RECOMMENDED GUIDELINES FOR SAMPLING MARINE MAMMAL TISSUE FOR CHEMICAL ANALYSES IN PUGET SOUND

For

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CONTENTS

Page

Page

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS

BHC	gamma hexachlorocyclohexane
CITES	Convention on International Trade in Endangered Species
EPA	U.S. Environmental Protection Agency
MARC	Marine Animal Resource Center
MMPA	Marine Mammal Protection Act
NMFS	National Marine Fisheries Service
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PSEP	Puget Sound Estuary Program
PSP	paralytic shellfish poisoning
QA/QC	quality assurance and quality control

ACKNOWLEDGMENTS

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This chapter was updated in 1994 by Mr. Michael E. Wheeler of the Puget Sound Water Quality Authority, which has taken on the task of maintenance of the Puget Sound Protocols. The update process included another round of review by the review committee to which two additional reviewers on the Canadian side of Puget Sound were added.

INTRODUCTION

Recommended guidelines for sampling and analyzing marine mammal tissue for chemical contaminants in Puget Sound are presented in this chapter. The guidelines are based on the results of a workshop sponsored by the Puget Sound Estuary Program (PSEP) and written reviews by representatives from most of the organizations that fund or conduct studies of marine mammals in the sound (Table 1). The purpose of developing these recommended guidelines is to encourage all Puget Sound investigators conducting monitoring programs, baseline surveys, and intensive investigations to use standardized methods whenever possible. If this goal is achieved, most data collected in the sound should be directly comparable, thereby allowing the data to be integrated into a soundwide database. Such a database is necessary for developing and maintaining a comprehensive water quality management program for Puget Sound.

Before the recommended guidelines are described, the background information that led to many of the recommendations in this document is presented. The *Background Information* section addresses the following topics:

- The rationale for studying tissue contamination in marine mammals
- The legal issues involved in marine mammal studies
- An overview of the marine mammals in Puget Sound and considerations for sampling these animals
- A prioritization scheme for sampling and analysis activities.

Following this background section, specifications are provided for the field, laboratory, quality assurance and quality control (QA/QC), and data reporting procedures that are recommended for studies of tissue contamination in marine mammals from Puget Sound.

Although the following guidelines are recommended for most studies conducted in Puget Sound, departures from these methods may be necessary to meet the special requirements of individual projects. If such departures are made, however, the funding agency or investigator should be aware that the resulting data may not be comparable with most other data of that kind. In some instances, data collected using different methods may be compared if the methods are intercalibrated adequately.

TABLE 1. CONTRIBUTORS TO THE MARINE MAMMAL TISSUE SAMPLING GUIDELINES

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BACKGROUND INFORMATION

This section presents background information and sources of this information that were used to formulate the recommendations provided in this report.

RATIONALE FOR MARINE MAMMAL TISSUE STUDIES

The potential impacts of chemical contaminants on marine mammals are a major concern for several reasons. Because the toothed cetaceans and pinnipeds feed high on the food chain, have relatively high lipid levels in some tissues (e.g. blubber), and spend all or parts of their lifes in contaminated areas, they have a high potential for bioaccumulating chemical contaminants such as lipophilic organic compounds in lipid reservoirs (e.g., blubber). In Puget Sound and elsewhere, relatively high levels of chemical contaminants have been found in marine mammal tissue (discussed in detail later). In addition, studies have documented relationships between chemical contaminants and adverse effects (e.g., reproductive problems and population declines in pinnipeds).

In general, the potential effects of chemical contaminants on marine mammals have been relatively difficult to evaluate. Major limitations include the logistical, legal, and ethical constraints that make experimental studies on captive animals or collection of animals difficult. An alternative to these direct assessment techniques is a comprehensive study of the biology of a marine mammal population coupled with analyses of contaminants in marine mammal tissues. To date, relatively few of these comprehensive studies have been conducted.

Analysis of tissues for contaminants can be costly; scans for a broad range of pollutants cost over $2,000 per sample. Analysis of all tissues available from marine mammals is not financially feasible. Before expensive analyses are performed, it is important to determine priorities to ensure that analysis is performed only for those chemicals that are most informative and to ensure that tissue samples have been collected in a manner that allows meaningful interpretation of the results.

SOURCES OF INFORMATION

The guidelines presented in this document were derived primarily from the information and procedures presented in several earlier documents. The most valuable sources of information include:

- National Status and Trends Program for Marine Environmental Quality Specimen Bank Project: Field Manual (Lauenstein et al. 1987)

- Recommended guidelines for analysis of organic compounds and metals in tissue samples from Puget Sound (PSEP 1989a,b)

- Field Manual for Research on Seals (DeLong and Risebrough in press)

- Alaskan Marine Mammal Tissue Archival Project: A Project Description Including Collection Protocols (Becker et al. 1988)

- National Marine Mammal Tissue Bank Sampling and Archival Protocols (NMFS, in preparation)

- A Field Manual of Procedures for Postmortem Examination of Alaskan Marine Mammals (Fay et al. 1979).

- Marine Mammals Ashore. A Field Guide for Strandings (Geraci and Lounsbury 1993).

The procedures that were described in these sources often varied substantially. Some procedures, such as those for long-term tissue banking, are necessarily more restrictive than typical collection procedures.

LEGAL ISSUES RELATED TO COLLECTION OF MARINE MAMMAL TISSUES

The Marine Mammal Protection Act (MMPA) of 1972 broadly prohibits the "taking" of marine mammals. "Taking" includes killing, injuring, or harassing live animals, as well as possessing or collecting marine mammals or parts of marine mammals. Specific exceptions are made for subsistence use by native Americans, public display [under a National Marine Fisheries Service (NMFS) permit], and scientific research (under an NMFS permit). Although collection of marine mammal tissues can be granted under an NMFS scientific research permit, NMFS more frequently grants members of regional stranding networks general authority to handle stranded marine mammals and collect samples. A scientific research permit is required for the direct killing of animals for research or biopsy sampling of live, healthy animals.

NMFS oversees regional stranding networks that investigate marine mammals that become stranded (either alive or dead). Along the U.S. coast, including Puget Sound, these networks consist largely of scientists from government agencies, universities, and private groups. Because limited funding is available for examination of marine mammal strandings, participants in these networks often are volunteers.

The Northwest Marine Mammal Stranding Network responds to strandings in Washington and Oregon. Calls from the public or federal, state, and local authorities generally are channeled

through the Washington State Patrol to the stranding response centers (Figure 1). Designated Primary Response Centers coordinate the investigations of marine mammal strandings within a designated area throughout the region. The team that responds to the stranding is required to follow the procedures developed by the NMFS network (Figure 2). All stranding reports or responses are reported to NMFS. In addition, any parts sampled from stranded marine mammals must be returned to or registered with NMFS. Examples of an NMFS stranding report and a registration form are shown in Figure 3.

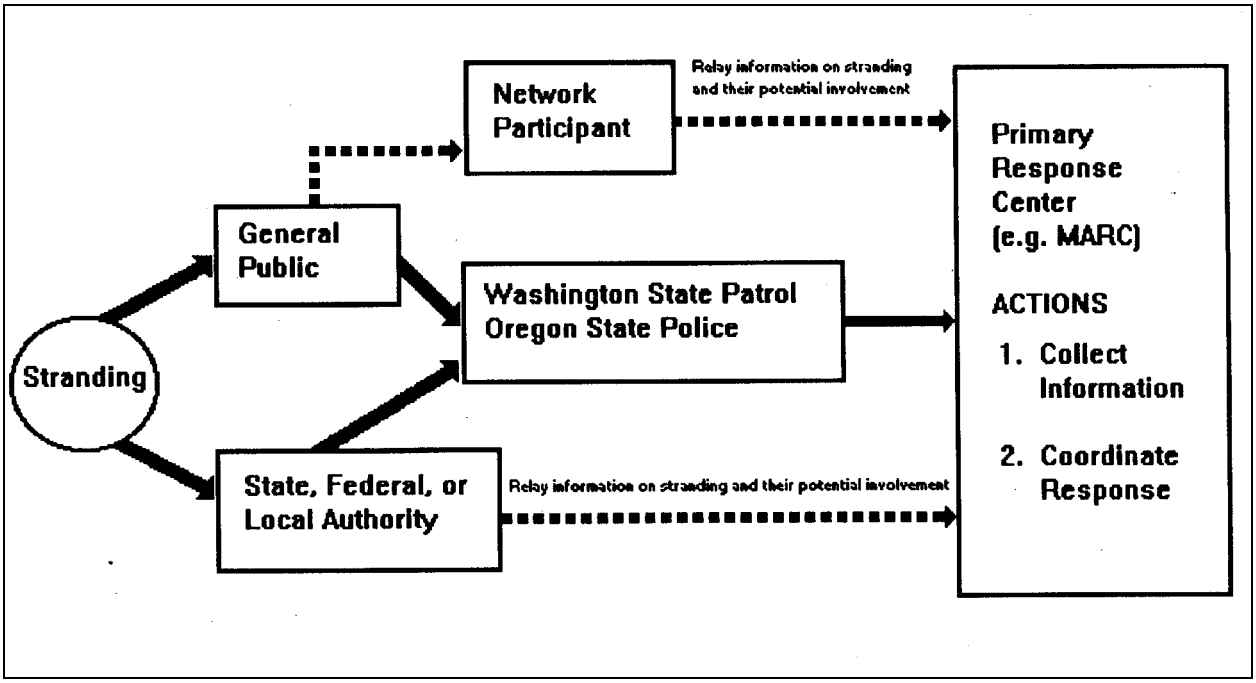


Figure 1. Northwest Marine Mammal Stranding Network notification procedures

OBJECTIVES FOR MARINE MAMMAL CONTAMINATION STUDIES

The objectives of research on chemical contaminants in marine mammals can encompass a number of areas. The number and kinds of samples collected and the analyses to be performed depend on the primary objective of each study. Previously, most studies have addressed the following general objectives (not prioritized):

- Evaluate potential effects of contaminants on marine mammals
- Evaluate temporal trends in contaminant concentrations
- Use marine mammals as indicators of contaminant levels in the marine environment
- Evaluate regional and worldwide patterns of marine contamination
- Provide information and insights into the biology of marine mammals (e.g., stock identification).

NEED FOR NATURAL HISTORY INFORMATION

The interpretation of contaminant levels in tissues of marine mammals requires knowledge of the natural history, anatomy, and physiology of marine mammal species. Some of this information can be gathered as ancillary data when the tissues of animals are examined and collected (see *Ancillary Data*). Other biological information (e.g., related to population trends, movements, and reproductive success) usually can be obtained from studies that address basic research. To achieve most of the objectives described in the previous section, it is essential that data on contaminant levels be interpreted with respect to available natural history information.

MARINE MAMMALS IN PUGET SOUND AND ADJACENT WATERS

Table 2 lists marine mammal species that have stranded on Oregon and Washington coasts in recent years. The number of strandings provides an indication of which species are likely to be available for sampling. A smaller number of marine mammal species are common to Puget Sound (i.e., south of Admiralty Inlet) (Table 3). Background information on these species is given in Osborne et al. (1988) and Angell and Balcomb (1982). This information is summarized below and in Table 3.

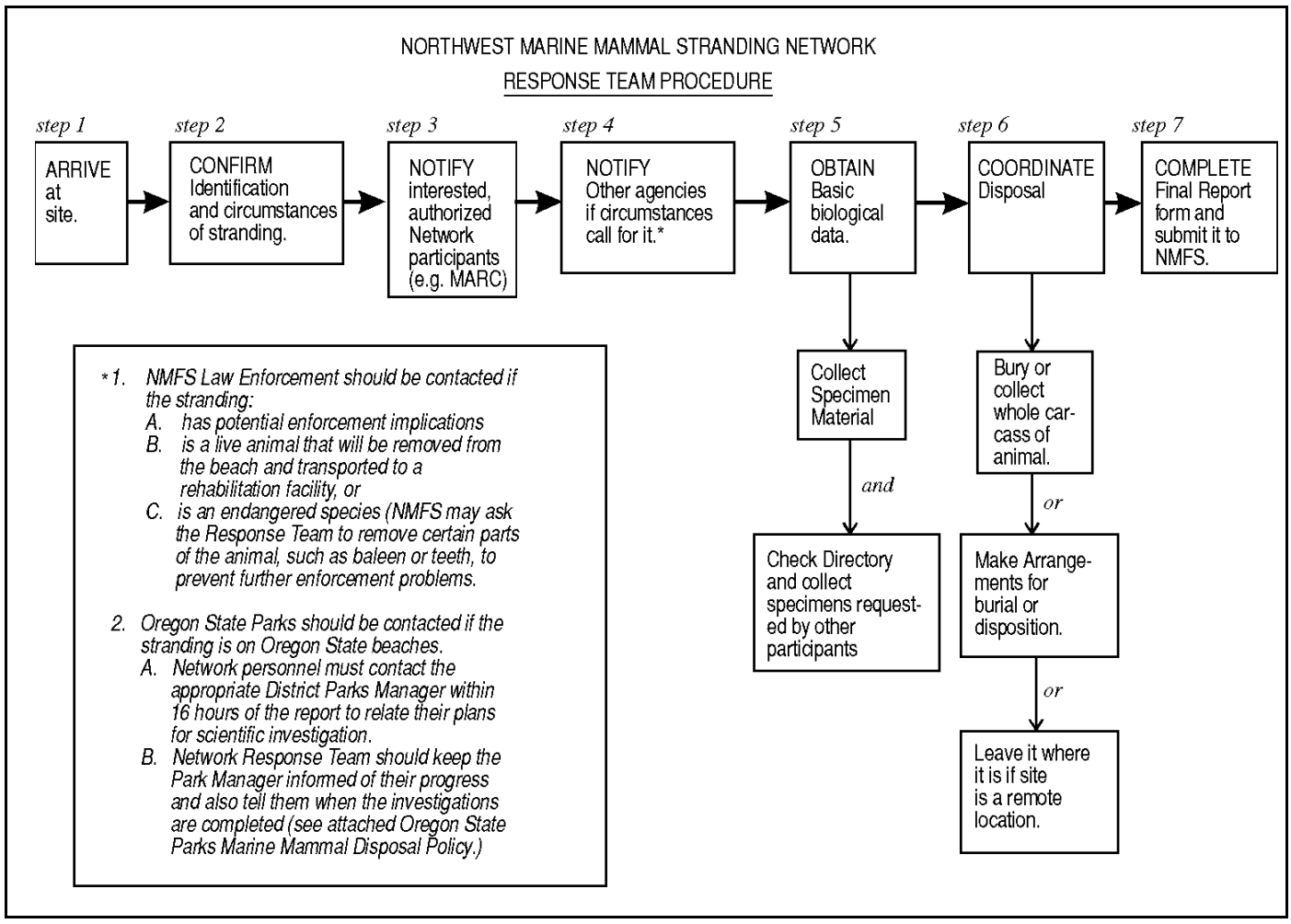
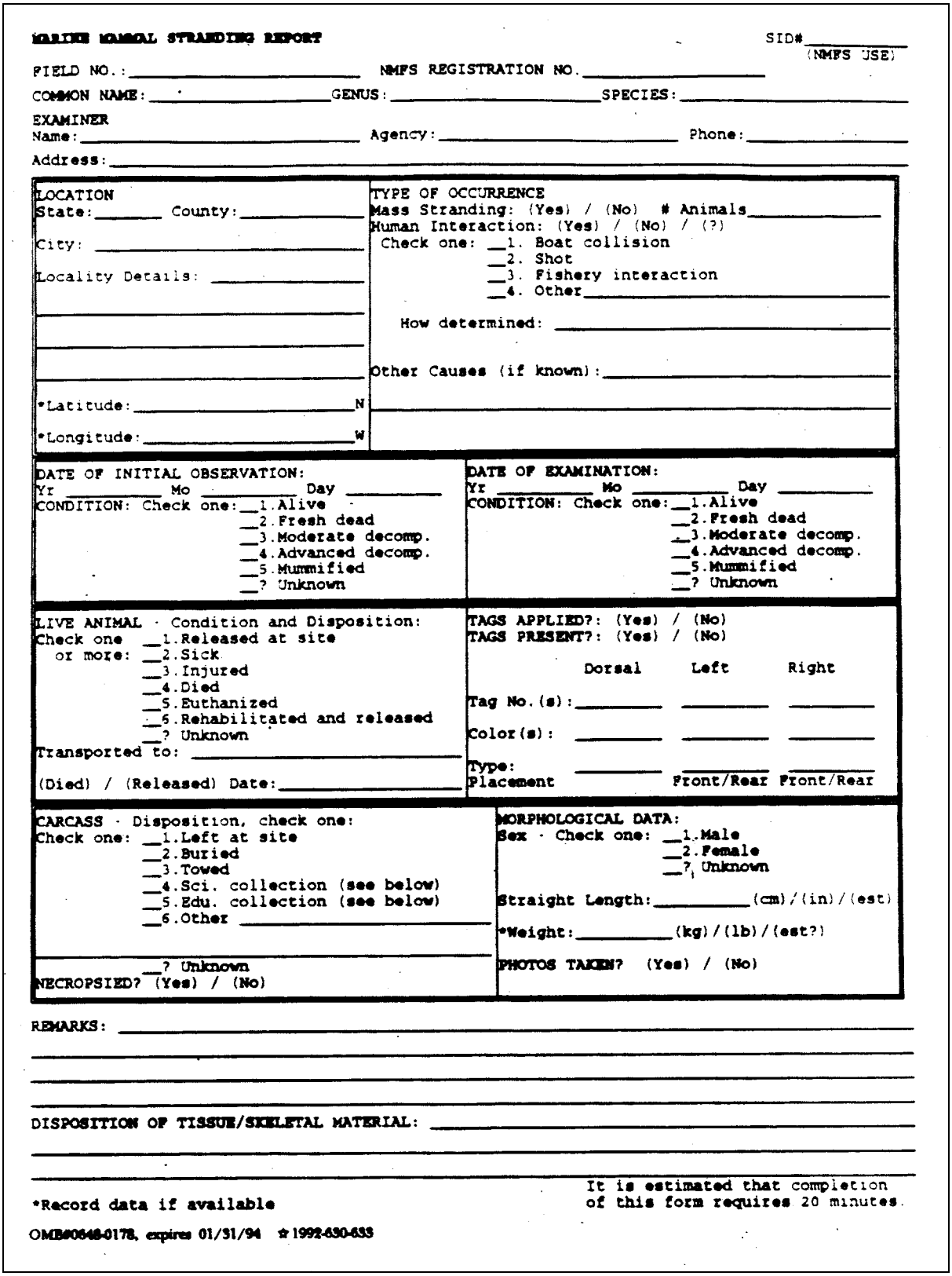


Figure 2. Northwest Marine Mammal Stranding Network response team procedure (from NMFS 1988)



MARINE MAMMAL STRANDING REPORT

SID#_____ (NMFS USE)

FIELD NO.:_____ NMFS REGISTRATION NO._____

COMMON NAME:_____ GENUS:_____ SPECIES:_____

EXAMINER
Name:_____ Agency:_____ Phone:_____

Address:_____

LOCATION
State:_____ County:_____

City:_____

Locality Details:_____

*Latitude:_____N

*Longitude:_____W

TYPE OF OCCURRENCE
Mass Stranding: (Yes) / (No) # Animals_____
Human Interaction: (Yes) / (No) / (?)
Check one: __1. Boat collision
__2. Shot
__3. Fishery interaction
__4. Other_____

How determined:_____

Other Causes (if known):_____

DATE OF INITIAL OBSERVATION:
Yr _____ Mo _____ Day _____
CONDITION: Check one: __1.Alive
__2.Fresh dead
__3.Moderate decomp.
__4.Advanced decomp.
__5.Mummified
__? Unknown

DATE OF EXAMINATION:
Yr _____ Mo _____ Day _____
CONDITION: Check one: __1.Alive
__2.Fresh dead
__3.Moderate decomp.
__4.Advanced decomp.
__5.Mummified
__? Unknown

LIVE ANIMAL - Condition and Disposition:
Check one or more: __1.Released at site
__2.Sick
__3.Injured
__4.Died
__5.Euthanized
__6.Rehabilitated and released
__? Unknown
Transported to:_____

(Died) / (Released) Date:_____

TAGS APPLIED?: (Yes) / (No)
TAGS PRESENT?: (Yes) / (No)

	Dorsal	Left	Right
Tag No.(s):			
Color(s):			
Type:			
Placement		Front/Rear	Front/Rear

CARCASS - Disposition, check one:
Check one: __1.Left at site
__2.Buried
__3.Towed
__4.Sci. collection (see below)
__5.Edu. collection (see below)
__6.Other _____
__? Unknown
NECROPSIED? (Yes) / (No)

MORPHOLOGICAL DATA:
Sex - Check one: __1.Male
__2.Female
__?, Unknown

Straight Length:_____(cm)/(in)/(est)

*Weight:_____(kg)/(lb)/(est?)

PHOTOS TAKEN? (Yes) / (No)

REMARKS:_____

DISPOSITION OF TISSUE/SKELETAL MATERIAL:_____

*Record data if available

It is estimated that completion of this form requires 20 minutes.

OMB#0648-0178, expires 01/31/94 ☆1992-630-633

Figure 3. Northwest Marine Mammal Stranding Network stranding report and tissue registration

TABLE 2. NUMBER OF STRANDINGS OF DIFFERENT SPECIES IN WASHINGTON AND OREGON

	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	Total
Harbor seal *Phoca vitulina*	92	99	48	88	95	68	114	210	220	207	113	1464
California sea lion *Zalophus californianus*	19	10	6	27	34	7	33	170	48	28	20	402
Northern sea lion *Eumetopias jubatus*	10	7	5	15	16	5	16	16	11	5	4	110
Northern elephant seal *Mirounga angustirostris*	2	4	4	7	1	5	11	8	12	12	4	70
Northern fur seal *Callorhinus ursinus*	4	6	0	53	4	2	7	4	4	1	0	85
Harbor porpoise *Phocoena phocoena*	3	1	7	9	13	14	8	16	22	15	31	139
Dall's porpoise *Phocoenoides dalli*	0	2	2	3	5	4	4	4	3	5	1	32
Pacific white-sided dolphin *Lagenorhynchus obliquidens*	1	2	0	2	1	5	5	4	0	2	0	22
Common dolphin *Delphinus delphis*	0	0	0	0	0	0	1	0	1	0	0	2
Striped dolphin *Stenella coeruleoalba*	0	0	0	1	0	1	0	0	0	0	1	3
Northern right whale dolphin *Lissodelphis borealis*	0	0	0	2	1	0	0	0	0	0	0	3
Pygmy sperm whale *Kogia breviceps*	1	0	0	1	0	0	0	1	1	0	0	4
Risso's dolphin *Grampus griseus*	0	0	0	0	0	0	0	0	1	0	1	2
Pilot whale *Globicephala macrorhynchus*	1	0	0	1	0	0	0	0	0	0	0	2
False killer whale *Pseudorca crassidens*	0	0	0	0	0	0	0	1	0	0	1	2
Killer whale *Orcinus orca*	1	0	0	0	0	0	1	0	0	0	1	3
Cuvier's beaked whale *Ziphius cavirostris*	0	0	2	1	0	1	2	1	0	2	2	11
Steineger's beaked whale *Mesoplodon steinegeri*	0	0	1	1	1	0	0	1	0	3	0	7
Hubbs' beaked whale *Mesoplodon carlhubbsi*	0	0	0	0	1	0	0	0	0	0	0	1
Gray whale *Eschrichtius robustus*	6	3	3	8	11	2	14	16	2	4	10	79
Humpback whale *Megaptera novaeangliae*	0	1	0	1	0	0	0	0	0	0	0	2
Sperm whale *Physeter catodon*	1	0	43	0	0	2	0	0	1	1	0	48
Minke whale *Balaenoptera acutorostrata*	0	0	0	5	1	1	0	1	2	1	1	12
Fin whale *Balaenoptera physalus*	0	0	0	0	0	0	0	0	0	1	0	1
Blue whale *Balaenoptera musculus*	0	0	0	1	0	0	0	0	0	0	0	1
Unidentified sea lion	57	42	17	4	0	6	11	311	67	38	28	581
Unidentified pinniped	22	0	0	3	7	4	0	29	75	48	26	214
Unidentified small cetacean	4	2	2	0	1	0	0	0	2	1	1	13
Unidentified large cetacean	2	0	0	0	0	1	0	0	0	2	1	6
Total	**226**	**179**	**140**	**233**	**192**	**128**	**227**	**893**	**481**	**376**	**246**	**3321**

[a] Includes strandings that were investigated and strandings that were reported but not investigated. Based on reports from the Northwest Marine Mammal Stranding Network as compiled and reported by Scordino (in press).

TABLE 3. SELECTED CRITERIA FOR DETERMINING THE SUITABILITY OF MARINE MAMMALS FOR CONTAMINANT-RELATED RESEARCH IN PUGET SOUND AND ADJACENT WATERS

	Species[a,b]										
	Pv	Ma	Zc	Ej	Cu	Oo[c]	Pp	Pd	Lo	Er[c]	Ba
Feed high on food chain	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
Year-round residents											
Puget Sound	Y	N	N	N	N	N	N	Y	N	N	N
Adjacent Waters	Y	N	N	N	N	Y	Y	Y	Y	N	N
Feed in Puget Sound	Y	N	Y	Y	N	Y	N	Y	N	Y	N
High accumulation of contaminants documented	Y	Y	N	Y	N	Y	Y	N	N	N	N
Impacts of contaminants	Y	N	N	N	N	Y	Y	N	N	Y	N
Biological information available for Puget Sound (population size, reproduction, and mortality)	Y	N	Y	N	Y	Y	Y	N	N	N	Y
Large population in Puget Sound	Y	N	Y	N	N	N	N	N	N	N	N
Fresh samples frequently available as incidental fishery entanglements	Y	N	Y	N	N	N	Y	Y	N	N	N
Suitable for biopsy sampling	Y	N	Y	Y	N	Y	N	N	N	Y	Y

[a] Pv *Phoca vitulina*, Harbor sea
Ma *Mirounga angustirostris,* Northern elephant seal
Zc ***Zalophus*** *californianus*, California sea lion
Ej *Eumetopias jubatus,* Northern sea lion
Cu *Callorhinus ursinus,* Northern fur seal
Oo *Orcinus orca,* Killer whale
Pp *Phocoena phocoena,* Harbor porpoise
Pd *Phocoenoides dalli,* Dall's porpoise
Lo *Lagenorhynchus obliquidens,* Pacific white-sided dolphin
Er *Eschrichtius robustus,* Gray whale
Ba *Balaenoptera acutorostrata,* Minke whale.

[b] Criteria scored as yes (Y) or no (N).

[c] This species has been regarded with special concern by the public in the Puget Sound region.

Harbor Seal (*Phoca vitulina richardsi*)

This species is the most numerous marine mammal in Puget Sound. Populations have been increasing in recent years at a rate of about 10-15 percent per year (Calambokidis et al. 1985, 1988). Over 5,000 harbor seals inhabit the inland waters of Washington state with about 1,000 of these in southern Puget Sound (Osborne et al. 1988). Harbor seal numbers are fairly low in the central Puget Sound areas near the most contaminated urban embayments, although the reason for this pattern is unknown (Calambokidis et al. 1985). Harbor seals are year-round residents of Puget Sound. Although tagged harbor seals along the outer coast of Washington have been shown to make long-distance moves (Jeffries 1985), animals in Puget Sound appear to be primarily full-time residents of the sound. Large seasonal shifts in seal numbers have not been noted and the pupping season of Puget Sound harbor seals is different from that in neighboring areas outside the sound (Calambokidis et al. 1978, 1979).

California Sea Lion (*Zalophus californianus*)

These animals are primarily seasonal visitors to Puget Sound, although a few individuals may remain throughout the year (Steiger and Calambokidis 1986). There are no California sea lion breeding areas north of California and only the males migrate to Washington state to feed on a seasonal basis. The Washington Department of Wildlife and NMFS have been conducting research on California sea lions, particularly related to their predation on steelhead near the Ballard locks in Seattle (Gearin et al. 1986, 1988, 1989). As many as 1,000 California sea lions are found in Puget Sound in winter and spring (Gearin et al. 1986, 1988). California sea lions numbers were low prior to 1979, when an increasing number began aggregating near Everett, Washington (Everitt et al. 1980). This pattern appears to be the result of a general increase in the population of these animals throughout their range.

Northern Sea Lion (*Eumetopias jubatus*)

Relatively few northern sea lions occur in Puget Sound compared with the more common California sea lions (Osborne et al. 1988; Steiger and Calambokidis 1986). Although northern sea lions breed in areas both north and south of Washington state, there are no breeding areas within the state. In Puget Sound, northern sea lions are often seen among aggregations of California sea lions. The largest numbers of these animals were seen in south Puget Sound in the vicinity of Fox Island in the mid-1980s (Steiger and Calambokidis 1986).

Harbor Porpoise (*Phocoena phocoena*)

Harbor porpoises were formerly considered one of the most abundant cetaceans in Puget Sound (Scheffer and Slipp 1948). With the exception of a few rare sightings or strandings of single animals, they currently are absent from the sound (Osborne et al. 1988; Calambokidis et al. 1985; Everitt et al. 1980). Harbor porpoises remain relatively common along the outer coast of Washington, in the Strait of Juan de Fuca, and among the San Juan Islands (Osborne et al. 1988; Calambokidis et al. 1985, 1987).

Dall's Porpoise (*Phocoenoides dalli*)

Dall's porpoises are observed throughout the year in Puget Sound north of Seattle (Osborne et al. 1988). They also are seen occasionally in south Puget Sound. Although the exact population size is unknown, the numbers of these porpoises are considered relatively low in Puget Sound. Dall's porpoises are more common in the Strait of Juan de Fuca and the San Juan Islands.

Gray Whale (*Eschrichtius robustus*)

Most of the gray whale population, which numbers just under 20,000, travels past the coast of Washington during migration between Mexican breeding areas and principal feeding grounds in Alaska. However, a small number of individuals spend prolonged periods feeding in waters south of Alaska, including the inland waters of Washington state. Residence times of up to 4 months have been documented for gray whales in the Strait of Juan de Fuca (Calambokidis et al. 1987). Gray whales sometimes move into Puget Sound and feed there, with the number of animals and the residence times varying among years. Gray whales are predominately bottom feeders. Although they generally feed on benthic invertebrates which are low on the food chain, they are exposed to contaminants through the sediments they engulf with their food.

Killer Whale (*Orcinus orca*)

Killer whales travel and feed in discrete, stable groups called pods. Two separate types of killer whales occur in the Puget Sound area (Bigg et al. 1987; Osborne et al. 1988). "Resident" pods are primarily piscivorous (Bigg et al. 1990). The three resident pods in the Puget Sound area include more than 95 animals and appear to occupy a fairly limited range along the outer coast of Washington, in the San Juan Islands, and in areas to the north as far as central Vancouver Island.

These whales also enter Puget Sound to feed, primarily in the summer and fall. "Transient" pods travel in smaller groups and occupy a wider range than the resident pods. The transient pods observed in the Puget Sound area range as far north as southeast Alaska. Killer whales that travel in transient pods have a diet consisting largely of other marine mammals, including harbor seals, California sea lions, harbor porpoises, Dall's porpoises, and Stellar sea lions (Bigg et al. 1990; Jefferson et al. 1991).

Minke Whale (*Balaenoptera acutorostrata*)

Minke whales are seasonal visitors to the Puget Sound area (Osborne et al. 1988). They occur most commonly in the summer months in the Strait of Juan de Fuca and around the San Juan Islands (Dorsey 1983). Their principal prey are small schooling fish. Although minke whales visit Puget Sound proper, their residence time appears to be relatively short.

STUDIES OF CHEMICAL CONTAMINATION IN PUGET SOUND MARINE MAMMALS

Several studies have examined contaminant concentrations in Puget Sound marine mammals. Most research has been conducted on concentrations of chlorinated hydrocarbons [primarily polychlorinated biphenyls (PCBs) and DDT)] in harbor seals from different regions of Washington. Arndt (1973) measured concentrations of PCBs and DDT in blubber and liver tissue of 32 harbor seals collected in 1972 from Gertrude Island in south Puget Sound, Smith Island in north Puget Sound, and Grays Harbor on the outer coast of Washington. Anas (1974a) reported extremely high concentrations of PCBs plus DDE (levels combined) in the blubber of two harbor seals from Puget Sound.

The number of analyses of marine mammals other than harbor seals has been relatively limited in Puget Sound. Calambokidis et al. (1984) found that concentrations of PCBs and DDT in other species of marine mammals were generally lower than the levels observed in harbor seals, with the exception of killer whales and harbor porpoises. Concentrations of chlorinated hydrocarbons in killer whales were generally higher than the levels observed in harbor seals from the same area (Calambokidis et al. 1985, 1990). Concentrations of those contaminants in harbor porpoises were similar to the levels observed in harbor seals (Calambokidis et al. 1984; Calambokidis and Barlow, in press).

Malins et al. (1984) reported concentrations of contaminants in tissues of a single gray whale that was stranded in the Strait of Juan de Fuca in 1984. With the exception of aluminum,

concentrations of contaminants were relatively low. The aluminum concentration in the brain was high relative to concentrations found in humans and laboratory animals, but there was no adequate baseline information on marine mammals to determine whether this level was unusually high for a gray whale.

EFFECTS OF CHEMICAL CONTAMINANTS ON MARINE MAMMALS

A relatively large number of studies have evaluated the potential effects of contaminants on marine mammals. Summaries of the observed effects are available in various reports (Risebrough 1978; Calambokidis et al. 1984, 1985; Wagemann and Muir 1984; Reijnders 1988; Addison 1989). Most of the suspected effects of contaminants on marine mammals have involved reproductive problems related to PCBs or DDT.

Evaluation of the impact of contaminants such as PCBs and DDT on marine mammals has been difficult because most studies have been conducted in the field, where the effects of contaminants cannot be isolated from other potential causative factors. Reijnders (1986) conducted the only controlled study with captive animals. In that study, harbor seals were fed fish from contaminated regions to examine potential effects on reproduction.

A number of possible contaminant-related effects have been suspected, but not confirmed, for marine mammals in Puget Sound. Chemical contaminants were suspected as the cause of high rates of premature births and birth defects in harbor seals at Gertrude Island in south Puget Sound in the early 1970s (Newby 1971, 1973; Arndt 1973). High rates of neonatal mortality were observed at several other sites in south Puget Sound in the late 1970s (Calambokidis et al. 1978). In 1984, however, increases were observed in the sizes of harbor seal populations in south Puget Sound. In addition, pup production in this area was similar to or higher than the rates observed in other, less contaminated portions of Washington state, and neonatal mortality rates were lower in south Puget Sound than in the other areas (Calambokidis et al. 1985; Steiger et al. 1989).

The death of several gray whales in the Puget Sound area in 1984 raised concerns that these animals were being exposed to toxic levels of some sediment-associated contaminants (Fouty 1984; Malins et al. 1984). Although there were media reports that these whales had been poisoned by contaminants, sufficient data were not available to justify that conclusion. Additional studies are needed to address this concern.

FACTORS AFFECTING CONTAMINANT CONCENTRATIONS IN MARINE MAMMALS

A number of factors have been shown to affect the contaminant concentrations found in marine mammal tissues. These factors should be considered when designing studies to examine contaminant concentrations in marine mammals.

Aguilar (1987) listed the following factors as sources of variation in pollutant loads in marine mammals:

- Fatness state (i.e., condition)
- Sex and age
- Differential metabolism and excretion
- Food chain level
- Variations in tissue sampling techniques
- Variations in tissue preservation and analytical procedures.

These factors can be grouped into two categories: 1) animal-specific characteristics (e.g., species, distribution, sex, age, and condition) and 2) sampling and analysis methods.

Animal-Specific Characteristics

Studies conducted on Puget Sound marine mammals have revealed some of these differences. Species feeding high on the food chain in coastal waters (particularly harbor seals, killer whales, and harbor porpoises) have exhibited some of the highest contaminant concentrations in marine food chains (Calambokidis et al. 1984, 1990; Calambokidis and Barlow, in press). Significant differences in concentrations of PCBs and DDT were found in harbor seals from different regions of Washington, with highest levels observed in south Puget Sound (Calambokidis et al. 1984). Contaminant concentrations also varied significantly by age and sex, with highest levels usually observed in adult males. This pattern is consistent with previous findings in marine mammals: in males, contaminant concentrations increase with increasing age throughout their lifetime, while in females, contaminant concentrations increase with increasing age only until reproductive maturity is reached (Addison and Smith 1974; Addison et al. 1973; Donkin et al. 1981). This

sex-related difference is the result of female marine mammals being able to eliminate contaminants through transplacental transfer and lactation.

Differences in contaminant concentrations relative to body condition of marine mammals have been reported, especially in relation to the degree of fatness or blubber thickness in the animals. The processes related to the mobilization of contaminants from the blubber of marine mammals are not well understood (Aguilar 1985, 1987). Some researchers have found an inverse correlation between blubber thickness and chlorinated hydrocarbon concentrations (Addison and Smith 1974; Donkin et al. 1981).

Sampling and Analysis Methods

Although sampling and analysis methods can influence the contaminant concentrations measured in marine mammals, relatively little research has been done on this subject. Major concerns include variations caused by 1) whether animals were collected live (and therefore presumed healthy) or found dead (stranded), 2) how and from what locations the samples were collected, 3) how the samples were stored, 4) the analytical methods, and 5) the methods used to quantify and report the data.

Tissue Sources—The tissue source was not found to be a significant factor influencing concentrations of chlorinated hydrocarbons in California sea lions or harbor seals, regardless of whether the source was animals killed during collection or stranded (i.e., sick or dead) animals (Le Boeuf and Bonnel 1971; Drescher et al. 1977). In Washington state, no significant differences were found between collected and stranded harbor seals from the outer coast of Washington (Calambokidis et al. 1984). However, Olsson (1978) found that ringed and grey seals found dead had significantly higher contaminant concentrations than animals that had been killed during collection in a previous year. The higher prevalence of emaciation in stranded animals compared to healthy animals would be the most likely reason for the differences between the two groups of animals.

Body Locations Sampled—The body locations from which blubber is sampled from marine mammals may affect the resulting measurements of contaminant concentrations. Calambokidis et al. (1978) found relatively low variation in PCB and DDT concentrations among samples taken from nine different locations on a harbor seal. A similar evaluation of seven body locations sampled from two harbor porpoises also revealed low variation, although the lowest values in both animals came from the same sampling location (Calambokidis 1986). Anas and Worlund (1975) found differences in concentrations of PCBs plus DDT in the blubber of northern fur seals, depending on how the animals had been subsampled.

Analyses of contaminants in cetacean blubber can also be affected by the layer of blubber from which samples are collected because of differences in the lipid composition between the inner and outer layers of the blubber of some cetaceans (Ackman et al. 1975a,b). For this reason, Aguilar (1985) recommended that the full thickness of blubber be sampled and analyzed.

Differences in the distribution of contaminants in other organs may also be a problem. Reijnders (personal communication) found differences in contaminant concentrations in different lobes of pinniped livers.

Storage and Preservation of Samples—For sampling of stranded marine mammals, preservation of tissues has three critical time periods: 1) the time from death to the time when the animal is examined and sampled, 2) the time from collection to storage, and 3) the time from storage to laboratory analysis. Clearly, it is most advantageous to minimize the temperature and length of time for each of these stages.

Postmortem alterations in chemical composition of some contaminants in liver can occur very rapidly. Two DDT isomers (p,p′-DDT and o,p′-DDT) are broken down rapidly after death in avian liver tissues (French and Jefferies 1969; Jefferies and Walker 1966). Freezing liver samples at –10°C and –20°C slowed, but did not eliminate, the breakdown of DDT in avian liver tissues (Walker and Jefferies 1978). Similar DDT breakdowns also occurred in frozen avian blood (Ecobichon and Saschenbrecker 1967) and frozen avian brain (Walker and Jefferies 1978). Wiemeyer et al. (1984) found losses of 35 percent of DDE in avian blood after freezing samples at –20°C for 2 months and 8 months. γ-Hexachlorocyclohexane also disappears from avian liver; the rate of disappearance was slowed, but not eliminated, by freezing samples at –20°C (French and Jefferies 1968).

The breakdown of contaminants has not been shown to be a problem in fat tissues. The breakdown of p,p′-DDT did not occur in frozen avian fat tissues (Walker and Jefferies 1978). Olsson et al. (1975) found no significant changes in levels of PCB and DDT compounds in extractable fat from ringed seal blubber before and after 5 months of outdoor exposure during the summer (in Sweden). Concentrations of PCBs and DDE in duplicate harbor seal blubber samples analyzed by two laboratories 6 years apart showed good agreement (Table 24b in Calambokidis et al. 1984; time difference from Calambokidis, unpublished data).

Metals do not appear to be as easily affected by storage time. No changes were noted in the concentrations of three metals in avian blood frozen at –20°C for 8 months (Wiemeyer et al. 1984).

Significant postmortem changes in weights of rat livers occurred after they were left at room temperature for up to 3 days and after they had been frozen and thawed (Iyengar 1980). Significant changes were also noted in the concentrations of various trace elements under the same conditions.

Chemical Analysis Considerations—Chemical analysis procedures are beyond the scope of this report and are addressed in other PSEP documents (PSEP 1989a,b). Procedures for analyses of bile for hydrocarbon metabolites are given in Krahn et al. (1986). The preferable methods for measuring PCB concentrations in marine mammal tissue are those based on congener-specific evaluations. These methods account for the selective uptake and metabolism of some PCB congeners by marine mammals. Congener-specific analyses also provide information on concentrations of the more toxic coplanar PCBs (e.g., 3,3′4,4′-tetrachlorobiphenyl).

Marine mammal tissues require special considerations compared to tissues of other aquatic species (e.g., clams, crabs, fishes). Blubber tissue has a higher percentage of lipids than is typically encountered in other aquatic species. The proportion of lipids in blubber tissue, however, can vary with the nutritional state of each marine mammal. The percent lipids in marine mammal tissue should be routinely determined as a part of all analyses for organic contaminants using a method comparable to the one described by Hansen and Olley (1963).

The quality control program incorporated with the analyses of marine mammal samples needs to be especially rigorous because of the special considerations required to analyze tissue samples with high lipid content (e.g. blubber). A control material should be analyzed with each set of samples to monitor the accuracy and precision of the analytical method. Two control materials suitable for use with marine mammal samples are presently available (National Institute of Standards and Technology's Whale Blubber QA Material, and a certified reference material SRM 1588 Cod Liver Oil). A certified reference material of whale blubber is being developed and when it is available should be analyzed with each set of marine mammal samples.

PRIORITIES FOR SAMPLE COLLECTION AND ANALYSIS

Typically, only a small proportion of marine mammal tissues is analyzed for contaminants, because of the relatively high costs of these analyses. Priorities for sample analysis depend on the specific objectives of the research. Given this limitation, guidance for prioritizing samples for collection and analysis is presented below.

Species

Selection of a target species involves consideration of a number of factors including species availability, the objectives of the particular study, the biological information available for that species, and the factors influencing the exposure of each species to contaminants (Table 3). In Puget Sound, the most suitable species for monitoring studies are harbor seals and killer whales because they are residents of the region, feed high on the food chain, can potentially accumulate high concentrations of contaminants, and are available for sampling (Calambokidis et al. 1984, 1990) (Table 3). The same criteria also make these species well suited for studies evaluating contaminant impacts. However, other species such as harbor porpoises and gray whales may be reasonable choices because pollutants have been suggested as playing a role in their mortality in Puget Sound.

Tissues

While health of an animal cannot be determined by tissue samples alone, liver and blubber are the highest priority tissues for collection and analysis for most contaminant research (Table 4). The broadest base of historical data is also available for these tissues. Although blubber has been the tissue most frequently used for analysis of long-term accumulation of organic contaminants, it is not suitable for metals and trace element analyses. Liver tissue is suitable for analysis of most contaminants, including chlorinated hydrocarbons and metals. Kidney tissue is suitable as a secondary tissue for evaluating metals and trace elements. The evaluation of contaminant concentrations in stomach contents is valuable for examining recent exposure to contaminants. Brain tissue is a difficult tissue to sample but is the best tissue for evaluating the acute toxic effects of contaminants. Exposure to petroleum hydrocarbons may be best determined by analyzing bile and urine for metabolites. Blubber and blood are the tissues of choice if live animals are sampled (see *Sampling Recommendations for Live Animals*).

TABLE 4. SUITABILITY OF DIFFERENT TISSUES FOR CONTAMINANT SAMPLING

	Tissue							
	Blubber	Liver	Kidney	Muscle	Brain	Bile	Stomach Contents	Colon Contents
Comparative data for chlorinated hydrocarbons	1[a]	1	3	3	3	2	3	3
Comparataive data for petroleum hydrocarbons	3	2	2	3	3	1	1	3
Comparative data for metals	3	1	2	3	2	3	3	3
Chronic exposure	Y[b]	N	N	N	N	N	N	N
Acute exposure	N	Y	Y	Y	Y	Y	Y	Y
Ease to sample	1	2	2	2	3	2	2	2
Indicative of acute toxicity	N	N	N	N	Y	N	N	N

[a] Criteria scored on scale from 1 (good) to 3 (poor).

[b] Criteria scored as yes (Y) or no (N).

Age and Sex

Because concentrations of contaminants vary according to the age and sex of the animal, variations due to these factors need to be considered when designing studies. Studies to determine long-term trends in contaminants are best conducted using only a specified age class. Older adult males represent the "worst case" examples to examine the highest accumulation of contaminants because males tend to accumulate contaminants with age. Because many of the chronic effects of chlorinated hydrocarbons have been related to female reproduction, adult females provide a sensitive age and sex class for examining sublethal effects.

Location

Multiple locations for sampling marine mammals are recommended because of geographical differences in contaminant distributions. The highest concentrations of contaminants have generally been found near centers of industrial and human activities, making these areas the ones best suited for examination of potential impacts of contaminants. In Washington state, for example, concentrations of chlorinated hydrocarbons were significantly higher in harbor seals from Puget Sound than from other areas (Calambokidis et al. 1978, 1984). Analysis of animals from at least one uncontaminated site is useful for providing reference conditions and for evaluating the levels of tissue contamination found in contaminated areas. However, caution should be exercised in selection of an uncontaminated site. Such a site should be away from agricultural drainage areas, and currents and the animal's potential movements should be taken into consideration. Also, collection locations for marine mammals are restricted by the availability of the animals. This factor may be a primary consideration when selecting a sampling location for long-term monitoring studies.

PRIORITY CONTAMINANTS IN MARINE MAMMALS

Studies of contaminant concentrations in marine mammals have involved a wide range of contaminants. Some contaminants, such as PCBs, have been extensively studied in marine mammals, while limited research has been conducted on identifying concentrations and possible impacts of other contaminants.

The specific contaminant analyses recommended for marine mammals depend on the objectives of the study. Contaminants considered of highest priority because of past concerns over impacts to marine mammals are PCBs, chlorinated pesticides (e.g., DDT and its derivatives), mercury, and selenium. However, historical evaluations of PCB concentrations have not quantified concentrations of specific congeners, such as the highly toxic coplanar PCBs. It is important to examine other stable chlorinated hydrocarbons, mostly pesticides, because these lipophilic compounds have a high potential for bioaccumulation in marine mammals. The highly toxic polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) have only been analyzed in a limited number of marine mammals and therefore may be a priority for additional study.

The following sections provide some background information relevant to sampling Puget Sound marine mammals for different contaminants.

Chlorinated Pesticides and PCBs

For marine mammals, the contaminants considered of greatest concern include PCBs and chlorinated pesticides . As described earlier, studies of suspected impacts of contaminants on marine mammals in the Wadden Sea and the Baltic Sea have implicated PCBs as a causative agent. DDT and its derivatives were associated with high rates of premature births in California sea lions in the Channel Islands (DeLong et al. 1973; Gilmartin et al. 1976). All of the above contaminants can accumulate in extremely high concentrations in the tissues of marine mammals, especially coastal pinnipeds and small cetaceans (Reijnders 1980; Risebrough 1978; Wagemann and Muir 1984). PCBs include numerous related chemical compounds (i.e., congeners) and were commercially produced in a variety of mixtures based on total chlorine content. Because many marine mammals feed high on the food chain, the proportion of different PCB congeners is not necessarily similar to those in the original commercial mixtures. Additionally, some of the PCB compounds (i.e., the coplanar congeners) are much more toxic than other forms (Tanabe et al. 1989). For these reasons, analyses of PCBs in marine mammals are best conducted by quantifying the different congeners present (Duinker et al. 1988).

Historical data on PCBs and DDT in tissues of Puget Sound marine mammals are more extensive than for any other contaminants (Calambokidis et al. 1978, 1984, 1988), with data available as far back as 1972 (Arndt 1973). A smaller number of harbor seal samples have been examined for other chlorinated hydrocarbons. Detectable levels of hexachlorobenzene, dieldrin, endrin, mirex, heptachlor epoxide, α-hexachlorocyclohexane, ß-hexachlorocyclohexane, γ-hexachlorocyclohexane, and a number of chlordane compounds were found (Calambokidis et al. 1984). A variety of chlorinated hydrocarbon pesticides were also identified in tissues of a gray whale sampled immediately outside Puget Sound (Malins et al. 1984) and in killer whales from Washington and British Columbia (Calambokidis et al. 1984).

Dioxins and Furans

PCDFs and PCDDs are considered to be among the most highly toxic pollutants. These chemicals occur as trace contaminants of PCBs, polychlorinated phenols, etc. Because PCDFs and PCDDs are present in commercial mixtures of PCBs, toxic effects of PCBs determined in experiments using commercial mixtures may, in part, be the result of PCDFs and PCDDs. Association between PCB concentrations and reproductive problems discussed in previous sections could reflect the effects of PCDFs, PCDDs, and PCBs. Toxic effects attributed to the ingestion of PCB-contaminated rice oil in Japan may instead have been caused by PCDFs occurring with the PCBs (Kuroki and Masuda 1978).

PCDFs and PCDDs were identified as contaminants of concern in Puget Sound by Konasewich et al. (1982) because of their high toxicity and potential for wide-spread distribution throughout Puget Sound. PCDFs have been detected in Puget Sound sediments (Malins et al. 1982).

Research on PCDFs and PCDDs in marine mammals has been limited. Analysis of samples collected through the Stranded Whale and Dolphin Program of British Columbia, and samples from the east and Arctic coasts of Canada have shown that harbor porpoise and killer whales from southwestern British Columbia have the highest levels of PCDFs and HxCDD (hexachlorodibenzo-p-dioxin) of 8 species of marine mammals in Canada (Muir and Norstrom 1990). Rappe et al. (1981) found 12 PCDF isomers totaling 0.04 μg/kg in the fat of a grey seal from the Baltic Sea and suspected that these contaminants were related to PCB contamination. Both PCDFs and PCDDs were identified in the blubber of seals from Scandinavian waters (Olsson et al. 1988; Oehme et al. 1988). PCDFs, which were apparently related to PCB contamination, were detected in the blubber of killer whales off the coast of Japan (Ono et al. 1987). The high cost of analyses for PCDFs and PCDDs may prohibit routine analysis for these compounds. For the reasons described above, however, analysis of either a subset or a composite of samples would provide valuable information.

Metals and Trace Elements

Unlike chlorinated hydrocarbons, metals and trace elements occur naturally in the ecosystem with varying amounts that can be attributed to human activities. Konasewich et al. (1982) identified seven metals and trace elements of greatest concern to Puget Sound, including mercury, arsenic, cadmium, copper, lead, selenium, and silver.

Mercury concentrations found in tissues of marine mammals from Puget Sound have been high compared with concentrations found in other organisms, prompting concerns about the impact of this metal. High concentrations have been found in tissues of harbor seals and killer whales from Puget Sound and neighboring areas (Anas 1974b; Northrup 1981; Calambokidis et al. 1984, 1990). Mercury levels in a false killer whale and a killer whale from southern British Columbia have been the highest levels recorded in a cetacean world-wide (Langelier et al. 1990). Marine mammal tissues generally contain a low proportion of mercury in the toxic methylated form (Koeman et al. 1975; Roberts et al. 1976; Smith and Armstrong 1978). Selenium appears to play a part in the detoxification of mercury because of the 1:1 molar ratio of concentrations found in tissues (Koeman et al. 1973, 1975; Kari and Kauranen 1978). Martin et al. (1976) suggested that reproductive problems in California sea lions off the coast of southern California may have been more related to ratios of mercury, selenium, and bromine than to the absolute concentrations. These findings indicate the importance of testing for methylmercury, selenium, and bromine to allow accurate interpretation of total mercury concentrations.

Other elements previously examined in Puget Sound harbor seals include aluminum, arsenic, cadmium, copper, lead, selenium, silver, chromium, manganese, zinc, bromine, iron, rubidium, nickel, gallium, and strontium (Calambokidis et al. 1984). Malins et al. (1984) reported high concentrations of aluminum in tissues of a single gray whale from the Strait of Juan de Fuca. Baseline concentrations of aluminum are not available for gray whales to evaluate whether these levels were elevated compared with the whole population.

Petroleum Hydrocarbons and Metabolites

The primary concerns over marine mammal exposures to petroleum hydrocarbons have been the impacts of direct contact of contaminants with fur, skin, baleen, and eyes (Geraci and St. Aubin 1980, 1982). Most of these compounds are metabolized by marine mammals and do not accumulate in tissues (Risebrough 1978; Engelhardt et al. 1977). Geraci and St. Aubin (1982), however, report recovering naphthalene from tissues of marine mammals that they examined. Metabolites of petroleum hydrocarbons were recovered from urine and bile of seals that were exposed to oil (Engelhardt et al. 1977; Engelhardt 1982). Metabolites of petroleum hydrocarbons have also been found in the bile of fish (Krahn et al. 1986).

Radionuclides

Risebrough (1978) reviewed levels of radioactive isotopes found in marine mammals throughout the world and reported low, although above background, levels of radioisotopes. He concluded that radioisotopes may slightly increase levels of mutation in marine mammals, but they probably have few other adverse effects. Tomilin and Smychlyoyov (1970) suspected that abnormalities that were noted in the baleen apparatus in some whales were the result of radionuclide exposure, although there was no conclusive evidence to support their suspicion. Thompson (1988) concluded that existing information is not adequate for evaluating the potential impacts of radionuclides on marine mammals.

Other Contaminants

A variety of other contaminants have been identified in marine mammals but their importance is either unknown or is considered minor. Walker et al. (in preparation) identified tris(chloro-phenyl)methanol in blubber tissues from Puget Sound harbor seals. Zitko (1972) found bis(2-ethylhexyl) phthalate esters in harbor seals, but concluded that phthalate esters probably have little effect on the environment. Polychlorinated terphenyls have been identified in a variety of wildlife, including seals (Jensen and Jorgensen 1983). Other contaminants may also be important, even if their role in the animal is not known at the present time.

A variety of metabolites and derivatives of PCBs and DDT have also been identified from marine mammals (Jensen et al. 1979; Sundstrom et al. 1975; Bergman and Wachtmeister 1977; Jensen and Jansson 1976; Jansson et al. 1975).

RECOMMENDED TISSUE COLLECTION PROCEDURES

A number of constraints exist for the collection of marine mammal tissue that are not typical of most other sampling situations. These constraints are as follows:

- A concerted short-term collection effort by killing marine mammals would be difficult because of permit restrictions, public opposition, and limited population sizes for some species
- Most procedures for collecting tissue from stranded marine mammals are currently conducted without funding; therefore, high material costs for sample collection supplies would not be practical
- Logistical problems related to access to and disposal of whales often restricts the type of sampling activities
- The inability to move large animals sometimes requires sampling to be performed under variable and often adverse conditions
- The need for background training in the anatomy of marine mammals.

For these reasons, the guidelines in this protocol attempt to allow some flexibility in the recommended collection materials and procedures outlined in this protocol, while still ensuring proper collection and storage. For each sampling aspect discussed below, recommended and alternate acceptable procedures are given.

CARCASS CONDITION

For many stranded marine mammals, accurate information is not available on the length of time between death and necropsy. The best sources of fresh samples for which this information is usually known include: 1) animals killed for scientific sampling or incidental to commercial fishing, 2) biopsy samples of live animals, 3) live stranded animals that later die, and 4) animals recovered from active beach searches at locations where deaths occur naturally (e.g., pinniped rookeries during the pupping season). Occasionally, information on time of death is also available from sightings of identified individual animals (e.g., gray whales) prior to their death.

The effect of postmortem changes on contaminant concentrations varies by tissue and contaminant. Chlorinated hydrocarbon concentrations in blubber, for example, have been found to be fairly stable even after a prolonged postmortem delay in sampling. Liver tissue, however, deteriorates

more rapidly (particularly in an ongoing disease state) and is the site of postmortem alterations of some contaminants.

A number of criteria can be used in evaluating the condition of an animal with an unknown time of death. However, these criteria do not identify the exact time of death and vary depending on factors such as species, condition, and temperature. Criteria generally indicating recent death include:

- No evidence of postmortem tissue degeneration, discoloration, or autolysis (tissue breakdown due to postmortem action of enzymes) on gross examination
- Good tissue condition revealed by histological examination (if histological analysis is subsequently performed in the lab)
- No signs of scavenging, especially of the eyes
- No bloating
- Presence of rigor mortis (usually occurs 6-24 hours after death)
- Skin/hair not sloughing
- Baleen intact and firmly attached (mysticete whales).

The condition of an animal is critical information that should be recorded for all animals sampled. Categories for defining the acceptability of animals for sampling are provided below:

- Preferred: Animal is alive or known to have died within 24 hours prior to necropsy.
- Acceptable: Time of death is not known or is greater than 24 hours prior to necropsy and animal meets all the factors that indicate recent death (listed above).
- Conditional: Animal does not meet all criteria that indicate recent death (listed above), however, organ sampled is intact and clearly identifiable. Analytical results from these animals should not be interpreted unless the interpretation considers any postmortem changes that may affect the contaminant concentrations. Concentrations of stable chlorinated hydrocarbons in blubber, for example, may be reliable even in these animals.
- Unacceptable: The sampled tissue does not meet any of the criteria listed above.

SAMPLE CONTAINERS

PSEP guidelines recommend containers made of borosilicate glass or polytetrafluroethene (Teflon®) for organic analysis (PSEP 1989a) and containers made of borosilicate glass or linear polyethylene for metals analysis (PSEP 1989b). Glass containers are recommended for marine mammal tissue samples because they are acceptable for both organic and metals analysis. Although glass containers should have a teflon-lined cap, foil-lined caps are acceptable for organic analysis. Sample jars should be cleaned with detergent, rinsed with tap water, soaked in acid (1:1 hydrochloric or nitric acid), rinsed with metal-free water, and rinsed again with high purity methylene chloride or methanol (PSEP 1989a,b). Sampling implements and containers for other tissue collections, such as biopsies of blood or blubber, should be cleaned in the same manner.

Containers should be kept capped and sealed after cleaning and prior to sample collection. Handling of containers should be kept to a minimum and the inside of the container should not be touched by anything other than the sample.

Sample container blanks should be kept for each series of samples collected in a given set of similar containers (see *QA/QC Activities*). Details on how sample containers were cleaned and handled prior to sampling should be recorded on the sample data sheet (see Appendix A).

SAMPLING EQUIPMENT

The following sample collection equipment is recommended for collection of most marine mammal tissue samples:

- Sample collection containers (see above)
- Large stainless steel knife or flensing knife for opening body cavity of large animals
- Stainless steel knife
- Stainless steel scalpel blades and handles
- Stainless steel forceps
- Stainless steel surgical scissors
- Gloves (non-powdered, vinyl).

Teflon-handled titanium knives are ideally suited for collection of tissues (Becker et al. 1988;

Lauenstein et al. 1987) but are not easily available.

Clean non-powdered vinyl gloves should be worn by all sampling personnel. Sampling gloves should be changed in between external examination and cutting (i.e., a new pair of gloves should be worn after opening the body cavity and before sampling internal tissues).

Extreme care should be taken to keep sampling implements clean prior to sample collection. Implements should be washed free of any adhering tissues and blood with water, rinsed with distilled/demineralized water, and then rinsed with methanol, isopropanol, or methylene chloride (this chemical should be handled with caution and recovered if used). New scalpel blades should be used and rinsed with methylene chloride prior to the collection of each tissue sample. After cleaning, gloves and sampling equipment should not come in contact with any surface (e.g., the ground, necropsy kit, etc.).

Cross-contamination between tissues should be avoided. This is particularly important after blubber tissue has been handled for chlorinated hydrocarbon sampling. The scalpel and forceps should be cleaned after taking each sample. All tissue surfaces that come into contact with implements that were not cleaned (e.g., blubber when the body was opened) should be cut away with clean implements. The sample should not come into contact with the outside of the sampling container or the ground.

SAMPLE COLLECTION

General Specifications

All sampling information should be written on field data sheets. This should include information on what samples were collected, the sampling conditions, and how samples were collected, along with all ancillary data. Examples of field data sheets are presented in Appendix A.

Animals should not be frozen prior to dissection. Freezing and thawing could cause damage to organs, fluid loss, and cross-contamination between tissues. Blubber samples should be taken from the mid-ventral region, preferably above the sternum. Blubber samples should be a cross section of the blubber layer (inside to outside). Orientation of the blubber sample may be needed for some analyses. A small piece of muscle or skin could be left on to accomplish this.

For all samples, it is important to sample the same portion of the liver because of possible variation in contaminant concentrations within this tissue. The posterior portion of the left anterior lobe of the liver is recommended for all pinniped samples, and the posterior portion of the left lobe is recommended for cetaceans. Sampling personnel should be careful not to rupture the gall bladder when taking the liver tissue from pinnipeds, and samples exposed to bile should be rejected. In some cases it may be desireable to take large enough samples so that subsampling can later performed in the laboratoary under controlled conditions.

Other organs selected for study should also be sampled at a constant location. Procedures and locations for sampling tissues should be described on the field data sheets.

Approximately 100 grams of each tissue should be collected for analysis. If different types of analyses are to be conducted by separate laboratories, then separate 100-grams samples should be taken from the same body location. For blood samples, a sample size of 40-60 cm^3 should be collected.

Procedural Steps in Sample Collection From Dead Specimens

Because of the range of species, specimen conditions, field conditions, and levels of ancillary data collected for marine mammal sampling, the following steps focus primarily on procedures for collecting samples for contaminant analyses.

1. Record species and location information.

2. Conduct external examination of carcass, determine sex, take external measurements (including standard length), and record all other ancillary data related to external condition.

3. Make an incision over the sternum, midway between the axillae, through the skin and blubber. Measure the thickness of the blubber. Note appearance of the blubber and whether oil is leaching from it. Cut open the abdominal cavity to determine carcass condition. Examination of cetacean samples may require incision and sampling through the left lateral body wall, as recommended by Becker et al. (1988).

4. Wearing clean gloves, sampling personnel should clean sampling implements. After cleaning, gloves and sampling equipment should not come into contact with any surface (e.g., the ground, necropsy kit, etc.) Remove blubber sample from the sternum region with a knife or with a scalpel and forceps. Place 100 grams of sample into a sampling container. The sample should not come into contact with the outside of the sampling container or the ground.

5. Label the sampling container, place the sample in a cooler on ice, note sampling location and time, and clean implements (wash with distilled water, change scalpel blade, rinse with distilled water, and rinse with methylene chloride).

6. Using a knife or clean scalpel, open the abdominal cavity to expose the liver. If using sampling implements to perform this task, then reclean them after use. Note the general appearance of the liver and examine it for abnormalities. If a necropsy is performed for determining the cause of death, it should be conducted by qualified personel.

7. Sample the posterior portion of the left anterior lobe of the liver in all pinnipeds and the posterior portion of the left lobe of the liver for cetaceans. Collect 100 grams of sample into the sampling container. The sampler should be careful not to rupture the gall bladder during sampling the liver; samples exposed to bile should not be collected. The sample should not come in contact with the outside of the sampling container or the ground.

8. Label the sampling container, place the sample in a cooler on ice, note sampling location and time, and clean implements (wash with distilled water, change scalpel blade, rinse with distilled water, rinse with methylene chloride).

9. To sample other tissues, follow the cleaning and sampling procedures described above. It is important to avoid cross-contamination among different tissues; sampling implements must be cleaned thoroughly after cutting and after each sampling. Note the condition and general appearance of the tissue, sampling locations, and the procedures specific to each tissue (e.g., if brain tissue is sampled, note what part of the brain and how the skull was opened).

10. During internal examination, evaluate carcass condition and confirm sex.

11. Collect other samples (e.g., histopathology and microbiology) and all other ancillary data specific to the project (see *Collection of Supporting Data*). For example, both stomach contents and fecal material might be sampled to determine contaminant loading in the digestive tract.

Sampling Recommendations for Live Animals

Several techniques are available for nonlethal sampling of live animals for contaminants. These methods have major limitations in the kind and amount of tissue that can be collected. However, several major advantages of sampling live animals as opposed to stranded carcasses exist, such as 1) apparently healthy animals can be sampled, 2) specific animals can be selected for

sampling, 3) a larger number of animals can be sampled, and 4) identified individuals can be sampled at a later date.

Tissues that have been sampled for contaminants from live marine mammals include blubber, blood, and hair. Other tissues may also be sampled from live animals, but these have not been sampled extensively in the past.

Blubber Biopsy—Two methods are available for biopsy sampling of blubber. For animals that can be handled, such as pinnipeds, a biopsy needle can be used to take a small sample of blubber (Slatter 1985). For larger animals, such as whales, a biopsy dart can be used to take a plug of skin and blubber (Lambertson 1987).

Recently, researchers have used whale biopsy samples for several different purposes. Baker et al. (1990) examined differences among stocks of marine mammals using mitochondrial DNA, while Lambertson et al. (1988) and Baker et al. (in preparation) determined the sex of individual whales. Hoelzel and Amos (1988) used newly developed DNA fingerprinting techniques for these purposes. These techniques have used the skin from the biopsy plug; blubber tissue is usually available from the same plug.

Blubber biopsy samples are most valuable for examining organic contaminants that accumulate in this tissue. Blubber samples can also be used for lipid analyses. Sampling considerations, containers, and precautions described above for sampling organics must be followed. Several additional concerns with this technique exist. For example, biopsy dart samples typically are exposed to salt water after they have hit the whale. This could present a contamination problem if the surface water microlayer is highly contaminated. Removing the outermost portion of the plug that comes into contact with the water would decrease this problem, but it would also reduce the already limited size of the available sample. Additionally, biopsy samples usually do not sample all layers of the blubber. This is a problem for sampling whales because variations in the lipid and contaminant contents in different layers of blubber have been found (see *Factors Affecting Contaminant Concentrations in Marine Mammals*).

Blood Samples—Whole blood from marine mammals has been used for examining both chlorinated hydrocarbons (Tanabe et al. 1981; Risebrough 1978; Kurtz and Kim 1976; Kawai et al. 1988) and metals (Kim et al. 1974; Honda et al. 1982). Concentrations of contaminants in blood were far lower than the concentrations found in most soft tissues. For some contaminants, values in blood were below detection limits even though concentrations of contaminants were detectable in other tissues. This problem is further complicated when sampling live animals due to the limited volume of blood that can be collected practically.

The low concentrations in blood require that special caution be taken to avoid contamination of these samples. One potential source of contamination is anticoagulants that are sometimes added

to blood and sampling syringes.

Other Tissue—Some limited examinations of other tissues have also been used that might be suitable for live animals. Hair has been used to examine concentrations of some metals in pinnipeds (Freeman and Horne 1973; Kim et al. 1974; Braham 1973; Sergeant and Armstrong 1973).

SAMPLE STORAGE

Sample storage and holding times should be in accordance with PSEP guidelines for organics (PSEP 1989a) and metals (PSEP 1989b). All samples should be stored in a freezer at –20°C or below until analysis. Storage time and temperature records, along with any variations or periods of storage at higher temperatures, should be maintained. Acceptability of periods of storage above –20°C depends on the tissue and contaminants being evaluated (see *Recommended Tissue Collection Procedures*).

The maximum holding times for tissues recommended by PSEP guidelines are 1 year for organics (with the exception of volatile organic compounds, which have a maximum holding time of 14 days), 28 days for mercury, and 2 years for all other metals. Samples held for longer periods may be suitable for analysis of some contaminants, but suitability should be evaluated based on the contaminants being tested and then described in a report presenting results for these samples.

LABELING

Each sample container should, at a minimum, be labeled with the following information:

- Animal ID number - including collector identification and number
- Species
- Tissue - duplicate samples should be numbered sequentially
- Date collected
- Collection site.

Animal ID numbers should provide an unambiguous reference to the data sheet that contains other pertinent information on the sample. Labels should be written with waterproof ink and securely attached to the outside of each sample container.

SHIPPING PROCEDURES

The goal of the shipping procedure is to prevent samples from thawing and sample containers from breaking during shipment. Preferably, samples enroute to the analytical laboratory would be packed in dry ice. However, if delivery time is short (less than 6 hours, depending on ambient temperatures), then samples could be delivered in coolers filled with ice. If sample delivery will take longer or if samples are sent by overnight courier, they must be packed with dry ice. If thawing occurs, it should be noted by the receiving laboratory, and procedures should be altered so that thawing will not occur in the future.

NMFS should be notified of any transfer of marine mammal tissues. Shipment into or out of the U.S. of any endangered or threatened species, as listed in appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), requires a CITES permit from the U.S. Fish and Wildlife Service in addition to the permits required under the MMPA and the Endangered Species Act.

COLLECTION OF SUPPORTING DATA

The interpretation of contaminant concentrations observed in marine mammals is facilitated by the collection of supporting data from the sampled organisms. The types of required supporting data depend on the objectives of each study. However, it is recommended that as much supporting data as possible should be gathered to aid in later interpretation of the observed contaminant concentrations.

ESSENTIAL INFORMATION

The minimum amount of data considered essential for sampled animals includes the following: date, species, location of stranding, condition of carcass (state of decomposition), standard length, sex, and blubber thickness. As discussed earlier, age class (derived through length and other aging techniques), sex, reproductive condition of females, and condition of stranded animals (pre- and postmortem) are relevant for interpretation of contaminant levels. Measurement locations are shown in Figure 4. Detailed explanations of this necessary information are provided below.

Date and Time

The date and time of death (if known) and tissue collection should always be recorded.

Species

If the species identification of the marine mammal sampled is uncertain, standard taxonomic guides should be used to correctly identify the animal. These guides should be particularly good in describing characteristics of dead marine mammals. For cetaceans, suggested guidebooks include *Whales, Dolphins, and Porpoises of the Eastern North Pacific and Adjacent Arctic Waters* by Leatherwood et al. (1982) and *The Sierra Club Handbook of Whales and Dolphins* by Leatherwood and Reeves (1983). For the more common marine mammal species in this region, the recommended guidebook is *A Guide to Marine Mammals of Greater Puget Sound* by Osborne et al. 1988. If species identification is still tentative after using guides, it is important to photograph the carcass and provide a detailed narrative description.

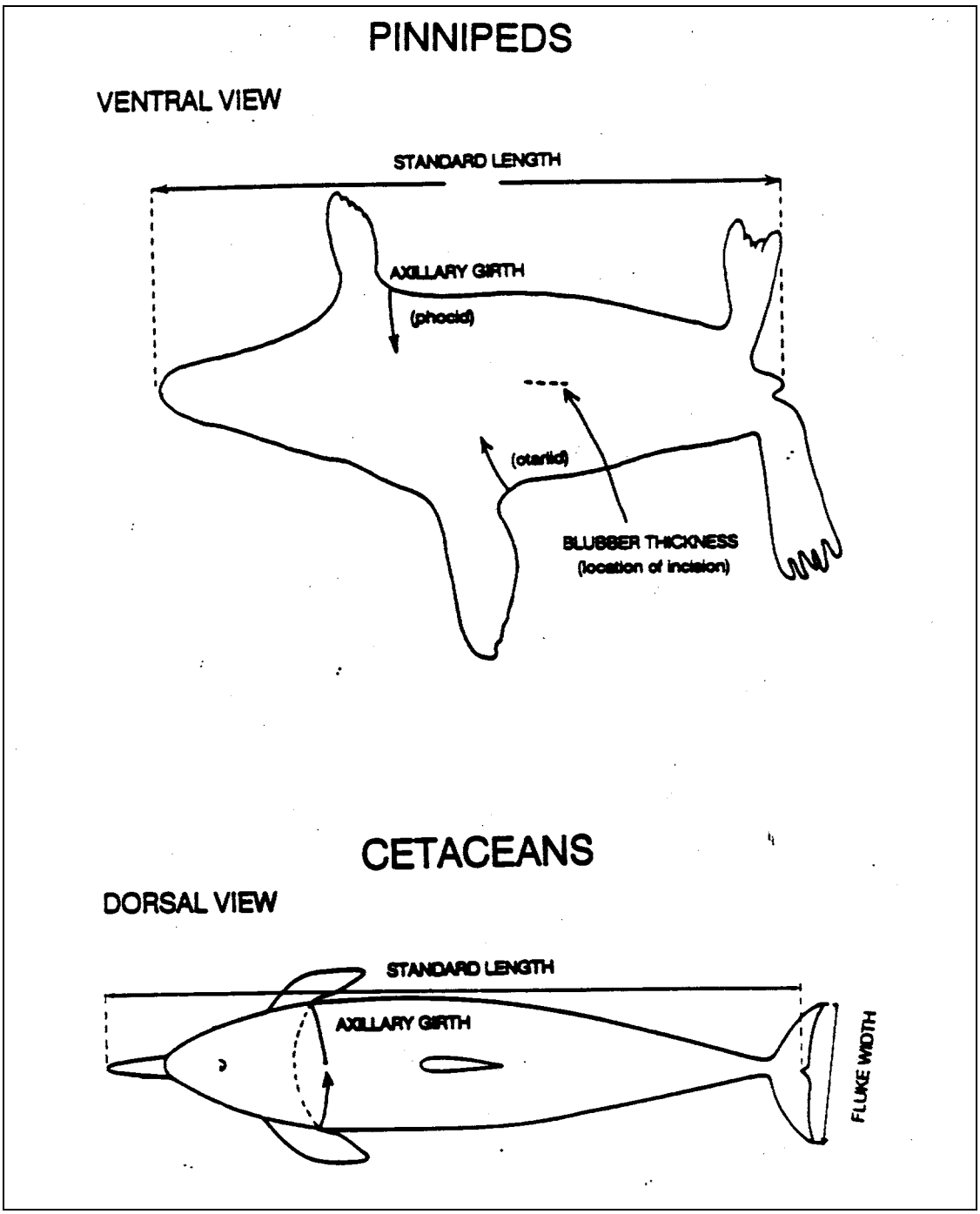


Figure 4 Page 35 Measurement locations for marine mammals

Sampling Location

The description of the location where the specimen was collected should be specific to allow accurate relocation, but it should also be general enough so that it is informative for investigators who are unfamiliar with the area. Latitude and longitude information are best, preferably to the nearest tenth of a minute. If that is not possible, it is acceptable to describe the name or physical description of the area, the distance and direction to the nearest town or well-known landmark, and the county.

Condition of Carcass

The condition of the carcass should be evaluated using the factors listed previously (see *Recommended Tissue Collection Procedures*).

Standard Length

For pinnipeds, sampling personnel should measure (in cm) the carcass on its back (ventrum up), then straighten the carcass as much as possible and measure the straight-line distance from the snout to the tip of the tail flesh (Figure 4; Scheffer 1967). For cetaceans, sampling personnel should measure the carcass on its stomach (dorsal side up), then measure the straight-line distance from the tip of the upper jaw to the deepest part of the fluke notch (Figure 4). For any species, if the carcass is too large to roll or straighten, this length should be measured the best possible way and the method used noted (Fay et al. 1979).

Sex

The sex of pinnipeds should be determined by examining the ventrum posterior to the umbilicus for the presence of two mammary nipples for females or a penile aperture for males. For cetaceans, the genital slit is closer to the anus in females than in males. Some male cetaceans have accessory mammary grooves, which can make using this trait for identification of gender inaccurate. However, the best diagnostic technique is to insert a probe into the genital slit; the probe will pass anteriorly into the slit for females and posteriorly only for males (Fay et al. 1979).

Blubber Thickness

The thickness (in cm) of the blubber (skin not included) over the posterior end of the sternum (xiphoid cartilage) should be measured using a ruler (Scheffer 1967; Fay et al. 1979). Again, it should be noted whether the blubber appears decomposed and if oil is leaching out. Geraci and Lounsbury (1993) present a method for measuring blubber thickness wich minimizes biases.

OTHER USEFUL INFORMATION

The collection of other information is strongly recommended when possible. Appendix A provides field data sheets that prompt the collector for this information and identify other samples for collection. These data are described in the following sections.

Photographs

Photographs are valuable to document species identification (particularly with unusual species), general condition, and gross abnormalities or lesions. Additionally, with species where photo-identification studies have been conducted, photographs of natural markings (if available) could reveal valuable information on the individual whale's history and feeding habits. For killer whales, photographs should be taken of the dorsal fin and saddle patch just posterior to the dorsal fin; for gray whales, photograph the sides near the dorsal hump; for humpback whales, photograph the ventral side of the tail-fluke and the dorsal fin; and for minke whales, photograph the dorsal fin region. Photo-identification studies of these species are currently being conducted by a number of investigators in Puget Sound.

Axillary Girth

The circumference of the body should be measured (in cm) just posterior to the fore flippers or fins (Figure 4). The girth measurement provides more information on the condition of the animal. When it is not possible to measure the girth of large cetaceans, an estimate or half girth should be recorded and measurement techniques should be noted.

Weight

Determine the weight (in kg) of marine mammals when possible. It should be noted whether the recorded weight is an estimate or an accurate value.

Fluke Width and Other Measurements for Cetaceans

Fluke width measurements can be used to double-check the length measurement in some cetaceans. Measure the straight-line distance between the flukes (Figure 4). Several other measurements should be taken on all cetaceans if time permits. A list of the measurements used by the Los Angeles County Museum of Natural History is presented in Appendix A.

Gross Abnormalities, Injuries, Lesions, and Parasites

This information may provide more information about the condition and cause of death of the animal sampled. Gross abnormalities, injuries, lesions, and parasites should be described and quantified when possible. In addition, abnormalities should be sampled for histological examination, if possible (see below). Carcasses should be examined for signs of entanglement (net markings) and bullet wounds. Any observed parasites should be collected, fixed in formalin, and preserved in ethanol for examination and identification by specialists. Parasites can also be preserved in alcohol for genetic analyses.

Histology

Samples collected for histopathological examination can provide information on the condition of the animal before death and the cause of death. Small sections of tissues, approximately 10×5×4 mm [tissues should be no thicker than 4 mm (Luna 1968)], should be sampled from carcasses that have not been frozen previously. Some investigators feel that in dealing with larger cetacea, a larger sample could be taken in the field and pared down in a laboratory setting providing the material is handled properly and expeditiously. Also, some samples collected for histology from frozen animals may be valuable for determining cause of death if gross lesions are present. Tissues should include skin, heart, liver, kidney, spleen, lung, stomach, intestine, lymph nodes, thymus, thyroid, adrenal gland, and brain. Multiple (3-4) samples should be collected from different areas of each organ and should include one or more cross sections of abnormalities (including both normal and abnormal tissue). Gross observations of all abnormalities should be described. All samples should be collected in a jar or whirl-pac and fixed with 10 percent buffered formalin, with a minimum of a 1:10 ratio of tissue to liquid volume (Fay et al. 1979; Luna 1968). Samples should not be allowed to dry. For prolonged storage, samples should be transferred to a 70-percent solution of ethyl alcohol after 48-72 hours of fixation. Most fluid can be drained after fixation is complete, usually 48-72 hours (Fay et al. 1979; Luna 1968). Histological samples should be stored at room temperature and should not be frozen. It is advisable that histological analysis be performed to validate tissues that are taken for chemical analysis.

Reproductive Condition

For adult females, notes should be taken on signs of pregnancy, lactation, and size and condition of the ovaries and reproductive tract. Ovaries can be examined and collected for more specific information on reproductive history (Miller et al. 1978; Bishop 1967; Bigg 1969; Melin et al. in press). For males, the testes and epididymis should be weighed and measured, and the epididymis should be examined for the presence of sperm (Bigg 1969; Green 1972; Melin et al. in press).

Stomach Contents

Information on any contents in the digestive tract can be useful; the degree of fullness should be noted. Additionally, contents can be examined, collected, and screened to determine prey items. For piscivorous marine mammals, all identifiable remains (e.g., whole fish or seaweed) should be identified. Other remains should be frozen until screening and then preserved either dry or in alcohol [formalin can destroy otoliths from fish and statoliths from squid (Heyning, in press)]. It may be worth considering collecting whole fish or stomach fluids for biotoxin analyses.

Sample Collection For Aging

For pinnipeds, an upper canine tooth should be collected for age determination. This can best be done by removing the entire skull for later tooth extraction or by using a hack saw to remove the snout, cutting just anterior to the eye orbit. Details of canine extraction from the upper maxilla are described in Becker et al. (1988). For odontocetes, the skulls should be collected and the teeth can be removed later for aging. For mysticetes, the length of the longest baleen plate should be measured and several plates should be collected by cutting at the gum line.

Information on Age of Neonates

For newborn animals, the following information is helpful in assessing age and condition and whether the animals were stillborn or born prematurely: length, blubber thickness, condition of the umbilical cord, a description of tooth development, description of pelage and the presence of any lanugo coat for harbor seals, signs of lung aeration, and stomach contents.

Cause of Death

Information from macroscopic and microscopic examinations should be used by a trained pathologist to try to determine the cause of death. This should include the primary and contributing causes; if none are apparent, the cause of death should be noted as undetermined. Studies on the cause of death of marine mammals in the wild have been reported by Stroud and Roffe (1979); Calambokidis and Gentry (1985); Steiger et al. (1989); and Dieter (in press). For harbor seals pups, Steiger et al. (1989) characterized premature pups using the early timing of birth, standard length, and presence of lanugo coat; emaciated pups were characterized by a blubber thickness of ≤5 mm.

ANCILLARY DATA

Microbiology

Samples for microbiological analyses (i.e., bacteriology, virology, and mycology) should generally only be collected from animals that are freshly dead, because postmortem spread of these pathogens occurs quickly. Acutely dead animals break down more slowly. If samples are collected from animals that are not freshly dead, these should be restricted to walled-off areas (i.e. from within abscesses). Aseptic techniques are necessary and are described by Fay et al. (1979). Special culture techniques may be required for particular microorganisms.

Examination for Natural Toxins

Recent marine mammal mortalities have been linked to exposures to naturally occurring dinoflagellate toxins. Humpback whales died in Cape Cod Bay after eating Atlantic mackerel that contained saxitoxin (Geraci et al. 1989), and the mass mortality of bottlenose dolphins along the U.S. Atlantic Coast in 1987-1988 was suspected to be caused by exposure to brevetoxin (Geraci 1989). In Puget Sound, paralytic shellfish poison (PSP) is present, but research has not been conducted to identify its potential effects on marine mammals. The primary method for identifying exposure to these toxins is through testing of stomach contents using mouse bioassay (AOAC 1984) and high performance liquid chromatography (Sullivan and Wekell 1988). Liver samples might also be used for biotoxin analysis.

Genetic Studies

Information from examination of skin, blood, eyes, and other tissues can be used in genetic studies of marine mammals (see, for example, Duffield et al. 1983 and Baker et al. 1990). The primary relevance of these studies with respect to tissue contaminants is in identifying the discreteness of marine mammal populations for interpretation of any geographic differences in contamination.

QA/QC ACTIVITIES

The following procedures should be performed to ensure sample quality:

- Maintain a record of all procedures used during sample collection as a part of the data sheet for the sample
- Record descriptions of sample containers and implements that came into contact with the sample, including how they were cleaned.
- Keep a container blank sample for each set of tissues collected, using the same procedures
- Subsample any interior (unexposed) portions of samples collected
- Maintain a record of the chain of custody of the tissue samples and the conditions under which they were transferred and stored (see Appendix A for an example of a chain-of-custody form).
- Follow PSEP (1989a,b) guidelines for chemical analyses.

DATA REPORTING

The following information should be reported when collecting marine mammal tissue samples:

- A record of all marine mammals examined, the tissues collected, and the disposition of animals and tissue, submitted to NMFS as part of the requirements of marine mammal research permits or for participants in the Northwest Marine Mammal Stranding Network.

- A log of all field activities to sample marine mammals.

- A marine mammal field data sheet (see Appendix A), completed for each animal examined. At a minimum, the field data sheets should include the ID number, date, location, sampling personnel, species, sex, standard length and other measurements, condition, results of external examination, tissues examined and condition, tissues sampled and sampling procedures used, sample storage conditions, and other ancillary data collected.

- The information on chemical analyses identified in PSEP (1989a,b) guidelines.

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RECOMMENDED GUIDELINES FOR SAMPLING MARINE MAMMAL TISSUE FOR CHEMICAL ANALYSES IN PUGET SOUND

I. APPENDIX A

Necropsy/Sampling Sheet

Cetacean Data Record

and Chain-of-Custody Record

Sample _____
Page _____ of _____

NECROPSY/SAMPLING SHEET

A. General Information

Species: _____ Common name:_____
Location: _____
Lat/Long: _____ County: _____
Date/time collected: _____ Sampled: _____
Contact name/# : _____ Referred by _____
Chief scientist: _____ Team: _____
Photographs: no yes: roll: _____ Frames: _____
Comments: _____
_____
_____

B. External Examination

Condition of carcass:
live_____/preferred_____/acceptable_____/conditional_____ /unacceptable_____
Evidence of scavenging?_____ rigor mortis?_____ bloating?_____
skin sloughing?_____ baleen intact? (for mysticetes)_____
Describe: _____
Weight: Weighed _____ kg Estimated _____ kg
Std length _____ cm
Axillary girth _____ cm
Sternal blubber thickness _____ cm
Notes on measurements: _____
Sex: Male _____ Female _____ Undetermined _____
Estimated age class: _____
External lesions/injuries/scars: post-mortem?_____
_____
Parasites: examined _____ Description and location: _____
_____
For pups or calves:
umbilical cord (length and condition): _____
tooth development: _____
presence of lanugo hair (harbor seals):% and loc _____
Other comments on external condition: _____
_____

C. Other Reporting Information

Samples collected: no yes: list _____
Carcass disposition: _____

D. Gross Internal Examination

[This section should only be filled out by personnel trained to recognize conditions and abnormalities.]

General appearance of organs: (note signs of post-mortem degeneration)

_____

_____

_____

Type of examination: Detailed_____ Cursory_____ None_____

Organs/tissues examined: Notes on conditions/abnormalities:

_____ umbilicus _____

_____ liver _____

_____ spleen _____

_____ stomach: contents _____

_____ intestines _____

_____ pancreas _____

_____ kidney _____

_____ adrenals _____

_____ gonads _____

_____ trachea: contents _____

_____ esophagus: contents _____

_____ thyroid _____

_____ lungs: for neonate: aerated _____

_____ heart _____

_____ lymph nodes: _____

list: _____

_____

_____

_____

_____

_____ skull _____

_____ brain _____

_____ other _____

Notes on reproductive condition: _____

for females: lactating_____ pregnant_____

CAUSE OF DEATH:

Primary: _____ Contributing: _____

How determined: _____

_____

Sample _____
Page _____ of _____

E. Samples for Toxicology

Sampling conditions (incl. temperature): _____
_____

Sample history: (list date,time,other information)
Estimated time dead: _____
Carcass found: Date: _____ Time: _____
Carcass on ice: Date: _____ Time: _____
Sampled: Date: _____ Time: _____
Samples frozen: Date: _____ Time: _____ Temp: _____
Samples moved to storage freezer:
Date: _____ Time: _____ Temp: _____

Type of containers/lids used:

Tissue Container type Body location Lab Remarks
_____
_____
_____
_____
_____
_____
_____
_____

F. Other Samples Collected: Storage Location:

Stomach with contents collected: _____
Stomach contents only collected: _____
Parasites: _____
Canines: _____
Skull: _____
Gonads: _____
Other notes on collection:_____
_____

Sample _____
Page _____ of _____

G. Histology (in 10% buffered formalin)

Pathology laboratory: _____

Tissue Describe (abnormalities, condition)

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

1.CETACEA DATA RECORD

Catalog No_____
Field No_____

Species _____ Sex_____ Length_____ Condition_____
Observer _____Date of occurrence _____ of data _____
Locality _____
Latitude and Longitude _____ Reported by _____
Photographs/Drawings _____
Circumstances, cause of death _____
_____
External description _____
_____
Tooth/baleen count: erupt _____ total _____ up L _____ up R _____ low L _____
low R _____
Diameter largest tooth/length longest baleen plate _____ baleen color __ _____

MEASUREMENTS (specify units _____)

1 total length.....	_____	26 flipper length, anterior*	_____
2 snout to anus.....	_____	27 flipper length, posterior*	_____
3 snout to genital slit.....	_____	28 flipper width, maximum*	_____
4 snout to umbilicus.....	_____	29 length mammary slits-right	_____
5 snout to throat grooves.....	_____	length mammary slits-left	_____
6 snout to dorsal fin tip.....	_____	30 number of mammary slits	_____
7 snout to ant. dorsal fin.....	_____	31 length genital slit	_____
8 snout to flipper.....	_____	length anal slit	_____
9 snout to ear.....	_____	32 perineal length (males)	_____
10 snout to eye.....	_____	33 fluke width*	_____
11 snout to gape.....	_____	34 fluke depth*, lobe*	_____
12 snout to blowhole(s).....	_____	notch*	_____
13 snout to melon apex.....	_____	35 fluke notch depth*	_____
14 eye to ear*.....	_____	36 dorsal fin height	_____
15 eye to gape*.....	_____	37 dorsal fin base length	_____
16 eye to blowhole edge, L*...	_____	38 girth at eye*	_____
17 eye to blowhole edge, R*...	_____	39 girth at axilla*	_____
18 blowhole length_____width*	_____	40 girth, maximum*	_____
19 diameter ear opening.....	_____	41 girth at anus*	_____
20 head diameter at eyes*....	_____	42 girth midway anus to notch	_____
21 length of eye opening..	_____	43 height same place*	_____
22 rostral width, melon apex*	_____	44 thickness same place	_____
23 projection up/lower jaw..	_____	45 blubber thickness, dorsal	_____
24 number of throat grooves	_____	46 blubber thickness, lateral	_____
25 length of throat grooves	_____	47 blubber thickness, ventra	_____

REPRODUCTIVE SYSTEM

Female
ovaries: weight R _____ L _____ dimensions (LxWxD) R _____ L
uterus: immature _____ mature _____ uterine horn width R _____L
number corpora albicantia _____, corpora lutea _____ diameter CL
mammary gland: color _____, length _____, width ____ , depth ____ ,
milk?____pregnant?
fetus: length _____, sex _____, weight
vagina length _____, number of vagina folds

Male
testes: weight with epididymis R _____ L _____ , without R _____ L _____
dimensions (LxWxD) R _____ L _____ ,
penis length
sperm in epididymis?

STOMACH CONTENTS

fore: volume ____ fish ____ bones ____ otoliths _____ squid _____ beaks

main: volume ____fish ____ bones ____ otoliths ____ squid ____ beaks

pyloric: volume ____fish _____bones ____ otoliths ____ squid ____ beaks_____ general remarks

CHAIN OF CUSTODY RECORD

DOCUMENT NO. 1996

PROJECT	**SAMPLERS:** *(Signature)*

SAMPLE NO.	**SITE**	**DATE**	**TIME**	**SAMPLE MATRIX**						NUMBER OF CONTAINERS		**REMARKS**
				WATER	SEDIMENT	TISSUE	AIR	OIL	OTHER			TAG NO.

RELINQUISHED B *(Signature)*	**RECEIVED B** *(Signature)*	**DATE / TIME**
RELINQUISHED I *(Signature)*	**RECEIVED B** *(Signature)*	**DATE / TIME**
RELINQUISHED I *(Signature)*	**REC'D BY MOBILE LAB FOR FIELD ANAL** *(Signature)*	**DATE / TIME**

DISPACTCHED B *(Signature)*	**DATE / TIME**	**REC'D. FOR LAB B** *(Signature)*	**DATE / TIME**

METHOD OF SHIPMENT:

DISTRIBUTION: ORIGINAL & ONE COPY - ACCOMPANY SHIPMENT

ONE COPY - SURVEY COORDINATOR FIELD FILES