL IN PERSON OTHER:

Appendix 10b. Chain of Custody Form, page 2

ITEM NO:	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	
				DELIVERED VIA: FEDEX
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE	RECEIPT DATE:	U.S. MAIL IN PERSON OTHER:
ITEM NO:	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: FEDEX
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE:	U.S. MAIL IN PERSON OTHER:
ITEM NO:	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: FEDEX
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE	RECEIPT DATE:	U.S. MAIL IN PERSON OTHER
ITEM NO:	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: FEDEX
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE:	U.S. MAIL IN PERSON OTHER:
ITEM NO:	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: FEDEX
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE	RECEIPT DATE:	U.S. MAIL IN PERSON OTHER:
ITEM NO:	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: FEDEX
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE:	U.S. MAIL IN PERSON OTHER:

Intentional Blank Page