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Alaskan Marine Mammal Tissue Archival Project: A Project Description Including Collection Protocols

Paul R. Becker

Alaska Office, Ocean Assessments Division
National Ocean Service
National Oceanic and Atmospheric Administration
Anchorage, Alaska 99513

and

Stephen A. Wise, Barbara J. Koster and Rolf Zeisler

U.S. DEPARTMENT OF COMMERCE
National Bureau of Standards
Center for Analytical Chemistry
Gaithersburg, Maryland 20899

March 1988



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U.S. DEPARTMENT OF COMMERCE, C. William Verity, *Secretary*
NATIONAL BUREAU OF STANDARDS, Ernest Ambler, *Director*

PREFACE

The Alaskan Marine Mammal Tissue Archival Project was initiated in 1987 with financial support from the Minerals Management Service, Outer Continental Shelf (OCS) Studies Program. Although the emphasis is on the collection of tissues for analysis of contaminants that may be associated with the petroleum industry, it is also recognized that the development of an archive of marine mammal tissues collected and stored using carefully controlled procedures provides a resource that goes beyond the immediate needs of the OCS Program. Such an archive developed over several years provides a resource of materials for future investigators addressing questions concerning the transport of elements and compounds (contaminants and non-contaminants) throughout the polar ecosystem, regardless of source. It is hoped that this resource will gain wide support from the many agencies involved in marine mammal research and management, environmental assessment and management, as well as organizations and individuals with interests in the polar ecosystem, as a whole.

This document provides the basic information on Project objectives and management, justification for the species, tissues, and contaminants of interest, and specific instructions for collecting, handling, and storing samples. At this time, the protocols have been employed only in the collection of northern fur seal tissues, therefore, the details are somewhat biased toward this species. As these procedures are applied to the sampling of other marine mammals, the protocols will probably have to be modified. Therefore, this document represents the first in a series of reports providing the most recent protocols used by the Project. Specific comments on the protocols and other contents of this and future reports which can be used to improve the project are welcome.

Paul R. Becker
Ocean Assessments Division
National Ocean Service, NOAA
701 C Street, Box 56
Anchorage, Alaska 99513

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The protocols described in this report were tested and refined during the July, 1987, sampling of northern fur seals on St. Paul Island. This work could not have been accomplished without the expert advice and aid provided by Dr. Steve Zimmerman, Chief, Alaska Office of Marine Mammals and Endangered Species, National Marine Fisheries Service. Dr. Zimmerman set the stage for logistic support and local cooperation that allowed us to sample the St. Paul Island fur seals. Staff from the National Marine Mammal Laboratory provided numerous pointers on the practicality of sampling this species in the field.

A special note of appreciation is extended to Dr. Terry Spraker, College of Veterinary Medicine, Colorado State University. Dr. Spraker provided expert consultation on the sampling of this species, removed the tissues from the animals in the field, and produced a necropsy report on each of the individual animals sampled. Additional support was provided by Dr. Pat Kozloff, St. Paul, and Sara Pumphrey, College of Veterinary Medicine, Texas A&M University.

The successful testing of protocols at St. Paul could not have been conducted without the cooperation and help provided us by the residents of St. Paul and the Tanadgusix (TDX) Corporation. Key individuals were Pat Kozloff, Darleen Melovidov, John R. Mercurief, and Gregory Fratis, seal harvest foreman, TDX Corporation.

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INTRODUCTION

The concept of archiving biological and environmental samples for retrospective analysis is recognized as a major component of systematic environmental monitoring. The long-term storage of carefully selected, representative samples in an environmental specimen bank is an important complement to the real-time monitoring of the environment. These archived samples permit:

1. The use of new and innovative analytical technology that was not available at the time the samples were archived, for clear state-of-art identification and quantification of analytes of interest, and
2. The identification and quantification of analytes that are of interest at the present but that were not of interest at the time the samples were archived.

The retrospective analysis of archived samples allows the comparison of present and past analytical techniques and values, thus providing continued credibility of past analytical values, and allowing flexibility in environmental monitoring programs.

Marine mammals are considered top predators in the marine environment. Chemical analysis of their tissues can be particularly useful in determining whether bioaccumulation of contaminants (and potential biological effects) associated with human industrial activities, including offshore petroleum and mineral extraction, is occurring in marine food chains. The collection of marine mammal tissues over a period of several years will provide an archive of samples that can be used to determine baseline contaminant levels against which future contaminant measures can be compared.

Collections of marine mammal tissues began in 1987. Protocols for collecting, handling, shipping, and storing the tissues were developed and are being tested through cooperative efforts by Minerals Management Service (MMS), National Oceanic and Atmospheric Administration (NOAA), and National Bureau of Standards (NBS) and through coordination with National Marine Fisheries Service (NMFS), U.S. Fish and Wildlife Service (USFWS) and Alaska Department of Fish and Game (ADF&G).

The Alaskan Marine Mammal Tissue Archive is maintained by NBS in the National Biomonitoring Specimen Bank (NBSB), Gaithersburg, Maryland. This facility, designed for long-term storage, is the result of 10 years development involving cooperative efforts between NBS and EPA, and several years of comparative studies with specimen archiving programs of West Germany, Japan, Sweden, and Canada. Other agencies using the NBSB include EPA, U.S Department of Agriculture (USDA), Food and Drug Administration (FDA), the National Cancer Institute (NCI), and the National Status and Trends Program of NOAA.

Goals and Objectives

The goal of the Alaskan Marine Mammal Tissue Archival Project is to archive a representative collection of Alaskan marine mammal tissues for future contaminant analyses and documentation of long-term trends in environmental quality. The Project has three objectives for the first two years:

1. Collect Alaskan marine mammal tissues that are suitable for determining levels of organic and inorganic contaminants.

Collections of tissues for archival are being limited to freshly killed animals taken by researchers or taken in subsistence hunts. When a sample archived by this project is analyzed, the researcher must have confidence that the sample was collected as prescribed in acceptable protocols (refer to Methods Section). No dead and stranded animals nor old specimens archived from past programs will normally be accepted by this project. As an additional task, however, tissue collections held by other individuals and organizations are being cataloged and their suitability for future contaminant analysis by this project evaluated.

2. Transport, catalog, and curate the tissues in a condition suitable for long-term storage and eventual contaminant analyses.

After collection, samples are packaged, transported, cataloged, and archived according to protocols consistent with those employed by the National Biomonitoring Specimen Bank. Storage is under liquid nitrogen vapor at -150°C , which is the best condition available for minimizing sample degradation. Samples will be selected by OCSEAP/MMS for future contaminant analysis. Emphasis will be on those contaminants associated with offshore mineral extraction. Requests by other researchers and agencies for archived samples will be considered on a case-by-case basis.

3. Determine the most appropriate collection protocols for long-term specimen banking of marine mammal tissues.

Field collection protocols were tested in July 1987 during sampling of the northern fur seals on St. Paul Island. The protocols were evaluated as to their practicality and suitability for obtaining uncontaminated samples of four tissue types (liver, kidney, blubber and muscle) and were revised as warranted. These revised protocols are found in the "Methods" section of this report; they will provide the guidelines for future tissue sampling.

Protocol evaluation will continue throughout the life of the Project as more species are sampled. Selected tissue samples may also be analyzed to determine the suitability of each tissue with respect to levels of inorganic and organic contaminants.

Management System

The collection, packaging, and shipment of samples are being conducted by OCSEAP/MMS researchers under existing separate programs, and through cooperative efforts with other management, survey and research programs of agencies such as NMFS, USFWS, and ADF&G.

Cataloging and archiving of samples is done by the National Biomonitoring Specimen Bank (NBSB), National Bureau of Standards at Gaithersburg, Maryland. Procedures are consistent with those employed by the NBSB in support of the National Status and Trends Program.

Requests for any tissues for analyses or other uses will be considered. Release of the tissues to outside investigators will be contingent upon the approval of the sponsor of this project, MMS, after consultation with OCSEAP, NBS, and the organization originally providing the sample. Release of these tissues will depend on a determination that a surplus of requested tissues exists beyond anticipated sampling or analytical needs and that such analyses or uses will be performed cooperatively by MMS and the requesting organization. Requests for samples will require a written proposal that includes the following information:

1. Individual/organization making the request,
2. Purpose of the proposed study,
3. Researcher/laboratory conducting the analyses,
4. Kind of analyses to be performed/purpose of analyses,
5. Procedure/instrumentation/detection level,
6. Accuracy/precision to be expected,
7. Quality control procedures to be used, and
8. Agreement to provide OCSEAP/MMS, NBSB, and the individual or organization originally providing the samples with the data resulting from the analyses.

The Project will publish Annual Tissue Inventory Reports. These reports will provide the current inventory of tissues maintained in the Archive and the results of any chemical analyses.

Opportunities for cooperative efforts and exchange of information with the Canadian Wildlife Service and the Canadian Department of Fisheries and Oceans are being pursued. An important source of contaminant data on species common to both Alaska and Canada is the Canadian Wildlife Service's National Registry of Toxic Chemical Residues and National Specimen Bank (Elliott 1987).

BACKGROUND

Species of Interest

The marine mammals of principal interest include: polar bears, bowhead and belukha whales, Dall's porpoise, walrus, Steller sea lions, northern fur seals, bearded seals, ringed seals, spotted seals, harbor seals, and sea otters. These animals represent a range of sizes, habitat use, and subsistence values. Table 1 presents a matrix of these animals and the factors most prominent in determining which species will be sampled, and when and where the sampling will probably take place. These factors are:

1. the geographic range of the species,
2. geographic range of a single population (whether local or migratory),
3. mode of potential contamination through the food chain (bottom or pelagic feeder).
4. whether it is a subsistence species,

Table 1a. Marine Mammals of Principal Interest

Factors	PLBR	BWHD	BLKA	DLPR
Geographic Range	Broad	Broad	Broad	Broad
Local vs Migratory	Migr.(ice)	Migr.	Migr.	Migr.
Feeding Mode	Top Pred.	Pelag.(Zoopl.)	Pelag.(Fish)	Pelag.(Squid, Fish)
Subsistence Mode	Major	Major	Major	None
Biological Information	Good	Adequate	Adequate	Adequate
Ease of Collecting	No	Yes	No	Yes
Ongoing Program	Yes(FWS)	Yes(MMS)	No	Yes(NMFS)
Collection Period (months)	?	4-8	5-7	5-8
Collection Area	?	Barrow Wainwright	Point Hope Kivalina	S. Bering (Gill Net Fishery)
Contaminant Information	Yes	Yes	Yes	?
PLBR, BWHD, BLKA, DLPR,	Polar Bear Bowhead Whale Belukha Whale Dall Porpoise			

Table 1b. Marine Mammals of Principal Interest

Factors	WLRS	STSL	HBSL	FRSL
Geographic Range	Broad	Broad	Broad	Broad
Local vs Migratory	Migr.(ice)	Migr.	Local	Migr.
Feeding Mode	Benthic (Clams)	Pelag.(Fish)	Pelag.(Fish)	Pelag.(Fish)
Subsistence Use	Major	Minor	Minor	Major
Biological Information	Good	Good	Good	Good
Ease of Collecting	Yes	Yes	No	Yes
Ongoing Program	Yes(FWS)	Yes(NMFS)	No	Yes(NMFS)
Collection Period (Months)	5-8	?	?	7-8
Collection Area	St. Lawrence I. Little Diomede Nome Point Hope Barrow	?	Icy Bay Tugidak I. Glacier Bay Izembek Lagoon	Pribilofs
Contamination Information	Yes	Yes	Yes	Yes
WLRS,	Walrus			
STSL,	Steller Sea Lion			
HBSL,	Harbor Seal			
FRSL,	Fur Seal			

Table 1c. Marine Mammals of Principal Interest

Factors	BDSL	RGSL	SPSL	SEOT
Geographic Range	Broad	Broad	Restricted	Broad
Local vs. Migratory	-----	Migratory (ice)	-----	Local
Feeding Mode	Benthic	Pelag.(Fish, Zoopl. Crustaceans)	Pelag.(Fish)	Benthic
Subsistence Use	Major	Major	Major	No
Biological Information	Adequate	Good	Adequate	Good
Ease of Collecting	No	Yes	Yes?	No
Ongoing Program	No	No	No	Yes(FWS)
Collection Period (Months)	5-8	5-8	5-8	?
Collection Area	-----	Nome	-----	Prince William Sound, Kodiak
	-----	Kotzebue	-----	
	-----	Wainwright	-----	
	-----	Barrow	-----	
	-----	Shishmaref	-----	
Contaminant Information	Yes	Yes	Yes	?
BDSL,	Bearded Seal			
RGSL,	Ringed Seal			
SPSL,	Spotted Seal			
SEOT,	Sea Otter			

5. availability of baseline biological information,
6. ease of collecting fresh samples where source is predictable and protocol can be followed,
7. whether agency programs exist that can provide collections,
8. periods when collections are feasible,
9. geographical areas of potential collection, and
10. availability of contaminant information.

Selection Criteria

Five of these 10 factors function as principal criteria for the selection of the species to be sampled. Each of these criteria are discussed below.

The Geographic Range of the Species. A species with a broad geographic range would be of more value for monitoring purposes and tissue archival than one of a restricted range since such a species would provide some measure of comparability on an international basis. Samples from a single species from a variety of locations throughout the Arctic or within the U.S. on a north-south or east-west gradient could provide the comparability which could be used in interpretation of contaminant levels found in that species. For example, a species such as the harbor seal, which occurs in both the Atlantic and Pacific, and from Alaska to Baja California would provide more comparative opportunities than the ribbon seal which occurs only in the Bering and Chukchi seas.

Mode of Potential Contamination through the Food Chain (Bottom or Pelagic Feeder). Selection of species to sample should be representative of the range of feeding modes which occurs in marine mammals. Rather than restricting the sampling to only pelagic fish eating mammals, species representing plankton feeders, benthic feeders, and top predators (e.g., feed on seals and walrus) will also be sampled.

Subsistence Use. A substantial portion of the diet of coastal residents of the Beaufort, Chukchi and Bering seas is marine mammals. Selection of marine mammals which are consumed by humans, or which utilize the same species as commercial fisheries, would not only provide information on environmental contaminant levels, but also data with possible implications regarding human health. Areas such as the Bering Sea export fish all over the world, yet there is no nearby human population to easily test for contaminants derived from these fish. Marine mammals are readily available indicators of unusual levels of contaminants that could cause human health concerns.

Availability of Baseline Biological Information. Interpretation of contaminant information requires biological knowledge regarding the species of interest. This knowledge includes life cycle information, behavior, distribution and movements of populations, age and reproductive status, feeding behavior, and basic physiology. Such information would be necessary in order to determine where and how contaminants might be accumulated, to explain the physiological response of the animal, and to determine how internal regulation of contaminants and biochemical byproducts might affect the health of individual animals and populations.

Ease of Collecting Fresh Samples where Source is Predictable and Protocol can be Followed. The archival of tissue samples and its contribution to the monitoring of contaminants require that samples be reliably and economically available on a long-term basis. The species chosen should not be so rare that future collection might be jeopardized by restrictive permit requirements. If samples are to be obtained from hunters, the hunt should occur predictably at a location which is near a commercial airport and where samples can be adequately processed and preserved. Meeting the standards of sampling protocols might be difficult at any collection location. Hunting often occurs at remote camps with no electricity, under less-than-sterile conditions, and under extreme weather conditions that hampers normal human activities.

Species Evaluation

Presented below are discussions of each of the marine mammal species of interest as related to the selection criteria for the tissue archival. With the exception of Dall's porpoise, these discussions are based on information provided by Kathy Frost (personal communication 1987).

Polar Bear (*Ursus maritimus*). This mammal is widely distributed throughout the Arctic. Its management is subject to international treaty, and it has been a species of interest relative to contaminant monitoring and archival programs in Canada. The polar bear is a top predator; contaminants present in its tissues may be reflective of those present in lower trophic forms (fish, seals, etc.) and passed up through the "food chain". Basic biological data are available for this mammal. USFWS research on this species is ongoing and involves monitoring the subsistence harvest and radio tracking.

Polar bears are eaten by humans, but to a lesser extent than seals or walruses. Tissue sampling would require development of a network among hunters, and coordination with the agency managing this species. If regulations are implemented that require the sealing of polar bear hides, it might be possible to build a sampling scheme to obtain necessary archival material. Sampling protocol would have to accommodate field collection of specimen material, most likely by standard hunting implements. Hunting does not occur within a predictable time frame but is spread over many months. Thus, in order to be economically feasible, sampling would have to be coordinated by on-site agency personnel rather than by a special sampling team.

Bowhead Whale (*Balaena mysticetus*). The bowhead whale occurs in the western Arctic of Alaska and Canada, and in the eastern Arctic. It feeds on zooplankton (primarily copepods and euphausiids), and in this regard has its closest trophic connection to the ringed seal which feeds primarily on euphausiids and arctic cod. Baseline biological information on the bowhead is more limited than for some other Arctic marine mammals, but the basics of seasonal migrations and food habits are known. At present, there is considerable controversy regarding age determination, but research is ongoing.

The bowhead whale is one of the most important marine mammals used by coastal residents from St. Lawrence Island to Kaktovik. Although the bowhead harvest is small, biologists are present (in Barrow) to obtain and care for samples; additional personnel should not be required. Because of high international visibility, funding and personnel will probably be available in future years to ensure a successful tissue archival program. There would probably be

considerable local interest. In the past, the North Slope Borough Department of Wildlife Management in cooperation with the Alaska Eskimo Whaling Commission have collected and analyzed tissues from this species.

Belukha whale (*Delphinapterus leucus*). This animal is widely distributed throughout the Arctic and Subarctic; the western Arctic population is shared by Canada and Alaska. The belukha eats primarily fish, cephalopods, and some crustaceans, and are trophically similar to spotted seals. They consume many of the same species taken by commercial fisheries (herring, salmon, pollock, etc). Although more data are needed, baseline information is available on distribution, reproduction, and food habits. This odontocete cetacean (toothed whale) presents a different taxonomic group than seals and walruses, which are pinnipeds, and may metabolize and store contaminants somewhat differently.

The belukha whale is a major subsistence species in Alaska and Canada. The logistics for obtaining tissue samples might be complicated. The largest hunts in Alaska occur at Point Hope or Kivalina in the spring, and in Kotzebue Sound during early summer. Since imposition of the bowhead quota, hunters at Point Hope spend less time on the ice and consequently take fewer belugas. The timing of this hunt is variable depending on weather, ice conditions, and other hunting activities. There are no ADF&G or USFWS offices in these communities, nor do agency personnel monitor the harvest. The Kotzebue Sound belukha hunts have traditionally been the largest in the state. However, they are unpredictable; during the past three years, the harvest has been near zero. The main belukha hunting camp (Elephant Point) can be accessed only by charter aircraft or small boat. Running water and electricity are not available, so it would be difficult to process and store tissue samples.

Dall's Porpoise (*Phocoenoides dalli*). Dall's porpoise occurs in the North Pacific within Japanese and Russian waters eastward to the North American coast. This species is especially abundant in the Sea of Okhotsk and the southern Bering Sea, ranging at least as far north in the summer as the Pribilof Islands. Although separation of the different stocks and details of migrations are poorly known, genetic evidence indicates that the animals of the southern Bering Sea are of single stock (Linda Jones, personal communication). This species feeds primarily on squid, pelagic fish and crustaceans. Background information is adequate.

Although Dall's porpoise is not a subsistence species, tissue samples could probably be obtained from the NMFS Cetacean Research Program. NMFS collects whole animals caught by the Japanese high seas gillnet fishery in the southern Bering Sea. These animals are quick frozen on board the Japanese vessels and then returned to the National Marine Mammal Laboratory for study. If proper arrangements are made ahead of time, application of appropriate protocols for obtaining tissues for archival from the specimens is quite feasible.

Pacific Walrus (*Odobenus rosmarus divergens*). The Pacific walrus does not occur throughout the Arctic. There is, however, an Atlantic subspecies which could be used in comparisons. The walrus is a benthic feeder, eating primarily bivalve molluscs which are known to bioaccumulate some environmental contaminants. It is a major subsistence resource to coastal residents of Alaska; the Atlantic walrus is eaten by the Canadian Inuit. Baseline biological data regarding distribution, movements, reproduction, and food habits are available, and studies are ongoing.

Hunting for walrus occurs every year in locations which provide access to air freight services and reasonable laboratory and freezer facilities. ADF&G maintains field offices in several walrus hunting villages. USFWS hires people each year who might assist in sample collection. Hunter participation could possibly be coordinated through the Eskimo Walrus Commission. There is already considerable interest in contaminants in walrus because of the recent attention drawn to the high cadmium levels in this species.

Steller Sea Lion (*Eumetopias jubatus*). The Steller sea lion occurs from the Bering Sea south to California. Its diet is similar to that of the harbor seal and includes commercial species such as salmon, herring, and pollock. Baseline information on sea lions is relatively good.

There has been a substantial decline in numbers of this species since the 1970's for unknown reasons and it might be declared depleted, which could make sampling difficult. However, it may be desirable to obtain archival material from a declining population. Tissues would probably have to be obtained through directed sampling efforts and in conjunction with other research by NMFS or ADF&G.

Northern Fur Seal (*Callorhinus ursinus*). The northern fur seal summers in Alaskan waters and winters south to California. It also occurs in Japanese and Soviet waters. This species eats primarily fish and squid, and utilizes species which are commercially harvested, particularly pollock. Extensive baseline data exists.

Fur seals are harvested annually (July-August) on the Pribilof Islands under very controlled conditions. There has been a steady 5-7% per annum decrease in the numbers of Pribilof animals in recent years. Causes for this decline are unknown. Efforts are underway to declare fur seals depleted. The current harvest should allow ready access to samples, but it includes only subadult (3-4 year old) males. Unless special additional sampling were done in conjunction with other research activities, there would be little opportunity to archive samples from females or reproductive-age animals. NMFS personnel currently conduct research on the Pribilof Islands and could aid in the sample collection.

Harbor Seal (*Phoca vitulina*). This species occurs from Bristol Bay south to Baja California, along eastern and western Atlantic coasts, and in Japan. Populations are localized and do not migrate to any extent. This would allow comparative sampling in different parts of its range. Harbor seals eat primarily fish and lesser amounts of invertebrates such as shrimp and octopus. Their diet includes many species which are also commercially fished, including salmon, herring, pollock, and smelt. They may compete quite extensively with fisheries. The data base on this animal is extensive.

Harbor seal populations in parts of Alaska are currently declining, for unknown reasons. This species is not a primary subsistence species; therefore, tissue samples would have to be obtained through collections conducted as part of other research, or through collections directed solely at obtaining archival material.

Bearded Seal (*Erignathus barbatus*). The bearded seal is circumarctic in distribution, but is less abundant than the ringed seal. It is similar to the

walrus in being a bottom feeder, but the bearded seal's diet is generally more diverse. It is a preferred human food species because of its relatively large size (up to 250 kg). Baseline biological information is available.

Bearded seals are hunted primarily in the spring, beginning not long after pupping. Because of their large size, almost all bearded seals are butchered on the ice during the hunt. Only the meat or other desired parts are returned to the beach. This would make it more difficult to ensure that sampling was accomplished according to prescribed procedures.

Ringed Seal (Phoca hispida). The ringed seal is circumarctic and the most abundant of the northern pinnipeds. It feeds pelagically on mostly non-commercial species, such as arctic and saffron cod. It is the mainstay in the diet of the polar bear and an important source of food for the northern Eskimo. Baseline biological data on this species are quite extensive.

Ringed seals are harvested throughout northern Alaska, including the larger population centers of Nome, Kotzebue, and Barrow, and obtaining samples should be straightforward. Local customs determine that whole seals are brought back to be butchered by the women, which should facilitate sampling of tissues. Seals are hunted primarily in the spring just after pupping and breeding.

Spotted Seal (Phoca largha). Although closely related, this species is not as widespread as the harbor seal. It occurs in the Bering and Chukchi seas and, in much smaller numbers, in the Beaufort Sea. Its diet is similar to that of the harbor seal and beluga whale. The biological data base is less complete than for the harbor seal but is probably adequate for selecting and characterizing archived specimen material. Spotted seals are hunted by coastal Eskimos in the spring and autumn but the largest and most predictable hunts occur in small remote villages.

Sea Otter (Enhydra lutris). The sea otter is abundant and is increasing in Alaska. It is widely distributed from the Bering sea south to California. It is a bottom feeder, often eating commercial species such as Dungeness crab and abalone. This species is not a significant food item for humans, nor for any other large carnivore. It would be difficult at present to obtain specimen material except through a directed collection program. Existing and proposed research plans do not include sacrifice of animals.

Contaminants

The contaminants of interest (Tables 2 and 3) are essentially those being addressed by NOAA's National Status and Trends Program (Lauenstein 1986). These include organic contaminants, consisting of aromatic hydrocarbons, chlorinated compounds, and polychlorinated biphenyls (PCBs), and trace elements, consisting primarily of heavy metals. Other contaminants will probably be added to these lists as the project develops. Candidates for inclusion are: additional cyclodienes such as toxaphene and endosulphan, polychlorinated dibenzodioxins and furans, nitrosamines and other nitrogen containing compounds, and organometallic compounds of elements such as arsenic, mercury, phosphorous, selenium, and tin.

The trace elements of Table 3 are listed in descending priority by groups, based on the classification of Goyer (1986). The high priority elements are

Table 2. Trace Organic Contaminants of Interest. These are Consistent with Those Being Analyzed by NOAA's National Status and Trends Program (Lauenstein and Young, 1986). Alternate names as listed in "The Merck Index".

<u>Aromatic and Heterocyclic Hydrocarbons</u>	<u>Alternate Name</u>
Acenaphthene	1,2-Dihydroacenaphthylene
Anthracene	Paranaphthalene
Benz[a]anthracene	1,2-Benzanthracene
Benzo[a]pyrene	3,4-Benzpyrene
Benzo[e]pyrene	1,2-Benzpyrene
Biphenyl	Dephenyl; phenylbenzene
Chrysene	1,2-Benzphenanthrene
Dibenz[a,h]anthracene	- - -
Dibenzothiophene	- - -
Fluoranthene	1,2-(1,8-naphthylene) benzene
Fluorene	o-Biphenylenemethane
Naphthalene	- - -
Perylene	Dibenz[de,kl]anthracene
Phenanthrene	- - -
1-Methylphenanthrene	- - -
Pyrene	Benzo[def]phenanthrene
<u>Chlorinated Compounds</u>	
Aldrin	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene
alpha-Chlordane	1,2,4,5,6,7,8,8-Octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene
o,p'-DDD	1-Chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene
p,p'-DDD	1,1-Dichloro-2,2-bis-(p-chlorophenyl)ethane
o,p'-DDE	1-Chloro-2-[dichloro-1-(chlorophenyl)ethenyl]benzene
p,p'-DDE	1,1'-(Dichloroethenylidene)-bis(4-chlorobenzene)
o,p'-DDT	1-(4-Chlorophenyl)ethyl]benzene
p,p'-DDT	1,1'-(2,2,2-Trichloroethylidene)bis[4-chlorobenzene]
Dieldrin	3,4,5,6,9,9-Hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth[2,3-b]oxirene
Endrin	1,2,3,4, 10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo,endo-1,4:5,8-dimethanonaphthalene
Heptachlor	1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene
Heptachlor epoxide	1,4,5,6,7,8,8-Heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
Hexachlorobenzene	- - -
Lindane (gamma-BHC)	Gamma-Hexachlorocyclohexane
Mirex	1,1a,2,2,3,3a,4,5,5a,5b,6-Dodecachlorooctahydro-1,3,4-metheno-1H-cyclobuta[c,d]pentalene
trans-Nonachlor	1,2,3,4,5,6,7,8,8-Nonachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene
<u>PCB's</u>	
Dichlorobiphenyls	- - -
Trichlorobiphenyls	- - -
Tetrachlorobiphenyls	- - -
Pentachlorobiphenyls	- - -
Hexachlorobiphenyls	- - -
Heptachlorobiphenyls	- - -
Octachlorobiphenyls	- - -
Nonachlorobiphenyls	- - -

Table 3. Trace elements of interest listed in descending priority by groups. This list is a modification of those being analyzed by NOAA's National Status and Trends Program (Lauenstein and Young 1986). Classification is based on that of Goyer (1986).

High priority elements:

A. Toxic metals; can result in multiple effects:

As Arsenic
Cd Cadmium
Hg Mercury
Pb Lead

B. Essential metals with potential for toxicity and/or related to toxicity of Group A, above:

Cu Copper
Cr Chromium
Se Selenium
Zn Zinc

Medium priority elements:

A. Essential metals with potential for toxicity:

Co Cobalt
Mo Molybdenum

B. Elements related to metal metabolism:

Ca Calcium (also Vitamins D and E)
Fe Iron

C. Minor toxic metals significant to oil industry:

Ni Nickel
V Vanadium
Ba Barium

Low priority elements:

A. Minor toxic metals:

Ag Silver
Al Aluminum
B Boron
Be Beryllium
Mn Manganese
Sb Antimony
Sn Tin
Te Tellurium
Tl Thallium

those most commonly associated with environmental contaminant problems. These consist of highly toxic, non-essential metals that can produce multiple effects, metals essential to mammals but with potential for toxic reactions at elevated levels, and metals that interact with other more toxic metals to modify their toxicity.

The medium priority elements consist of essential metals that have moderate potential for toxicity, elements related to metals metabolism and important to the interpretation of trace element concentrations, and minor toxic metals significant due to their elevated levels in crude oils.

The low priority elements consist of elements of low to moderate toxicity. These will probably not be considered in routine tissue analysis. Although beryllium is considered to be a highly toxic metal, it is included in the low priority group since the principal mode of uptake is via the respiratory system and is associated with heavy levels of fossil fuel combustion.

The contaminant load of an organism is the result of many factors associated with the contaminant, the organism, and the environment. Among these factors are the physiochemical characteristics of the contaminant, the relative quantities of the contaminant released into the ecosystem, the transport rate within the environment, the detoxification/degradation processes operating in the environment, and the uptake, accumulation, and internal regulation processes of the organism, itself. The availability and toxicity of a contaminant is related to its organic/inorganic form, valent state, reaction and competition with other elements or compounds, and lipid solubility.

Uptake, Transport, and Compartmentalization

The basic mammalian routes of absorption, distribution and excretion of xenobiotics are conceptualized in Figure 1. In this model, a whole animal is visualized as consisting of peripheral components (organs) which are linked to a central component (the circulatory system), but not to each other. The circulatory system, consisting of blood, lymph, and extracellular fluid, is the component that receives the contaminant from and returns it to the exterior of the organism and distributes the contaminant to all of the organs.

Of the three major routes of contaminant uptake (ingestion, inhalation, and dermal absorption), the one of most importance to marine mammals is probably ingestion. For example, food contributes over 90% of the organochlorine intake by mammals (Campbell et al. 1965).

Regardless of method of uptake, substances have to move across biological membranes in order to enter the circulatory system and in order to be transported from the extracellular fluid into the individual tissue cells. There are many absorption mechanisms including: filtration, active transport, facilitated diffusion by association with carrier molecules, pinocytosis (formation of lysosomes), and passive diffusion. For the majority of compounds that might be of significance from an environmental standpoint, however, the primary mechanism of absorption is passive diffusion (Tinsley 1979; Klaassen 1986).

Although small water-soluble molecules can diffuse through aqueous channels in the biological membrane, for the majority of xenobiotics which have relatively

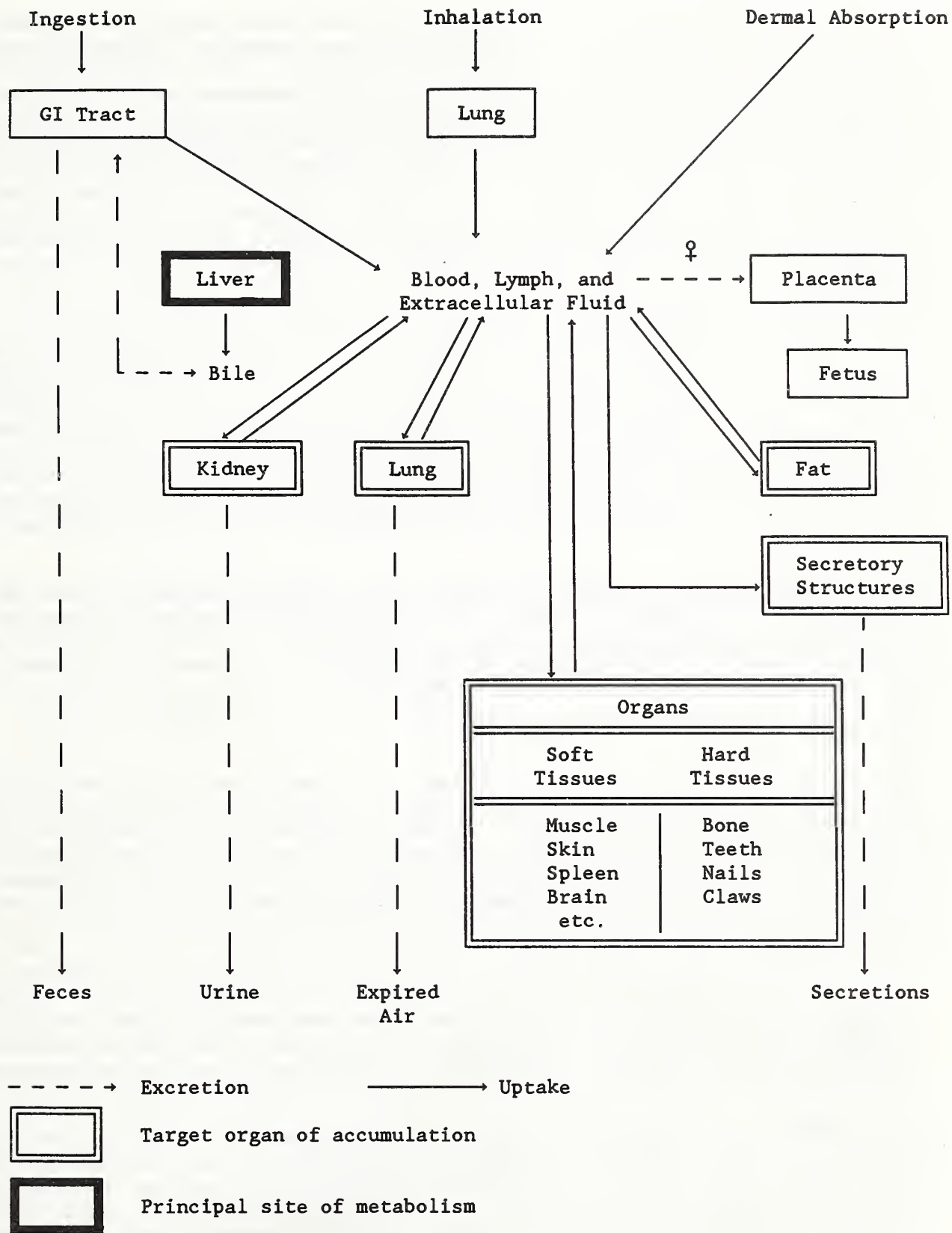


Figure 1. Absorption, Distribution, and Excretion of Xenobiotics. Modified from Klaassen (1986).

large molecular structure (the organic compounds), the rate of absorption is controlled by the partition coefficient of that substance. This is usually expressed as the octanol/water partition coefficient. The larger this coefficient, the more hydrophobic (and less polar) the compound, and the greater the tendency for the compound to pass through biological membranes. The lipid fraction determines the membrane permeability to hydrophobic, lipid soluble (lipophilic) substances. The tendency of organic acids and bases to be absorbed is also influenced by the PKa and pH of the environment in which absorption takes place (Tinsley 1979). Some compounds that have structures similar to normal metabolites may move by other pathways and thus their absorption characteristics can be different from those absorbed via passive diffusion.

Transport and distribution of an absorbed substance usually occurs rapidly, and the rate of distribution to the tissues of each organ is determined by the blood flow through the organ and the ease with which the chemical crosses the capillary bed and penetrates the cells of the particular tissue (Klaassen 1986). After entering the blood, xenobiotics may be associated with the blood cell proteins or with the plasma. Several plasma proteins can bind foreign compounds, the most important being serum albumin. While bound, the serum albumin/xenobiotic complex can not cross capillary walls into tissues; however, the binding is a reversible process and contaminants can be released at target tissues.

The natural environment contains many types of toxic substances that can be absorbed by the tissues of an organism. The survival of an organism depends on its ability to mediate this stress by: (1) excretion of the toxic substance, and (2) storage of the xenobiotic in a non-toxic form (in a storage depot) away from the site of toxic action (target organ).

Excretion

Excretion of a lipophilic xenobiotic depends on conversion of the compound to a water soluble form and elimination in urine, feces and other excretory fluids. Lipophilics in excretory fluids are usually reabsorbed by diffusion into cellular membranes and, therefore, are not easily eliminated. An exception to this is the lipophilic secretions produced by the scent glands of mammals (Larsson and Lindegren 1987). Considering the relatively small volume of these secretions, however, little elimination probably occurs via this route.

One route of excretion which occurs through secretory structures in mammals and which has an important bearing on differences in contaminant levels between mature males and females is lactation. The consistently higher burdens of organochlorines (as well as many heavy metals) in males as compared to female mammals has been linked to the loss of contaminants through lactation and parturition in the females.

The transfer of lipophilic xenobiotics from maternal fat stores to the offspring through secretion of lipid-rich milk has been suggested by Addison and Brodie (1987) to be particularly important in marine mammals. At least for the grey seal (*Halichoerus grypus*), the transfer of organochlorines from the female to fetus has been shown to occur to a greater extent via lactation than across the placenta (Addison and Brodie 1977); and Honda, et al. (1986) showed

the importance of lactation in excretion of lead, nickel, and selenium in striped dolphin (*Stenella coeruleoalba*).

Organisms have many biotransformation processes (mostly involving enzymatic reactions) for converting lipophilic xenobiotics to hydrophilic metabolites. Of the organs involved in biotransformation of xenobiotics (liver, lung, kidney, intestinal gut flora, skin, and gonads), the liver is the most important (Sipes and Gandolfi 1986).

Unfortunately, the metabolites resulting from the biotransformation or intermediate compounds leading to the metabolites are more toxic in many cases than the original xenobiotics. This is particularly true for some chemical carcinogens, organophosphates and a number of compounds that cause cell necrosis of lung, liver and kidney (Sipes and Gandolfi 1986).

Accumulation

As is suggested in Figure 1, contaminants may accumulate in any tissue types or organ: liver, kidney, lung, fat, bone, teeth, nails/claws, hair, muscle, skin, spleen, brain, etc. The concentration potential for any single contaminant, depends on the chemical properties of that contaminant and the physical and chemical properties of the tissue or organ in question.

Because certain organs appear to store large amounts of specific contaminants in non-toxic form, these have been termed "storage depots". The major storage depots in mammals are fat, bone, kidney, liver, and plasma. Although not commonly thought of as a storage depot, the plasma proteins bind certain xenobiotics preventing them from crossing capillary walls. This is usually a temporary and reversible reaction, depending upon the equilibrium established between the xenobiotic/protein complex and the free xenobiotic in the plasma, and also depending on competition from other chemicals for binding sites on the proteins.

Fat and bone are relatively long-term storage depots. Accumulation in fat is primarily determined by the lipophilic nature of the xenobiotic. Bone, however, is a relatively complex tissue and accumulation here can be quite complicated. Bone is an important storage depot for heavy metals, such as lead and zinc. Accumulation in bone can involve incorporation within the lipid component, association with collagen (copper), incorporation within the red marrow (iron and cadmium), or deposition with calcium (lead, manganese, zinc) (Yamamoto, et al 1987).

The liver and kidney are the principal organs involved in the metabolism, detoxification and excretion of contaminants. As such, they are also important storage depots for trace elements and organic contaminants. The latter is probably directly related to the fat content of these organs. Metal accumulation is related to the existence of metal-binding proteins, especially the thioneins, which are low molecular weight proteins containing a sulfhydryl group. The thiol group of these molecules has two outer orbitals with unpaired electrons, easily shared with a IIb group metal cation with vacant external orbitals, such as cadmium, mercury, and zinc (Roesijadi 1981). The result is the formation of metallothioneins which bind and store many metals in a non-toxic form in the kidney and liver.

One would expect the storage depot to be independent of the site of toxicity (target organ). In many cases this is true. The principal site of toxic response for lead and for organic pesticides is the nervous system; however, the major storage depot for lead is bone and for organic pesticides it is fat.

This is not so straight forward in the case of the kidney. Although the formation of metallothioneins allows for the storage of many metals in a non-toxic form, it also appears that at certain elevated metal levels, this system may be overwhelmed and irreversible damage to renal tubules and nephrons can occur (Goyer 1986).

Choice of Tissues for Archival

Ideally the tissue to be sampled in any monitoring program should be a main target of the contaminant, preferably a target organ or tissue which is indicative of the major toxic effect of the contaminant. Levels detected in this way would correspond directly to the effect caused. However, as is pointed out by Aguilar (1983), some target tissues may be more difficult to collect than others and may deteriorate more quickly (example, nerve tissue).

It may be more appropriate for practical reasons to collect tissue which is not the main target, but gives a representative picture of residue levels present in the rest of the body (total body burden). This has been successfully done for some birds (Dindal 1970; Capen and Leiker 1979; Friend et al. 1979; Larsson and Lindegren 1987) and significant research on this approach for marine mammals has been conducted by Aguilar (1985, 1987), Bergman et al. (1981), Honda et al. (1986), Reijnders (1984, 1986), and Tanabe et al. (1981). As Moriarty (1985) points out, most animals are exposed to fluctuating levels of contaminants. Since the levels in the various compartments of an organism are controlled by the dynamic equilibrium established between the circulatory and peripheral compartments, strong correlation between contaminant levels in the various tissues of accumulation is difficult without sustained constant exposure.

The accumulation of organic contaminants (organochlorines, organometallic compounds, aromatic hydrocarbons, etc.) is directly related to the lipid solubility of the compound. Tissues containing large amounts of lipids are, therefore, candidates for organic contaminant analysis. Such tissues include blubber, visceral fat, liver, muscle, brain, and bone.

Figure 1 is loosely based on the compartmental models commonly used in animal physiology and pharmacology (Moriarty 1983). However, the compartments of such models are not necessarily defined by organ or organ systems, but by the chemistry of the contaminant. A compartment is a mass of a contaminant that has uniform kinetics of transformation and transport, and whose kinetics are different from those of all other compartments (Moriarty 1983). Based on this definition, a compartment would be best represented by a cellular component (such as lipid) rather than an organ (such as liver) or even a tissue type (such as adipose).

Defining a compartment based on the chemistry of the contaminant is significant relative to analysis and data interpretation. A good example of this is the analysis and interpretation of contaminants associated with blubber. The blubber in some marine mammals has been found to have consistently higher

levels of organic contaminants as compared to other organs, but with relatively large variations between individual animals and between replicated samples. Much of this variation is dependant upon the body condition of the animal. Since most of the pollutant is incorporated into the body of mammals through food, a change in the relationship between food intake and fat deposition may account for changes in the concentrations of a given chemical in the body tissues. Metabolism of reserved fatty tissue during periods of little feeding may result in fat loss and an increase in the concentration of organic contaminants in the remaining blubber. Dilution of residue concentration due to the rapid expansion of the fat compartment during growth has been found in neonates and juveniles of man and other mammals (Aguilar 1985).

More significant than the total volume of fat tissue may be the amount of stored lipids. The blubber does not increase or decrease from cellular gain or loss, but by reduction of adipocyte vacuoles which means that there may exist a minimum thickness from which the blubber can continue freeing lipids without an appreciable reduction in its thickness (Aguilar 1985). Several investigators have found that, except for brain tissue, there is no marked difference between the organochlorine concentration in the different tissues of an individual when the pollutant concentrations are expressed on a lipid weight basis (Jensen et al., 1969; Linko et al., 1974; Perttinen et al., 1986). As will be discussed below, the exception of brain tissue may be related to the molecular structure of the brain lipids, as compared to the storage lipids of other organs.

The normalization of organochlorine pollutants in different tissues of cetaceans through reporting on the basis of lipid content was discussed by Aguilar (1985) relative to compartmentation of the pollutant and reliability of tissue sampling procedures. Most past studies of organochlorines in marine mammal tissues have compared concentrations on wet weight basis. Concentrations have been found to be highest in the blubber, but with also the greatest variability in the blubber. Since the apolar organochlorines are lipophilic they would tend to concentrate in the lipid portion of the tissue. Reporting of the concentrations based on lipid content should reduce this variability.

Lipids are also found in liver, kidney, muscle and brain, and, therefore, higher concentrations of lipophilic organic contaminants should also be found in the lipid portion of these tissues. Aguilar (1985) supposes that the organochlorines are shared among different organs and tissues in proportion to their fat content (fat compartment). In his review of organochlorines in whales, Aguilar (1985) also points out that lipid material consists of many different compounds with varying degrees of apolarity. The less polar triglycerides and non-esterified fatty acids (NEFA) should favor accumulation of organochlorine compounds over more polar lipids, such as phospholipids (Kawai and Fukushima 1981; Aguilar 1985). Therefore reporting concentrations based on triglycerides plus NEFA might reduce the variability of the measurements even further.

In comparison to blubber levels, the brain concentrations of xenobiotics have been found to be lower by an order of 10:1 than in other tissues, such as lung, liver, kidney, blubber, and muscle (Hayes 1975) with lesser variation between individuals and between replicated samples (DeLong et al. 1973; Robert DeLong, personal communication, 1987). This has been the case even when concentrations are expressed on a lipid basis. This could be due to the blood-brain barrier to passage of materials between the circulatory system and brain. This would be particularly effective relative to the transport of ionized compounds;

however, this barrier might not be as efficient relative to liposolubles (Aguilar, 1985). In fact, Walker (1975) indicates that the barrier is very permeable to organochlorines. The basic constituents of the cerebral lipids are the phospholipids, which are less conducive for accumulations of liposoluble compounds and, therefore, these compounds may explain the lower levels of organochlorines found in brain tissue. The brain levels of these contaminants probably remain relatively independent of body conditions since phospholipids are maintained even during starvation.

Statistically, the brain might be better for analysis than blubber; however, there are still questions regarding the ability of different contaminant compounds to cross the blood-brain transport barrier (Kurtz, nd.). There is also the problem of obtaining brain tissue without contaminating the sample, since one would either have to cut through the skull under field conditions without disturbing the brain or return the entire head to the lab for dissection.

The liver is a principal candidate for tissue sampling since it is the major detoxification site for the contaminants of interest, and for some marine mammals it is an item of human consumption. The liver generally has sufficient lipid content that it is suitable as an accumulator of organic as well as inorganic compounds and may also represent a higher proportion of metabolites than remaining tissues.

Tissues commonly sampled for trace elements include liver, heart, kidney, muscle, and bone. Although all of these organs appear to accumulate different trace elements to different degrees, both the liver and kidney appear to be principal organs of concentration for most elements. For example, Goldblatt and Anthony (1983) reported that, in the northern fur seal, the largest concentrations of most metals have been found in the liver and kidney while elevated levels of lead and nickel have commonly been found in bone as well as liver.

Considering all of the factors discussed above relative to the advantages and disadvantages of certain tissue types for contaminant analysis, six rather simple but practical criteria were followed for initial selection of tissues to be included in the Archive. These criteria are:

1. The minimum two 150 g samples can be obtained from the tissue (see Methods Section).
2. The tissue will provide a homogeneous sample.
3. The sample is conducive to precise anatomical description.
4. The tissue is accessible to sampling techniques.
5. The tissue has a potential for concentrating both inorganic and organic contaminants.
6. The tissue is commonly consumed by humans.

Based on these criteria, tissues will be routinely collected from liver, kidney, blubber, and skeletal muscle. Other tissues will be considered on a special case bases and as the sampling protocols are developed.

Variability in Contaminant Measurements

The preceding section of this report discusses several factors contributing to the variability in contaminant data in marine mammals. However, emphasis has been on normalization of the data through selection of appropriate units for

expressing concentrations (wet weight, lipids, triglycerides, etc.) Other factors are also involved in the variations among individuals of the same species and among individuals of the same populations that have been found in past studies of contaminants in marine mammals.

An examination of the variation in organochlorine levels in marine mammals by Aguilar (1987) resulted in his listing of eight major sources of variability. These sources, which probably apply to contaminants in general are:

1. Nutritional state of the animals.
2. Sex and age.
3. Differential metabolism and excretion.
4. Food chain level (varies depending on age and sex).
5. Distance from contaminant source (variation in exposure).
6. Variation in tissue sampling.
7. Selection of appropriate units for expressing concentrations (wet weight, dry weight, etc.).
8. Tissue preservation and analytical procedures.

Aguilar (1987) points out that, ideally, any comparison of pollutant concentrations between different marine mammal populations should use only individuals of the same age, sex, reproductive category and fattening state. He also suggests that sampling within a given size range of only one sex (preferably males because of the usual higher tissue loads) on a limited time period would help to overcome many of the variability problems. Within a program such as the Archival Project, the ability to direct the sampling such that the above restrictions are applied will be difficult. The Archival Project will be relying upon samples collected in cooperation with ongoing research and management programs of other agencies and such control may not be possible. However, for some pinniped species sex and age may be controllable. For example, northern fur seal samples obtained during the subsistence harvest will come from 2-3 year old males, since these are the only animals taken during the harvest. Sex and age may also be controllable sampling factors for walrus, and perhaps other species.

All sources of variation can not be controlled during sampling. However, as samples are collected and archived, knowledge of variability factors can be used to establish guidelines on what comparisons can be made and what interpretations can be derived from these comparisons.

Collection, storage, and analytical conditions should be exactly the same for all samples in order for comparisons between samples to be valid. Samples preserved under different conditions are not directly comparable unless the possibility of differential pollutant derivation or loss can be reasonably excluded (Aguilar 1987). The Archival Project can control some of the factors contributing to measurement variations, such as the variation in tissue sampling, the selection of appropriate units for expressing concentrations, and tissue preservation and analytical procedures. The collection protocols presented in the Methods Section of this report are intended to provide this control over sampling and preservation procedures. Analytical procedures and the selection of concentration units for variability reduction will continue to be investigated by the Project.

METHODS

Sample collections will be conducted in cooperation with research and/or resource management programs of federal, state and local agencies and organizations. Samples to be archived, including those obtained incidentally, will be collected, documented and handled in accordance with the standard protocols developed for the project in 1987, and which are consistent with those employed by the NBSB, NBS. Sample storage and inventory procedures will be in accordance with those routinely performed at the NBSB. This will include specimen storage under liquid nitrogen vapor at -150°C .

Collection Protocols

The collection protocols for this project are modifications of those used by the NBSB for the collection of human liver samples (Harrison, et al. 1981; Zeisler, et al. 1983a), and protocols used by NOAA's Status and Trends program for the collection of fish, bivalves, and sediment samples for the purpose of specimen banking and environmental contaminant analysis (Lauenstein 1986; Lauenstein and Young 1986).

The quality of logistic and support facilities vary within Alaska. The necessity for collecting materials in remote areas under relatively primitive conditions, will probably require the development of more than one protocol. Within the limitations imposed by the conditions under which collections are made, the intent of each of the collection protocols is to obtain fresh well-defined tissue samples uncontaminated by extraneous sources of trace elements and organic compounds, and to package and transport these samples as quickly as possible under conditions which eliminate or minimize chemical changes within the tissues prior to storage.

Materials

The following materials are used for collecting marine mammal tissue samples:

- Container with blue ice for transporting bagged samples from collection site to processing site,
- Teflon sheeting with adhesive backing for covering processing surfaces (usually lab benches),
- 1 Dry shipper (LN_2 cooled) with shipping container,
- LN_2 in container for freezing samples on site,
- Dewar and lid,
- Lab coats (disposable),
- Insulated gloves, face shield, and tongs for handling the LN_2 and frozen samples,
- Balance for weighing samples (triple-beam balance is suitable),
- Surgical scissors, forceps and screwcap vials with buffered formalin for histological samples,
- Labels for exterior of sample jars,
- Tape for securing exterior jar labels,
- Shipping labels,
- Data recording forms,
- 5' x 10' polyethylene sheet, and
- Garbage bags for the collection of used materials in the field for later disposal.

In addition, the collection of two replicates of a single sample requires:

- 4 pairs non-talced vinyl gloves,
- 2 Teflon FEP (Fluorinated ethylene propylene) bags to be used as sheets for clean working surfaces,
- 2 Teflon FEP bags for transporting samples from the field to the processing facilities,
- 2 Teflon jars (180 mL, 49 mm diameter, 120 mm length) with lid labels,
- 2 500-mL Teflon bottles containing high purity distilled or best available water for rinsing samples,
- 1 Titanium blade/Teflon TFE (Tetrafluoroethylene) handle knife, and
- High grade ethanol for rinsing knife (1 L).

For the collection of associated biological/environmental data, the following equipment will also be required:

- Thermometer for air temperature,
- Measuring tape (metric),
- Heavy duty scales for weighing animal (where practical),
- Short metric ruler for measuring blubber thickness,
- Camera for recording field procedures (optional)

General

The collection protocol consists of three stages: tissue removal from the individual animal, tissue processing to obtain representative samples, and packaging/shipping of the samples to the archive (Figure 2). The division of the protocol into stages is used as an aid in organizing and simplifying the collection procedures.

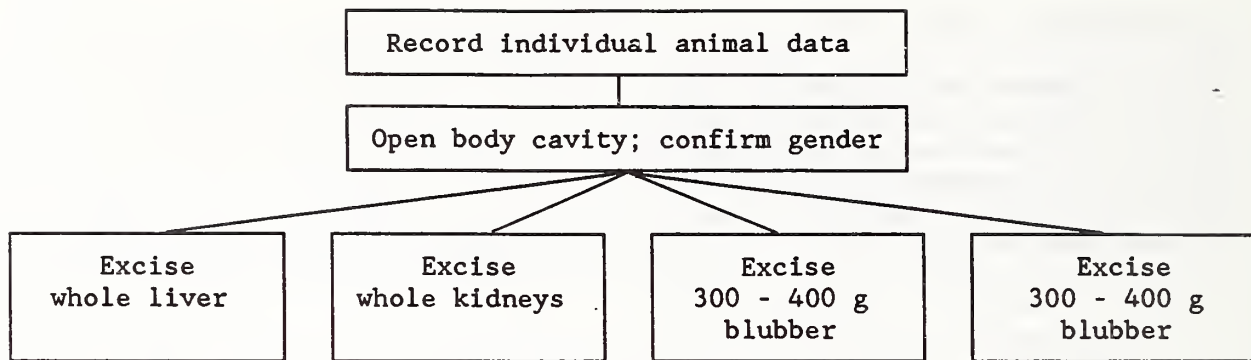
Stage I, tissue removal, will occur out-of-doors in the field under conditions of limited control. Procedures for tissue removal will vary depending upon the group of mammals being sampled (e.g., pinnipeds vs cetaceans), but should be the same for individuals of the same species.

Stage II, tissue processing, will occur indoors in the majority of cases and under laboratory conditions where possible; this includes shipboard laboratories.

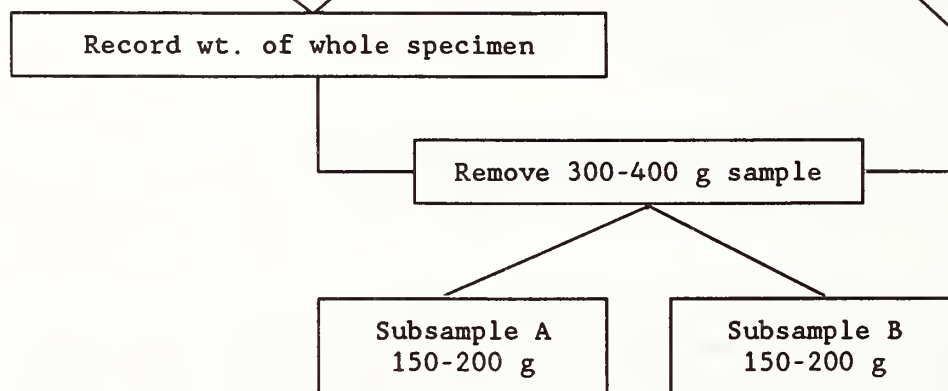
Stage III, sample packaging and shipping, should be relatively standard for all tissues and should not vary, while the processing of tissues might vary depending on the availability of laboratory facilities near the collection site and the tissue type to be sampled.

Standard forms for recording information pertinent to the sample collections are presented in Appendix A.

Stage I



Stage II



Stage III

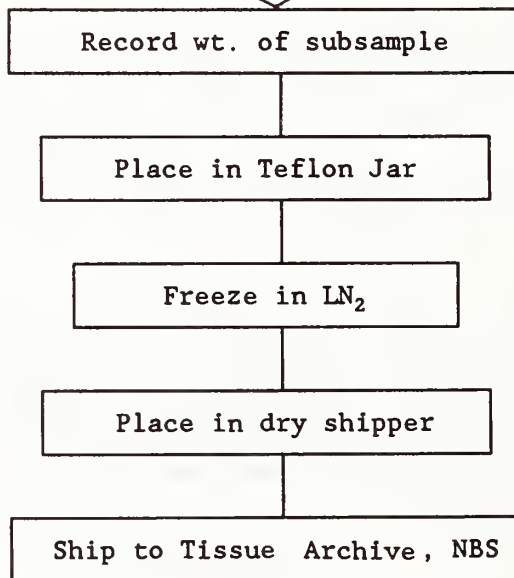


Figure 2. Generalized Collection Protocol

Stage I. Tissue Removal

1. The size of the tissue sample removed from an animal should be sufficient to provide two subsamples of 150-200 g each for archival. This amount of material will be required in order to maintain long-term archived samples plus aliquots for periodic analysis.
2. The anatomical location of tissue removal is specified in order to maintain consistency and comparability between the same tissue types.
3. Sterile, cleaned, non-talced vinyl gloves will be used by all personnel involved in sample removal and handling. Precaution must be taken throughout the procedures to reduce the risk of chemical contamination of the sample. Contamination may originate from the individual performing the work (do not smoke during the procedure), the atmosphere, the skin of the animal, the instruments used in the dissection, and any chemicals that happen to be in the area where the work is being performed. (refer to "Contamination Sources", Appendix B)
4. No animal will be considered a candidate for sampling, if tissues cannot be collected within 10 hours after death (air temperatures <40 °F) and if handling of the carcass between time of death and tissue removal can not be documented.
5. Procedures for Pinnipeds and Sea Otters (from Fay, et al. 1979):
 - a. Record the weather information and individual animal data on the data recording forms.
 - b. For carcasses that can be moved, place carcass ventral side up on 5' x 10' polyethylene sheet; straighten the spine (e.g., by grasping the head and pulling). Measure the length (tip of snout to tip of tail flesh, in a straight line) and axillary girth (cm). For animals that can not be moved, place the polyethylene sheet on the left side of the carcass to provide a working surface beside the animal.
 - c. Make an incision 4-5 cm long over the sternum, midway between the axillae, cutting through the skin and blubber. Measure the blubber thickness (mm) at this point.
 - d. Remove the ventral body wall from chin to anus, cutting through the costal cartilages, and lay it out, skin side down, to one side as a work area. Confirm the gender.

Note! In the case of the northern fur seals, the pelt is traditionally removed from the animal before the body cavity is opened. For this species the animal is placed on its back on the polyethylene sheet and incisions are made around the neck and flippers, and through the skin from the chin to anus. The animal is then turned over on its ventral side (still on the polyethylene sheet), the head is held in place by a forked steel bar, and the skin of the back of the head is grasped and pulled posterior to remove the entire pelt. The body cavity is then opened and the internal organs removed as described below.

6. Procedures for Cetaceans (from Fay, et al. 1979):
 - a. Record the weather information and individual animal data on the data recording forms.
 - b. For animals that can be moved, place carcass left side up on 5' x 10' polyethylene sheet. For animals that can not be moved, place the polyethylene sheet to the side of the carcass to provide a working surface beside the animal. Measure the length (tip of snout to fork of tail, in a straight line) and axillary girth (cm).
 - c. Make an incision through the skin and blubber about midway along the side and measure the blubber thickness (mm). The incision should be small enough to prevent distortion, but its size will depend on the size of the animal and thickness of its blubber.
 - d. With the animal lying on its side, remove the skin and blubber from the left lateral body wall and lay it out, skin side down, to one side as a work area. Confirm the gender.
7. The tissue samples are removed as soon as possible after opening the body cavity. Opening of the body cavity and initial cutting of the skin to expose muscle and adipose tissue may be performed with high quality stainless steel dissection tools previously rinsed in the high purity water.
 - a. Liver. Note the general appearance of the liver before removal, including any unusual coloration, texture, shape, etc.
 - (1) For the smaller species (northern fur, ringed, spotted, and harbor seals and sea otter: Remove the entire liver from the animal and place in a clean Teflon bag for immediate transport to the processing area, or place it on a Teflon sheet on a wooden board for dissection in the field (see Stage II.4 for the dissection instructions). Wetting the wooden surface enhances the adherence of the Teflon sheet. Ligaments of the liver may be cut using surgical scissors which have been previously rinsed. (If liver membrane is ruptured or stabbed, the sample is not acceptable).
 - (2) For the larger species (walrus, bearded seal, and cetaceans): Using the titanium knife, remove a 300-400 g section from the liver and place in a clean Teflon bag for immediate transport to the processing area. For animals with multi-lobed livers (pinnipeds), this section is taken from the posterior portion of the left anterior lobe. For animals with liver divided into two lobes by a shallow indentation in the posterior edge (cetaceans), this section is taken from the posterior portion of the left lobe.
 - b. Kidney. Note the general appearance of the kidney before removal, including any unusual coloration, texture, shape, etc.

- (1) For the smaller species (northern fur, ringed, spotted, and harbor seals and sea otter): Remove both kidneys from the animal and place in a clean Teflon bag for immediate transport to the processing area. Both kidneys are required in order to provide a total sample size of 300 g. (Note which is the left and which is the right kidney). Attachments of the kidney may be cut using surgical scissors which have been previously rinsed (If the kidney membrane is ruptured, the sample is not acceptable).
- (2) For the larger species (walrus, bearded seal, and cetaceans): Remove the left kidney from the animal and place in a clean Teflon bag for immediate transport to the processing area. Attachments of the kidney may be cut using surgical scissors which have been previously rinsed (If the kidney membrane is ruptured, the sample is not acceptable).

If the entire kidney is too large to be transported back to the processing area, remove a 300-400 g. section from the posterior end of the left kidney using the titanium knife and place in a clean Teflon bag for transport.

c. Adipose Tissue (Blubber). The anatomical site of blubber removal will depend on the distribution of fat layers on the animal and the butchering procedures used during subsistence harvests; it will, therefore, be rather species specific.

- (1a) For animals with relatively thin blubber layers, such as northern fur seals and sea otters: Using the titanium knife, remove sections of blubber from along the backbone until the required 300 g are obtained. Any small portions of muscle tissue will be removed at the processing site. Avoid collecting blubber from near the areas where the initial cuts through the skin were made since these areas are contaminated with loose hair.
- (1b) For animals with relatively thick blubber layers: In the region of the sternum (or to the left of the point where the blubber thickness measurement was made) remove a rectangular section (300 g) of blubber using the titanium knife previously rinsed in high purity water. This section should be a vertical section from just below the skin to the surface of the muscle.
- (2) Place the blubber sample in a clean Teflon bag for immediate transport to the processing area.

d. Muscle.

- (1) Using the titanium knife, remove strips of muscle tissue from along the backbone until approximately 300 g are obtained. Avoid collecting muscle tissue from near the areas where the initial cuts through the skin were made since these areas are contaminated with loose hair.

- (2) Place the muscle sample in a clean Teflon bag for immediate transport to the processing area.
8. For most of the pinnipeds the upper canine teeth are used to determine age. The procedures for extracting and preparing the teeth for examination are given in Appendix B.

Stage II. Tissue Processing

1. Tissue processing will take place indoors within a laboratory facility and under a clean air hood, if possible. At a minimum, the tissue processing will take place in a covered and enclosed area free of obvious sources of contamination such as cigarette smoke, fuel oil fumes and smoke, laboratory formaldehyde, etc. The processing area of the laboratory should be cleaned to remove dust and the working surfaces covered with Teflon sheeting.
2. Only titanium knives are to be used to cut samples during Stage II. Two knife cleaning procedures are presented in Appendix B. Cleaning Procedure I can be performed at the processing site. Procedure II will be performed only at the NBS facilities, or at another suitable laboratory.
3. If tissue sections are taken for histological work, this information is to be recorded in the appropriate space on the data reporting forms. The results of the histological analysis should be reported to the NBSB. Any change in the physical location of the histological slides should be reported to the NBSB. Investigators who intend to dispose of histological slides after analysis are encouraged to transfer these materials to the NBSB, where they will be cataloged, cross-referenced to archived tissue samples, and maintained in the NBS archives.

4. Liver

a. If the whole liver is transported to the processing facility:

- (1) Weigh the Teflon bag containing the liver specimen. Use an empty bag as a tare. Record the weight on the Data Recording Form.
- (2) Cut open the Teflon bag containing the specimen so that it provides a flat sheet for rinsing the liver.
- (3) Rinse the surface of the specimen with water from the Teflon bottle. Pour approximately 100 mL or more of the water from the Teflon bottle over the specimen to wash off blood and other fluid. Rub the specimen with gloved hand, if necessary, to remove blood, etc. Pick up the specimen and, while held in the air, rinse the Teflon surface with the water. Place the specimen back on the Teflon sheet, unwashed side facing up. Rinse this side with another 100 mL of water. Allow the specimen to drain for several minutes.
- (4) The seal liver consists of four to eight long finger-like lobes. Using the titanium knife, remove the two anterior lobes to give

two samples (Sample A and Sample B) of 150-200 g each. Each sample must fit in a Teflon jar with a volume of 180 mL (49 mm diameter and 120 mm length).

- (5) Rinse the titanium knife with the water and rub with the gloved fingers to remove all blood and fluids from the knife before they have time to become dried on. Rinse the knife with ethanol and air dry.
 - (6) Continue to Stage III, Tissue Packaging and Shipping.
- a. If a sample of the liver (300-400 g) has been taken in the field and transported to the processing facility:
- (1) Cut open the Teflon bag containing the specimen so that it provides a flat sheet for rinsing the liver.
 - (2) Rinse the surface of the specimen with water from the Teflon bottle. Pour approximately 100 mL or more of the water from the Teflon bottle over the specimen to wash off blood and other fluid. Rub the specimen with gloved hand, if necessary, to remove blood, etc. Pick up the specimen and, while held in the air, rinse the Teflon surface with the water. Place the specimen back on the Teflon sheet, unwashed side facing up. Rinse this side with another 100 mL of water. Allow the specimen to drain for several minutes.
 - (3) Using the titanium knife divide the specimen into two equal samples (Sample A and Sample B) of 150-200 g each. Each sample must fit in a Teflon jar with a volume of 180 mL (49 mm diameter and 120 mm length).
 - (4) Rinse the titanium knife with the water and rub with the gloved fingers to remove all blood and fluids from the knife before they have time to become dried on. Rinse the knife with ethanol and air dry.
 - (5) Continue to Stage III, Tissue Packaging and Shipping.

5. Kidney

- a. If both kidneys were collected from the animal:
- (1) Weigh the Teflon bag containing the kidneys. Use an empty bag as a tare. Record the weight on the Data Recording Form.
 - (2) Cut open the Teflon bag containing the specimens so that it provides a flat sheet for rinsing each kidney.
 - (3) Rinse the surface of each kidney with water from the Teflon bottle. Pour approximately 100 ml or more of the water from the Teflon bottle over each kidney to wash off blood and other fluid. Rub the specimen with gloved hand, if necessary, to remove blood, etc. Pick up the specimens and, while held in the

air, rinse the Teflon surface with the water. Place the specimens back on the Teflon sheet, unwashed side facing up. Rinse this side with another 100 mL of water. Allow the kidneys to drain for several minutes.

- (4) In some cases each kidney will approach the weight of the required specimen size for each subsample (150 g) and no subsampling will be necessary. The right kidney will provide Sample A and the left kidney will provide Sample B. If subsampling is required (or if cutting of the sample is necessary to fit the sample into the jar), use the titanium knife to remove a 150 g section from the kidney. Each sample must fit in a Teflon jar with a volume of 180 mL (49 mm diameter and 120 mm length).

b. If the left whole kidney was collected from the animal:

- (1) Weigh the Teflon bag containing the kidney. Use an empty bag as a tare. Record the weight on the Data Recording Form.
- (2) Cut open the Teflon bag containing the specimen so that it provides a flat sheet for rinsing the kidney.
- (3) Rinse the surface of the kidney with water from the Teflon bottle. Pour approximately 100 mL or more of the water from the Teflon bottle over the kidney to wash off blood and other fluid. Rub the specimen with gloved hand, if necessary, to remove blood, etc. Pick up the specimen and, while held in the air, rinse the Teflon surface with the water. Place the specimen back on the Teflon sheet, unwashed side facing up. Rinse this side with another 100 mL of water. Allow the kidney to drain for several minutes.
- (4) In some cases the entire kidney may approach the weight of the required specimen size and no subsampling will be necessary. If subsampling is required (or if cutting of the sample is necessary to fit the sample into the jar), use the titanium knife to remove a 150 g section from the kidney. Each subsample must fit in a Teflon jar with a volume of 180 mL (49 mm diameter and 120 mm length).

c. If samples of kidney tissue were collected from the animal:

- (1) Cut open the Teflon bag containing the specimen so that it provides a flat sheet for rinsing the kidney sample.
- (2) Rinse the surface of the specimen with water from the Teflon bottle. Pour approximately 100 mL or more of the water from the Teflon bottle over the specimen to wash off blood and other fluid. Rub the specimen with gloved hand, if necessary, to remove blood, etc. Pick up the specimen and, while held in the air, rinse the Teflon surface with the water. Place the specimen back on the Teflon sheet, unwashed side facing up. Rinse this side with another 100 mL of water. Allow the sample to drain for several minutes.

- (3) Using the titanium knife, remove two subsamples of 150 g each from the kidney sample. Each subsample must fit in a Teflon jar with a volume of 180 mL (49 mm diameter and 120 mm length).
- d. Rinse the titanium knife with the water and rub with the gloved fingers to remove all blood and fluids from the knife before they have time to become dried on. Rinse the knife with ethanol and air dry.
- e. Continue to Stage III, Tissue Packaging and Shipping.

6. Blubber

- a. Cut open the Teflon bag containing the specimen so that it provides a flat sheet for rinsing the blubber.
- b. Pour approximately 100 mL or more of the water from the Teflon bottle over the specimen to wash off blood and other fluid. Allow the specimen to drain for several minutes.
- c. Use the titanium knife to remove any remaining portions of muscle attached to the blubber. Excise a 300-400 g section from the specimen.
- d. Using the titanium knife divide the specimen into two equal samples (Sample A and Sample B) of 150-200 g each. Each sample must fit in a Teflon jar with a volume of 180 mL (49 mm diameter and 120 mm length). Rinse the titanium knife with the water and rub with the gloved fingers to remove all blood and fluids from the knife before they have time to become dried on. Rinse the knife with ethanol and air dry.
- e. Continue to Stage III, Tissue Packaging and Shipping.

7. Muscle

- a. Cut open the Teflon bag containing the specimen so that it provides a flat sheet for rinsing the muscle.
- a. Pour approximately 100 mL or more of the water from the Teflon bottle over the specimen to wash off blood and other fluid. Allow the specimen to drain for several minutes.
- b. Use the titanium knife to remove any portions of blubber attached to the muscle tissue. Excise a 300-400 g section from the specimen.
- c. Using the titanium knife divide the specimen into two equal samples (Sample A and Sample B) of 150-200 g each. Each sample must fit in a Teflon jar with a volume of 180 mL (49 mm diameter and 120 mm length). Rinse the titanium knife with the water and rub with the gloved fingers to remove all blood and fluids from the knife before they have time to become dried on. Rinse the knife with ethanol and air dry.

- d. Continue to Stage III, Tissue Packaging and Shipping.

Stage III, Tissue Packaging and Shipping

1. Place each sample in individual pre-cleaned Teflon jars. Tare the weight of the jar and weigh each individual sample. Record the weights on the Data Recording Form and sample labels. Affix the sample labels to the jars with and place the lid labels in the jar lids.
2. Freeze each sample by immersing in the LN₂ for 10 minutes.

Liquid nitrogen should not be stored in sealed containers. Personnel handling LN₂ are cautioned to wear boots, cuffless trousers, non-absorbent aprons, loose insulating gloves, and face shields.
3. The LN₂ shipper should be filled with liquid nitrogen for at least 6 hours to fully prepare it for shipping. This is required to fully saturate the absorbent inside the shipper. Pour off the excess LN₂ and place the frozen samples in the shipper (10-12 sample boxes per shipper).
4. Once full transport the shippers to the NBS; do not store in intermediate freezers.
5. Double check the Data Recording Forms for completeness and accuracy. Any deviations or modifications of the protocol must be noted on the sampling form.
6. Place a copy of the completed forms in the shipper; another copy is retained by the collector for project records.
7. Samples should be shipped within 48 hours or as soon as possible after sample collection using 24 hour express package service to:

National Bureau of Standards
Route 270 and Quince Orchard Road
Building 235, Room B118
Gaithersburg, Maryland 20899
Attn: Barbara Koster (301) 975-6291

In most cases, samples will have to be shipped commercial-air to Anchorage and then 24 hour express package service to the Archive. The shippers must not contain LN₂ when shipped. Maximum holding time for the shippers is 10-12 days. Shipping expenses will be borne by OCSEAP. Do not ship late in the week, i.e., Thursday, or Friday, or before holidays, unless special arrangements have been made with the shipping service, OCSEAP, and NBS.

8. The Specimen Bank personnel should be notified by telephone as soon as possible after the specimens are shipped:

Barbara Koster	(301) 975-6291 (FTS, 879-6291), or
Rolf Zeisler	(301) 975-6290 (FTS, 879-6290), or
Steve Wise	(301) 975-3112 (FTS, 879-3112)

Sample Archival

Samples are received at NBS and transported to the Specimen Bank facility. The shippers are unpacked and samples are inspected for any packaging problems and for unsuitable temperature. Sample data forms and samples are compared to insure that they correspond and that all information has been included. These samples are stored in a temporary storage LN₂ freezer and are logged into the temporary storage log book. They remain in temporary storage until assigned an NBS number and permanent LN₂ freezer space.

When the samples are moved into the permanent freezer location, a storage form is completed and the information is entered into the inventory form on the IBM-AT computer. Depending on the geometry of the samples, various types of containers are used to hold them in the LN₂ freezer. The samples may be placed in long cylindrical tubes containing several samples or small square freezer boxes in metal racks. These samples will remain stored in the LN₂ freezer and the conditions monitored until they are requested for analysis.

Monitoring of Sample Stability During Storage

In order to evaluate the stability of the archived tissues, NBS monitors the concentrations of selected contaminants in 20% of the tissue specimens. Aliquots of those specimens selected for monitoring are initially analyzed to establish the baseline levels. Repeated analyses of aliquots of these tissues on a regular basis (every one or two years) provides a measure of any change from the initial baseline concentrations. Besides providing a baseline to evaluate sample storage stability, these analyses also serve two other purposes:

1. They provide some real-time measure of contaminant concentrations for monitoring purposes.
2. They provide a baseline for comparing contaminant levels using present analytical techniques with those measured in the future by other laboratories using different methodologies.

In selecting the contaminants to be monitored, emphasis has been placed on those elements and compounds which may be linked to the oil and gas industry in Alaska. These are the heavier molecular weight aromatic hydrocarbons and trace elements, such as Ba, Cr, Ni, and V (Tables 2 and 3). Other contaminants which are known to occur in elevated levels in the tissues of marine mammals (Cd, Hg, Pb, Zn, and selected chlorinated hydrocarbons) will also be monitored on a routine basis.

Of the two subsamples (Samples A and B) of each tissue which are archived, sample "A" is maintained in long-term storage in the LN₂ freezers while sample "B" is used for the initial baseline analyses and storage stability evaluation. Samples to be analyzed are homogenized using a cryogenic homogenization procedure designed to reduce the likelihood of changes in sample composition due to thawing and refreezing (Zeisler et al. 1983b). The sample homogenate is then aliquoted into small Teflon jars (7-10 g) for analysis and for storage as the homogenate.

The analytical approach for the baseline determinations of the trace elements focuses on the use of a multi-element analytical technique [neutron activation analysis (NAA)] to provide data on a large number of trace elements using only a limited amount of sample. Additional analytical techniques (voltammetry and atomic absorption) are being used to provide data on elements of high priority that are not routinely measured by NAA (e.g., Pb and Ni) and to provide quality control data for selected elements by comparing data from two different analytical techniques.

Capillary gas chromatography with electron capture detection is used to measure selected chlorinated hydrocarbons and high performance liquid chromatography with fluorescence detection is used to measure aromatic hydrocarbons.

During the initial year of analyses, all four tissue types from the first year collection of northern fur seals will be analyzed to determine the suitability of each tissue for monitoring of contaminant levels and analytical problems associated with each tissue.

Quality Assurance

NBS is an active participant in the OCSEAP's Quality-Assurance Program for Trace Petroleum Component Analysis, which is coordinated with the Quality-Assurance Program of NOAA's National Status and Trends Program.

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APPENDIX A

DATA RECORDING FORMS: INSTRUCTIONS

Examples of the data recording forms are provided on pages 42 and 43. Standard biological/environmental information is recorded on the first page (see page 42). The boxes at the top of this page provide space for the NBS identification codes. These are assigned to the samples when they arrive at the NSBS. Other information recorded on this page are:

1. OCSEAP ID Number. This is a 10 digit alphanumeric code assigned by the OCSEAP office before collections are made. The first three numbers (692) refer to the OCSEAP Research Unit Number. The next four letters are abbreviated common names for the species sampled (FRSL = Fur Seal). The last three numbers are ascending numbers that identify the individual animal sampled.
2. Species. Genus/species name.
3. Sample Source. Information pertinent to identifying the agency, person(s), research or management program providing or aiding in providing the samples is entered here.
4. Site ID. This the most common name of the location where the animal is sampled (killed). This should be as specific as is practical.
5. Lat. Long. The latitude and longitude of the location where the animal is sampled. This should be to the nearest tenth of a minute, if possible.
6. Time of Death. Recorded as the day, month, year and hour. The month should not be numbered, but should be written in abbreviated form (Jan, Feb, May, Apr, May, Jun, Jul, Aug, Sep, Oct, Nov, Dec) and the hour should be on a 24-hour basis (example, 6:00 pm is reported as 1800).
7. Method of Collection. The method by which the animal is sacrificed.
8. Intermediate Storage. Method of storage of animal carcass before removal of tissues. If tissues are taken immediately after death, no entry is necessary.
9. Weather Conditions. Space is provided for the notation of weather conditions occurring during the sampling procedures (wind, temperature, precipitation), particularly anything that would be pertinent relative to contamination sources.
10. Sex, Age, and Method of Age Estimation. Blocks are provided for indicating the sex of the animal and the age (in years). Method of age estimation and the name of the individual making that determination should also be recorded here.
11. Weight. Estimated or measured weight of the animal sampled should be recorded in the space provided.

12. Length. This is measured as snout to tail tip length along a straight line for pinnipeds, sea otters, and polar bears, and as snout to fluke notch length along a straight line for cetaceans.
13. Axillary Girth.
14. Sternal Blubber Thickness.
15. For Females. Blocks are provided to note if the animal sampled is lactating or pregnant.
16. General Appearance of Individual. Any comments which describe the healthy or unhealthy appearance of the animal, external parasites, evidence of trauma, or body condition of the animal.
17. General Appearance of Organs. Any unusual appearances of any of the internal organs, particularly those to be sampled. If they all appear normal, note this also.
18. Stomach Contents. Food items if present are identified and some impression of degree of fullness recorded. If internal parasites are present, they are also noted.
19. Additional Samples. Samples collected from the same animal for other research purposes are recorded in this space. This information includes the kind of sample(s), for what purpose, and the name and location of the individual receiving the samples.

The second page of the data recording form (see page 43) provides for the entry of data and information specific to the tissue samples themselves.

20. OCSEAP ID Number. This is the same number as found on the first page (Item #1). It is entered here in case the two pages are accidentally separated.
21. Sample Type. The type(s) of tissue samples collected from the individual animal are indicated by circling the appropriate category or by writing the name in the space provided.
22. Time of Collection. Entered as in Item #6 on the first page of the data recording form. This is the time at which the tissues are removed from the animal in the field.
23. Collected by. Name of the individual actually removing tissue samples from the animal in the field.
24. Intermediate Storage. Space is provided to indicate how tissue samples collected in the field are stored during transport to the processing site (method of cooling, transport containers, etc.).
25. Time of Preparation. Entered as in Item #6 on the first page of the data recording form. This is the beginning time at which tissues are processed into subsamples and placed in containers in preparation for LN₂ freezing.

26. Time of LN₂ Freezing. Entered as in Item #6 on the first page of the data recording form. This is the time at which the samples are frozen in LN₂. It is basically concurrent with the time at which preparation of the subsamples are completed.
27. Time Shipped From Site. Entered as in Item #6 on the first page of the data recording form. This is the time at which the samples leave the sample processing site enroute to the NSBS, Gaithersburg, Maryland.
28. Time Received at Archive. Entered as in Item #6 on the first page of the data recording form. This is the time at which the samples are received at the NSBS, Gaithersburg, Maryland.
29. Processor. The name of the individual(s) processing the samples for LN₂ freezing and shipment to the NSBS.
30. Shipper. The name of the person responsible for shipping the samples to the NSBS.
31. Receiver. The name of the individual receiving the samples at NSBS.
32. Protocol. If the standard or modified protocol was used to collect or process the samples, this is noted here. If a modified protocol, the nature of the modification is recorded.
33. Remarks. Space is provided for any additional remarks pertinent to the collection, processing, or archiving of the tissue samples.
34. Histological Samples. If samples are collected for histological slides, these are recorded here. In all cases, histological samples are to be taken from B subsamples only. The Individual/Organization making the slides, the Final Destination of the slides, the kind of Tissues Sampled, and the method of sample preservation (if appropriate) are recorded here.
35. Sample Weight. The weights of subsamples are recorded here. Note, that in some cases the collection of whole organs might be necessary in order to provide enough tissue for a subsample. If that is the case, and if these tissues are paired, the location of the specific organ should be noted by the subsample space (In the example, the entire right kidney is subsample A and the left kidney is subsample B).
36. Prepared by. The name of the individual filling out the data recording form.

All of the appropriate information for express shipping the samples are provided in the lower right hand corner of the second page. This includes the street, building, and room address of the NSBS, as well as the name and telephone number of the individual responsible for receiving the samples.

NATIONAL BIOMONITORING SPECIMEN BANK

Sampling Data - OCSEAP/MMS

Alaskan Marine Mammal Tissue Archival Project

OCSEAP ID Number _____ Species _____

Sample Source _____

Site ID _____ Lat. _____ Long. _____

Time of Death:

--	--	--

--

 day mo yr hr Method of Collection _____

Intermediate Storage (Temp/remarks) _____

Weather Conditions (wind, temp., precip.) _____

Sex: Male Female Age Method of Age Estimation _____

Weight: Estimated _____ Weighed _____ kg. For Females:

Length: Snout-Tail Tip _____ cm. Lactating

Snout-Fluke Notch _____ cm. Pregnant

Axillary Girth: _____ cm.

Sternal Blubber Thickness _____ cm.

General Appearance of Individual:

General Appearance of Organs:

Stomach Contents:

Additional Samples:

OCSEAP ID Number _____

Sample Type: Liver Kidney Muscle Blubber Other _____

Time of Collection: Collected by _____
 day mo yr hr

Intermediate Storage (Temp/remarks) _____

Time of Preparation:
 day mo yr hr

Time of LN₂ Freezing:
 day mo yr hr

Time Shipped From Site:
 day mo yr hr

Time Received at Archive:
 day mo yr hr

Processor _____

Shipper _____

Receiver _____

Protocol: Standard Modified (Please note modification below)

Remarks:

Histological Samples:

Individual/Organization _____

Final Destination _____

Tissues Sampled _____

Sample Weight:	A	B	A	B
Liver	<input type="text"/> g.	<input type="text"/> g.	Blubber	<input type="text"/> g. <input type="text"/> g.
Kidney	<input type="text"/> g.	<input type="text"/> g.	Other	<input type="text"/> g. <input type="text"/> g.
Muscle	<input type="text"/> g.	<input type="text"/> g.		

Prepared by: _____
 Name (print)

 Signature

APPENDIX B

SPECIAL INSTRUCTIONS

Contamination Sources

The following is taken from a discussion of contamination sources one might encounter during the sampling of human liver tissue in a hospital autopsy room (Harrison, et al. 1981). Many of the sources are also pertinent to the Alaskan Marine Mammal Archival Project.

The control of contamination requires diligent attention to detail. A lack of contamination control has made existing banks of samples useless for general trace constituent analyses. Since it is impossible to point out every source of contamination that may be encountered, some of the most obvious sources will be described to provide a general awareness of the problem. The following example will illustrate the effect of contamination. A 1 μg flake of stainless steel contains approximately 100 ng of nickel. The natural occurrence of nickel in human liver is at levels of 1 - 2 ng/g of sample. As we generally use about 1 g of sample for analysis, a tiny flake of stainless steel in our sample could produce an analytical result which was 100 times higher than the true value.

In an autopsy room, the air, the implements, counter tops, and working personnel are sources of contamination. The air may contain trace vapors of formalin, xylene, and hair spray. It may also contain particles dust, cigarette ash, and wear particles from motors. Implements and working surfaces may be contaminated with chemicals used for cleaning and salt and oils from human contact (via hands, etc.). A common contaminant introduced by human hands is gold from jewelry. Cigarette ash contains relatively high amounts of cadmium and organic compounds. Cigarette smoke contains trace quantities of hundreds of organic compounds.

The dust-free gloves should be used liberally as they are easily contaminated. Picking up a pen to record a weight contaminates them, grasping the Teflon bottle to wash the liver contaminates them, adjusting eyeglasses, touching your face, touching the outside of the bag which contains the clean Teflon sheets or bags contaminates the gloves.

Recent research has pointed out other sources of contamination. One trace organic study found some interesting and unusual compounds in human tissue. They were later found to have been introduced when the sample was cut on a styrofoam board. In another case, the calcium content of a liver was found to be unusually high. It was traced back to contamination from the contents of the stomach. Liquid nitrogen contains oil and dust. The two sealed bags are provided to protect the sample as it is frozen in the LN_2 .

The examples above will give the reader an appreciation of the potential chemical contamination problem.

Since marine mammal tissues will be collected under conditions of less control than the sampling referred to above, contamination sources may be an even greater problem in the Alaska Marine Mammal Archival Project. Sources would include wind-blown dust, rainfall, boat, aircraft and vehicle fuel and exhaust fumes, fuel oil and gas fumes from indoor heating units, and tools used in native subsistence harvest, to name just a few.

Procedures for Cleaning Titanium Knife

There are two cleaning procedures for the titanium knives and other reusable implements: the first is to be completed after finishing a sample and the second is used after knife sharpening or after excessive contamination. The titanium knives should be sharpened only with the silicon carbide stone provided and only these knives should be sharpened with this stone.

Cleaning Procedure I

After placing tissue sections in bags and before leaving the sample preparation area, the knife should be rinsed using high-purity water. While rinsing, and with gloved hands, run fingers over the blade and handle of the knife to help remove any adhering blood or tissue. This is best done before any fluid or tissue has a chance to dry on the knife. In the laboratory, the knife should be rinsed again, as above, with water and then with ethanol. The knife is then placed on a clean surface (do not touch the blade) and allowed to air dry, preferably in a laminar flow hood. The knife should then be placed in a Teflon bag, made from the Teflon sheets, for storage and transported to the next sampling site. The implements should at no time be touched with ungloved hands.

Cleaning Procedure II

This procedure should be applied after excessive contamination of the implements and always after a knife is sharpened. Rinse the implement as described in cleaning procedure I. The knife may be disassembled to clean if necessary. In the laboratory, the implement is placed in a clean container and covered with 99.5% ethyl alcohol for one to two hours. The implement is then covered with high purity water overnight. The implement is covered with dilute hydrochloric acid (one part hydrochloric acid and ten parts high-purity water) for half an hour. The implement is then removed from the acid, rinsed with high-purity water, and covered with dilute nitric acid (one part nitric acid and ten parts high-purity water) for half an hour. The implement is again removed from the acid and rinsed with high-purity water. The implement is removed from the washing container and placed on a clean surface to air dry, preferably in a laminar flow hood. Only the knife handle should touch the drying surface. The clean, dry implement should be stored in Teflon bags made from Teflon sheets.

Procedures for Preparing Pinniped Teeth for Aging

Using bone saw, bolt cutters, or other appropriate tool remove the upper maxilla of the animal containing the upper canine teeth. Boil the upper maxilla for 20 minutes to soften the bone. Remove the two upper canines and extract the pulp from each tooth. Boil the teeth for 10-15 minutes in a solution of one tablespoon sodium triphosphate per one liter of water. Dry and label each tooth with following information:

OCSEAP ID Number
Species
Sex
Collection Date

U.S. DEPT. OF COMM. BIBLIOGRAPHIC DATA SHEET <i>(See instructions)</i>	1. PUBLICATION OR REPORT NO. NBSIR 88-3750	2. Performing Organ. Report No.	3. Publication Date APRIL 1988
4. TITLE AND SUBTITLE Alaskan Marine Mammal Tissue Archival Project: A Project Description Including Collection Protocols			
5. AUTHOR(S) Wise, S.A., Koster, B. J., Zeisler, R.			
6. PERFORMING ORGANIZATION <i>(If joint or other than NBS, see instructions)</i> NATIONAL BUREAU OF STANDARDS U.S. DEPARTMENT OF COMMERCE GAITHERSBURG, MD 20899		7. Contract/Grant No.	8. Type of Report & Period Covered
9. SPONSORING ORGANIZATION NAME AND COMPLETE ADDRESS <i>(Street, City, State, ZIP)</i>			
10. SUPPLEMENTARY NOTES <input type="checkbox"/> Document describes a computer program; SF-185, FIPS Software Summary, is attached.			
11. ABSTRACT <i>(A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here)</i> <p>The Alaskan Marine Mammal Tissue Archival Project was initiated in 1987 with financial support from the minerals Management service, Outer Continental Shelf (OCS) studies Program. Although the emphasis is on the collection of tissues for analysis of contaminants that may be associated with the petroleum industry, it is also recognized that the development of an archive of marine mammal tissues collected and stored using carefully controlled procedures provides a resource that goes beyond the immediate needs of the OCS Program. Such an archive developed over several years provides a resource of materials for future investigators addressing questions concerning the transport of elements and compounds (contaminants and non-contaminants) throughout the polar ecosystem, regardless of source.</p> <p>This document provides the basic information on Project objectives and management, justification for the species, tissues, and contaminants of interest, and specific instructions for collecting, handling, and storing samples. At this time, the protocols have been employed only in the collection of northern fur seal tissues, therefore, the details are somewhat biased toward this species. As these procedures are applied to the sampling of other marine mammals, the protocols will probably have to be modified. Therefore, this document represents the first in a series of reports providing the most recent protocols used by the Project.</p>			
12. KEY WORDS <i>(Six to twelve entries; alphabetical order; capitalize only proper names; and separate key words by semicolons)</i> Specimen Banking, collection protocols, marine mammals, pollution, trace elements, organic contaminants,			
13. AVAILABILITY <input checked="" type="checkbox"/> Unlimited <input type="checkbox"/> For Official Distribution. Do Not Release to NTIS <input type="checkbox"/> Order From Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. <input checked="" type="checkbox"/> Order From National Technical Information Service (NTIS), Springfield, VA. 22161		14. NO. OF PRINTED PAGES 53	15. Price \$13.95

