Marine Mammal Health and Stranding Response Program: Program Development Plan

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Office of Protected Resources

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Abstract

The Marine Mammal Health and Stranding Response Program (MMHSRP) was established under the authority of Title III of the Marine Mammal Protection Act to facilitate the collection and dissemination of reference data on health trends in marine mammal populations, correlate the health of marine mammals with environmental parameters, and coordinate effective responses to unusual mortality events. The components of the Program are: the National Marine Mammal Tissue Bank (NMMTB), the Stranding Network, Monitoring, and Quality Assurance. The NMMTB was established to provide a resource of research materials for future retrospective analyses. The NMMTB provides for the long-term cryogenic preservation of tissues using standard protocols for collection and archival.The Stranding Network consist of regional teams that respond to the stranding of marine mammals and are equipped to collect biological information and samples that can be used to understand the health, population dynamics, and life histories of marine mammals. The Monitoring Component conducts routine analysis of tissues in order to determine the baseline concentrations of chemical contaminants, biochemical components, and biotoxins in marine mammals. The Quality Assurance Component is a program that insures accuracy, precision, level of detection, and inter-comparability of data resulting from chemical analyses of marine mammals tissues. This program consists of interlaboratory comparison exercises and the production and distribution of control and standard reference materials for chemical analysis.

The MMHSRP includes marine mammal species from all marine waters of the United States. In the case of Alaska, the Program is being conducted through the Alaska Marine Mammal Tissue Archival Project, which is sponsored by the Minerals Management Service, U. S. Department of the Interior. In order to provide a mechanism for consultation and coordination with recognized scientists in the fields of research pertinent to the MMHSRP, the program has two scientific working groups who provide input during program planning: the National Marine Mammal Tissue Bank Team of Scientists and the Task Group on Unusual Marine Mammal Mortalities.

Because of the costs associated with collection and archival of specimens in the NMMTB and the increasing value of these specimens after years of storage, a formal Tissue Access Policy has been established to guide the release of specimens for research. A computer-based Information and Data Management System is in the planning stage. It will consist of several components including: sample, analytical data, and biological information tracking, as well as report bibliography and a specimen banks database. Additional avenues of research and development which are being considered for the Program are a blood serum bank, the development of biotoxin methodologies, and the evaluation of biomarker techniques for marine mammals.

Preface

This Program Development Plan is the basic document setting forth a multi-year program to develop the organizational and physical resources and a database that can be used to facilitate a better understanding of the effects of human activity on the health of marine mammals and their environment. Presented is the basis of planning and implementation strategy for the Marine Mammal Health and Stranding Response Program conducted by the National Marine Fisheries Service (NMFS) Office of Protected Resources. Acronyms used in this document are listed below:

AMMTAP - Alaska Marine Mammal Tissue Archival Project
ECD - Environmental Conservation Division (of Northwest Fisheries Science Center)
MMHSRP - Marine Mammal Health and Stranding Response Program
MMS - Minerals Management Service
NBSB - National Biomonitoring Specimen Bank
NIST - National Institute of Standards and Technology
NMFS - National Marine Fisheries Service
NMMTB - National Marine Mammal Tissue Bank
NMMTB&SN - National Marine Mammal Tissue Bank and Stranding Network
NOAA - National Oceanic and Atmospheric Administration
NWFSC - Northwest Fisheries Science Center

Introduction

Background

Hundreds of marine mammals are stranded each year on the beaches of the United States. Apparently most of these are animals that die at sea and then are carried onshore by winds and currents. In the contiguous United Sates most of the stranded cetaceans are bottlenose dolphins, *Tursiops truncatus*; pygmy sperm whales, *Kogia breviceps*; harbor porpoises, *Phocoena phocoena*; and common dolphins, *Delphinus delphis*. Stranded pinnipeds consist mostly of California sea lions, *Zalophus californianus*; harbor seals, *Phoca vitulina*; and northern elephant seals, *Mirounga angustirostris* (Wilkinson, 1991).

NOAA's National Marine Fisheries Service (NMFS) has established a marine mammal stranding network in each coastal region. The network consists of volunteers who respond to strandings of both dead and live animals to gather basic biological information and, in the case of live strandings, to attempt to rehabilitate and return the animals to the sea. At a minimum, the stranding network has been required to obtain information on location of stranding, species, length, condition, and sex of the stranded animals. This data is contributing to the development of a baseline of information for detecting unusual mortality events. To coordinate stranding network activities and ensure consistency among the Regions, a National Marine Mammal Stranding Network Coordinator has been appointed within the Office of Protected Resources.

In 1987-88 a massive mortality of bottlenose dolphins occurred on the east coast of the United States. Although the red tide organism, *Gymnodinium breve*, was implicated as a factor in causing this die-off, high levels of organochlorine contaminants (PCBs, chlorinated pesticides, etc.) were also found in these animals (Geraci, 1989). It was not possible to determine whether or not these contaminants had any role in the die-off. Since no comparable data on chemical contaminant concentrations in marine mammals existed for the United States and there was no way to determine whether those levels found in the bottlenose dolphins were "normal". It was apparent that there was a real need for establishing the baseline levels of anthropogenic contaminants in marine mammals in the United States and world-wide. It was also acknowledged that additional biological data should be collected by the stranding networks beyond that usually required and that stranding network procedures should be refined and standardized for all regions. This additional data should provide information which can be useful in monitoring the health of populations, including contaminant burdens.

The monitoring of contaminant burdens in marine mammals, if carried out over many years, requires that one consider the possibility of the appearance of "new" presently unknown contaminants and the future development of new and better analytical techniques. Analytical chemistry, particularly analytical organic chemistry, is a rapidly developing field. Methods are continually improving in accuracy, precision, and the ability to identify and separate closely related compounds.

Data from samples analyzed today may be obsolete and have limited usefulness for future considerations. It is therefore necessary that during the design of a contaminant monitoring program or a baseline database, some system for carefully collecting and storing environmental samples for future retrospective analysis be included. Environmental specimen banks established for such purposes are in operation in Canada, Europe, and the United States, and many other countries are planning such systems. The German environmental specimen bank system consists of two banks, one for human samples located at the University of Muenster and the second for other types of environmental samples located at the Nuclear Research Center (KFA) in Jülich (Boehringer, 1988). Both banks have operated for many years. The Canadian Wildlife Service

maintains a large specimen bank in Hull, Quebec. This system has been in operation for many years and has been an integral part of contaminant monitoring in Canadian wildlife (Elliott et al., 1988). The present Swedish system is for wildlife research and a bank for human toxicological research is being planned. Other banks are located in Denmark, Finland, and Norway, and the International Atomic Energy Agency is evaluating what role it might play in establishing similar facilities for use by developing countries in eastern Europe, the former Soviet Union states and countries in Asia and South America.

Within the United States, the National Biomonitoring Specimen Bank (NBSB), which is located at the National Institute of Standards and Technology (NIST), is designed to archive and store a wide variety of environmental samples under cryogenic conditions for long periods of time (decades). The NBSB has supported environmental research and monitoring programs of several agencies including: the Food and Drug Administration (FDA), Environmental Protection Agency (EPA), National Cancer Institute (NCI), National Oceanic and Atmospheric Administration (NOAA), and the Minerals Management Service (MMS) (Wise et al., 1988).

Program Development

In 1989, the NMFS Office of Protected Resources initiated the development of the National Marine Mammal Tissue Bank (NMMTB) at the NBSB, NIST. The NMMTB is designed to contain selected marine mammal tissues that have been collected and processed using rigorous well-documented protocols. These tissue samples are stored under the best conditions currently available (-150 °C) for maintaining sample integrity.

Enhanced support for the Marine Mammal Stranding Network also began in 1989. As part of its activities, the stranding network collects tissues for anthropogenic contaminant analyses and the NMMTB. Additional sources of animals are those caught incidentally during commercial fishing and animals taken for subsistence by Alaska native hunters.

Early in the development of the program, NMFS established a Team of Scientists, with expertise in the fields of marine mammal biology, toxicology, chemical contaminants, and specimen banking , to serve an advisory role to the NMMTB. Through the efforts of the Team of Scientists a protocol for collection, preparation and storage of tissue samples was developed based on an existing protocol that had been developed for a similar program in Alaska, the Alaska Marine Mammal Tissue Archival Project (AMMTAP) (Becker et al., 1988, 1991). In addition, the Team recommended (1) the tissues most appropriate for specimen banking (liver and blubber), (2) the selection of species to be sampled, (3) items to consider in a tissue access policy, (4) the development of a standard, "general" protocol which could be used by recognized research facilities involved in marine mammal research, (5) an inventory and location of tissues collected (sample tracking system), and (6) the development of a database that would include information on the tissues collected for the NMMTB.

A demonstration phase (Pilot Program) was initiated in 1990 to evaluate the practical aspects of obtaining suitable samples for the NMMTB from both incidental catches and strandings. This operation was conducted on the northeast coast of the United States in cooperation with scientists at the New England Aquarium. The species sampled were harbor porpoise, *Phocoena phocoena* (a coastal species), from incidental catches, and pilot whale, *Globicephala melaena* (a pelagic species), from mass strandings. Based on the successful sampling of these two species in the northeast, it was decided that the operation in this region should continue with the addition of the Atlantic white-sided dolphin, *Lagenorhynchus acutus*, from mass strandings as a target species. It was also decided to expand the program to the southeast coast of the United States and the Pacific coast. The NMMTB presently contains 30 samples of blubber and liver tissues collected from six

harbor porpoise and nine pilot whales.

In 1991 NMFS combined the NMMTB and the Marine Mammal Stranding Network into a single broader program and added both a contaminant monitoring and a quality assurance component. This larger program was named the National Marine Mammal Tissue Bank and Stranding Network Program (NMMTB & SN Program). The monitoring component, conducted by the Environmental Conservation Division (ECD) of the Northwest Fisheries Science Center (NWFSC), NMFS, includes real-time analyses to determine current levels (based on current methods) of biotoxins and chemical contaminants in marine mammals, as well as research on ways to enhance methods and procedures used by the program. The quality assurance component, conducted by the Chemical Science and Technology Laboratory, NIST, includes the development and application of quality assurance procedures and materials (such as SRMs) for chemical analysis of marine mammal tissues.

The NMMTB & SN Program has coordinated its work in Alaska with the AMMTAP, a project funded by the MMS, U.S. Department of the Interior. AMMTAP has been archiving samples at the NBSB from marine mammals taken in Alaska Native subsistence hunts. During these collections, the AMMTAP periodically has provided subsamples to the contaminant monitoring component of the Program. A closer coordination of AMMTAP with the Program came about in January 1992, when the management of AMMTAP was transferred from the Office of Ocean Resources Conservation and Assessment, National Ocean Service, NOAA, to the NMFS Office of Protected Resources.

On January 3, 1992, U.S. Congress passed the Oceans Act of 1992. This legislation amends the Marine Mammal Protection Act by adding Title III which establishes the Marine Mammal Health and Stranding Response Program the purposes of which are to: (1) facilitate the collection and dissemination of reference data on the health of marine mammals and health trends in marine mammal populations in the wild; (2) correlate the health of marine mammals and marine populations, in the wild, with available data on physical, chemical, and biological environmental parameters; and (3) coordinate effective responses to unusual mortality events. Most of the items required in Title III are already in place in the NMMTB & SN Program. However, some additional requirements were stated, particularly regarding information /data management and contingency planning for major strandings. The ability to respond to these additional requirements is uncertain, since funding for the program has not been appropriated.

In response to Title III, the NMMTB & SN Program has now been designated as the Marine Mammal Health and Stranding Response Program (MMHSRP). This document outlines the basic program within limitations of present funding. Appropriations will be required to expand this basic program to cover adequately all requirements of Title III.

Authority

The authority for NOAA, NMFS, to conduct the MMHSRP is provided in Marine Mammal Protection Act of 1972, 16 U.S.C. 1361-1407, as amended (Title III) by the Oceans Act of 1992. The primary purpose of this legislation is to protect and preserve marine mammals in order to maintain the health and stability of the marine ecosystem as a whole. NOAA's National Marine Fisheries Service has the responsibility for enforcing this act, except for manatees, polar bears, sea otters, and walrus, which are the responsibility of the U.S. Fish and Wildlife Service.

Program Components

The MMHSRP consists of four components: 1) Specimen Bank, 2) Stranding Network, 3) Monitoring, and 4) Quality Assurance. The goals of each of these components are discussed below.

Specimen Bank

Goal: Provide a resource that can be used for future retrospective analyses and documentation of long-term trends in environmental quality

The National Marine Mammal Tissue Bank (NMMTB) is maintained at the National Biomonitoring Specimen Bank (NBSB), NIST, Gaithersburg, MD. The purpose of the NMMTB is the long-term preservation of specimens that are representative of the state of the animal immediately prior to death. It is necessary that samples be collected, processed and stored under conditions that avoid or minimize contamination or other changes in the chemical composition of the specimen.

The sample collection and archival protocols for the NMMTB (see Appendix I, pages 25-32) are consistent with the standard procedures of the NBSB which focus on collection of duplicate samples, use of materials that minimize contamination from any contact with the sample, and freezing samples in liquid nitrogen (LN_2) as soon as possible after collection. A sample amount of about 150 g was selected as sufficient quantity of material to allow multiple analyses over a long period of time. The collection of two equivalent samples of 150 g provides one specimen for analyses as required and one for more permanent storage. Materials such as titanium and Teflon are used for all contact with the sample to minimize potential sample contamination. During sample preparation, contact with the specimen is generally limited to clean, dust-free Teflon surfaces. The samples are stored at the NBSB facility, NIST, in LN_2 vapor phase freezers at -150 °C. This temperature appears to be the best for long-term storage (Wise et al., 1989).

Liver and blubber are the two principal tissues chosen for routine banking. Blubber, due to its high lipid content, concentrates several organic toxicants to relatively high levels. The liver is a major site for detoxifying chemical contaminants and has a relatively high lipid content, thus, this tissue also accumulates chemical contaminants and may retain relatively high levels of their metabolites.

The archival of a representative collection of well preserved and documented marine mammal tissues will provide scientists with samples that have been collected and stored using standardized procedures so that results for samples collected at various times can be compared to determine whether environmental trends exist. Scientists will also have the opportunity to conduct retrospective analyses using improved analytical techniques or to measure currently unknown contaminants.

The NMMTB is not intended to serve as a substitute for activities that are currently being carried out by independent contaminants researchers. It is assumed that other tissues will continue to be collected, banked, and analyzed for ongoing monitoring and research. The NMMTB will not have the capacity to displace such activities and should not be viewed as a primary source of samples for real-time analyses. However, by providing protocols and quality assurance measures, the NMMTB can improve ongoing activities and provide valuable material for future retrospective study.

Stranding Network

Goal: Upgrade the capacity of the regional stranding networks

Research conducted on stranded marine mammals has been the source of a significant part of our knowledge on marine mammals. Data collected from stranded animals can serve as a baseline from which comparisons can be made. Marine Mammal Stranding Networks have been set up within each of the NMFS Regions. The Networks are primarily composed of volunteers who hold Letters of Authorization issued by the NMFS Regional Offices. They include marine mammal scientists, aquarium personnel, wildlife rehabilitation specialists, veterinarians, and wildlife management specialists. Network members are required to collect basic biological information including the species, sex, length, and evidence of human interaction. Many of the Network members collect additional information and provide tissues to scientists for research. Information collected from stranded animals can contribute to an understanding of marine mammal life histories, population dynamics, and diseases affecting marine mammal populations, to name just few avenues of biological research.

A program review of stranding network operations concluded that with a little effort, baseline data could be improved and that stranded animals could be the source of even more information. To realize the full potential of the Stranding Networks, an effort needs to be made to ensure that Network members know how to collect tissues for various analyses and that they have the required equipment.

Stranded animals are currently the most accessible source of tissues from marine mammals, and numerous researchers utilize the tissues using a variety of protocols. In an effort to improve baseline data collected from stranded animals, NMFS has published one technical document for use by Stranding Network members and is preparing a second. The first is a handbook for marine mammal strandings (Geraci and Lounsbury, 1993). It contains a species identification key, treatment protocols for live stranded animals, and basic information on tissue collection.

The second document is a forenisc laboratory manual for dolphins. This manual, which is being prepared by the NMFS Southeast Fisheries Science Center, will address a variety of scientific disciplines including methodologies for life history, gross necropsy, histopathology, pathogen detection, and collection of tissues for contaminant and toxicological analysis.

To enhance the capabilities of the Networks, the Program funding will provide equipment, supplies, and protocol training.

Goal: Detect and determine the cause of unusual marine mammal mortalities

To respond adequately to unusual mortality events, a systematic approach covering a range of possible causes needs to be developed. Available resources and gaps in either knowledge or Network coverage need to be determined in advance. As a first step in this process, NMFS established a Task Group on Unusual Marine Mammal Mortalities in 1991. This group is composed of experts from a number of scientific disciplines. They have established a series of criteria for determining when an unusual mortality is occurring based on information collected from marine mammal strandings. The Task Group was consulted five times between April 1991, and April 1992.

Funds are required to respond adequately to such events. In the past, it has been necessary to reprogram funds from base programs. In order to avoid reprogramming, it is the goal of NMFS to establish a permanent contingency fund that can be used when needed.

Goal: Maximize the use of the Regional Stranding Network data for management decisions

As the collection of baseline data improves, information from strandings can be used to understand the cause of mortalities, detect unusual mortalities, and understand fisheries interaction. Additional research can help to determine stock definitions, population dynamics, and genetic information. In order to make use of such data, the data collected must be more reliable, consistent, and comparable between regions. Upgrading the Regional Stranding Networks and combining the Network Program with the National Marine Mammal Tissue Bank Program should help improve this data collection.

Monitoring

Goal: Determine the baseline concentrations of chemical contaminants, biochemical components and biotoxins in marine mammals

High concentrations of persistent toxic substances in marine mammals is a phenomenon that is often reported in the literature. These substances include both naturally occurring toxicants, such as heavy metals, as well as anthropogenic substances, such as polychlorinated biphenyls (PCBs) and chlorinated hydrocarbon pesticides. Evidence to support the relationship between high contaminant levels in body tissues and detrimental effects to these animals has been shown in a few cases (DeLong et al., 1973; Helle, 1980, 1981; Bergman et al., 1981; Reijnders, 1980, 1984, 1986). The tendency of marine mammals to bioaccumulate contaminants can be explained by several factors including: relative position in the food web, tendency to accumulate large energy reserves in the form of body fat, relatively long life span, and relative ability to metabolize and excrete toxic substances (Tanabe et al., 1988; Walker, 1983).

Information on the baseline levels of environmental contaminants and toxins in marine mammal tissues is necessary in order to determine environmental trends related to the health of these animals. The results of the real-time analyses conducted by the Monitoring Component of the Program will add to the needed database on levels of various contaminants and toxins in different species of marine mammals. Real-time analyses will also help to answer questions about the effects of chemical contaminants and toxins on different species as well as on the disposition of contaminants and toxins among tissues.

Marine mammal sampling by the stranding networks, observer program, and others associated with the Program will be coordinated to collect tissues for banking and monitoring from the same animals. Following this approach, analytical results will be available for all the samples within the NMMTB, either as determined on actual subsamples from the bank (e.g., by NIST as part of their analyses) or on samples from the same animal as part of the monitoring component. As questions arise concerning long-term trends, new pollutants, or the validity of previous results, tissues from the NMMTB, which were collected years earlier, can be withdrawn to address these issues.

The NMFS Northwest Fisheries Science Center (NWFSC), Environmental Conservation Division (ECD), conducts the Monitoring Component of the Program. In addition to liver and blubber routinely collected for the NMMTB, the ECD analyzes other tissue and fluid samples including, but not restricted to: bile, kidney, and brain tissue. Analytes include: 1) organic chemicals and lipids for blubber, liver, and brain; 2) metals for liver, kidney, and brain tissue; 3) estimate of metabolites of polycyclic aromatic hydrocarbons; and 4) xenobiotic-DNA adducts for liver tissue. Certain

compounds appear to be particularly important contaminants in marine mammals. These include methylmercury, coplanar PCBs, chlordane isomers, and polychlorinated camphenes (PCCs). As part of the Monitoring Component, the ECD will evaluate analytical methods used for routine monitoring to determine concentrations of these substances as well as others of importance.

Goal: Develop guidelines for minimizing tissue sample variability

Selective partitioning of environmental contaminants within the tissues of marine mammals can affect the variability of the resulting analytical data and may be important in determining what sample locations are best for estimating body burdens. Such bias must be identified and steps taken to design sampling protocols to minimize and remove such bias and variability from the analytical results. Studies will be conducted to estimate the distribution of chemical constituents in marine mammal tissues and its effect on sampling variability and error, and to recommend tissue sampling procedures that provide the best samples for accurately determining the concentration of environmental contaminants in these tissues and providing the best estimate of body burdens.

Before stranded animals can be fully utilized as a resource for contaminant analysis, a basic question that needs to be answered is how long an animal may be dead before it is considered unsuitable for sampling for contaminants and other chemical constituents. What chemical changes occur in tissues and organs between the time of death and tissue collection, and during various conditions of storage prior to analysis? Such changes probably depend on the chemical constituent in question, type of tissue, the physiological state of the animal at time of death, and environmental conditions. Studies will be conducted to determine the temporal changes in selected chemical constituents of marine mammal tissues following death and to determine the maximum time that can elapse between animal death, sample removal, and sample freezing before change in selected chemical constituents become significant and do not reflect the original conditions in the animal at time of death.

Quality Assurance

Goal: Insure the accuracy, precision, level of detection, and intercomparability of data resulting from chemical analyses of marine mammal tissue samples

As part of the MMHSRP, analytical results for chemical contaminants in marine mammal tissues will be generated by laboratories in both the Monitoring and the Specimen Bank Components. To provide accurate analytical data, a quality assurance (QA) program has been developed for assessing the accuracy and comparability of results among the laboratories for both the organic and inorganic analyses. This QA program, which is administered by the Chemical Science and Technology Laboratory, NIST, includes the following:

1) Interlaboratory comparison exercises among laboratories involved in NOAA projects related to marine mammal tissue analyses.
2) Preparation, analysis, and distribution of marine mammal tissue control materials.
3) Development of Standard Reference Materials (SRM's) for use in the analysis of marine mammal tissues.

Following a comparison and evaluation of analytical methods for organic contaminants (particularly for lipid-rich tissue matrices) between NIST and the ECD, an interlaboratory

comparison exercise was conducted. Participants included NIST, the ECD, and several other laboratories involved in measurements of chemical contaminants in marine mammal tissues. This exercise consisted of the analyses of two "control" materials (whale blubber for organic analysis and whale liver for inorganic analysis) prepared by NIST from tissues obtained from two pilot whales. Approximately 2 kg of each tissue type were prepared as frozen tissue homogenates for distribution as subsamples of 15-20 g, each. The list of analytes for the first interlaboratory comparison exercise was based on input from researchers involved in chemical contaminant measurements in marine mammal tissues.

Recommended reference values will be determined through continued analyses of these materials, which will then be distributed for use as control material during routine analyses of marine mammal tissues. The experience gained from the analyses of the control materials is being used by NIST to develop both blubber and liver Standard Reference Materials (SRMs) for use in marine mammal tissue analysis. An SRM is a homogeneous material that has been analyzed by two or more independent analytical methods to certify concentrations of selected organic or inorganic constituents. These SRM's will then be available to any researchers involved in the analysis of marine mammal tissue for use in the validation of analytical methods.

Target Species

The NMMTB protocol is designed to collect a few selected tissues from a limited number of representative, "indicator" species. Since each region of the United States is unique in the potential availability of acceptable marine mammal samples, the specific species to be sampled for the Tissue Bank are determined on a regional basis. The selection of the "indicator"species has been based on the following considerations:

1) Available source of a large number of animals on a regular (ideally annual) basis;
2) Representatives of both coastal and pelagic species are included;
3) Representatives of both cetaceans and pinnipeds are included;
4) Potential for accumulating anthropogenic contaminants to relatively high levels;
5) Trophic levels should be considered (bottom feeder, pelagic fish feeder, pelagic plankton feeder); and
6) Relative value as food or subsistence species for humans (applicable to Alaska).

Indicator species selected for the NMMTB based on these considerations are summarized in Table 1. This species list may expand in the future, since Title III of the Marine Mammal Protection Act requires coordination with the USFWS. Four marine mammal species are under the management authority of the USFWS: manatee, *Trichechus manatus*; walrus, *Odobenus rosmarus*; sea otter, *Enhydra lutris*; and polar bear, *Ursus maritimus*. The manatee could function as an indicator species in the southeast United States, walrus and polar bear in Alaska, and sea otter in Alaska and Pacific Coast. Manatee, walrus, and polar bear are of particular interest since they represent trophic levels not represented in the species given in Table 1.

Table 1.–Indicator species for the National Marine Mammal Tissue Bank.[1]

Region	Coastal	Pelagic
Northeast	Harbor porpoise, *Phocoena phocoena* (I)	Pilot whale, *Globicephala malaena* (S)
	Harbor seal, *Phoca vitulina* (I)	
	*Atlantic white-sided dolphin, *Lagenorhynchus acutus* (S)	
Southeast	Bottlenose dolphin, *Tursiops truncatus* (S)	Pilot whale, *Globicephala malaena* (S)
Pacific	Harbor porpoise, *Phocoena phocoena* (I)	Dall porpoise, *Phocoenoides dalli* (I)
	Harbor seal, *Phoca vitulina* (I, N)	Northern fur seal, *Callorhinus ursinus* (N)
	California sea lion, *Zalophus californianus* (I)	
Alaska	Ringed seal, *Phoca hispida* (N)	Northern fur seal, *Callorhinus ursinus* (N)
	Beluga whale, *Delphinapterus leucas* (N)	Bowhead whale, *Balaena mysticetus* (N)

[1]Northeast = northeastern U.S., Southeast = southeastern U.S. (Atlantic and Gulf of Mexico), Pacific (including the Gulf of Alaska), Alaska = Bering Sea and Arctic Ocean; I = incidental catch, S = stranding, N = Native subsistence harvest. * = considered to be both a coastal and a pelagic species.

Management Plan

Organization

The diverse expertise and resources required by the MMHSRP has made it necessary that several NMFS organizations as well as organizations outside of NMFS be involved in the conduct of the Program. The primary organizations involved in the Program are: the NMFS Office of Protected Resources, the NMFS Environmental Conservation Division (ECD) of the Northwest Fisheries Science Center (NWFSC), the NMFS regional offices and their associated stranding networks and observer programs, and Chemical Science and Technology Laboratory of the National Institute of Standards and Technology (NIST). The responsibilities of each of these primary organizations are reflected in the Program structure which is organized to address six basic functions: program management, response to mortality events, necropsies and sampling, contaminant and biochemical monitoring, quality assurance, and specimen banking (Table 2).

Program management is the responsibility of the NMFS Office of Protected Resources. Program management includes establishing program policy, overall planning, budget formulations and allocations, coordination of program activities including international coordination and cooperation, establishing and maintaining a system for information and data management, and the dissemination of program information and results to the scientific community, government agencies, educational organizations, and the general public. The Office of Protected Resources will maintain a Program Manager and associated staff for facilitating these activities.

Response to mortality events is the responsibility of the NMFS regional offices through their regional stranding networks. The regional stranding networks include participants from various universities and non-governmental organizations. The interface between the Program and the regional stranding networks is provided through the Regional Stranding Network Coordinators and the National Stranding Network Coordinator located in the Program Office. One of the major goals of the Program is to upgrade the capacity of the regional stranding networks to respond to mortality events. Therefore, a very important responsibility of Program management will be to provide resources and training necessary for the stranding networks to successfully perform their function.

Necropsies and sampling for the NMMTB and the monitoring component of the Program is performed through arrangements made with the NMFS Observer Program and several individuals and organizations operating under the authority of the regional stranding networks. The interface between the Program Manager and the regional stranding networks and observer programs is provided by the Regional Stranding Network Coordinators and Observer Program Coordinators. In the case of Alaska, other agencies, local researchers, and management organizations provide additional necropsy and sampling support for animals taken during Alaska native subsistence harvests. These organizations presently include the MMS Alaska Office, the NMFS Western Alaska Field Office, the North Slope Borough Department of Wildlife Management, the Eskimo Walrus Commission, and Kawerak, Inc.

Contaminant and biochemical monitoring is conducted by the NMFS, NWFSC, ECD. This work requires careful coordination and cooperation with the specimen banking and quality assurance activities of NIST. Collaboration with similar monitoring programs being conducted by marine mammal investigators from other countries is encouraged in order to insure comparability of analytical data and to develop comprehensive research approaches.

Quality assurance for chemical analyses is provided by NIST, Chemical Science and Technology Laboratory, through interlaboratory comparison exercises with the ECD and other investigators

involved in research on contaminants in marine mammals, as well as the production and distribution of standard reference materials specifically designed for marine mammal tissue analyses.

Specimen banking for the Program is the responsibility of the National Biomonitoring Specimen Bank (NBSB) at NIST. The NBSB uses standard and state-of-the-art procedures for maintaining environmental samples under cryogenic conditions for long periods of time and routinely monitors the conditions of the samples during storage.

Table 2.--Organization structure of the National Marine Mammal Tissue Bank and Stranding Network Program.

Functions	Performing organization
Program Management	National Marine Fisheries Service Office of Protected Resources Silver Spring, Maryland
Response to Mortality Events	National Marine Fisheries Service Regional Offices through their Regional Stranding Networks & National Marine Fisheries Service Office of Protected Resources National Stranding Network Coordinator, Silver Spring, Maryland
Necropsies and Sampling	National Marine Fisheries Service Regional Offices through their Regional Stranding Networks and Fishery Observer Programs; Other organizations
Contaminant and Biochemical Monitoring	National Marine Fisheries Service Northwest Fisheries Science Center Environmental Conservation Division, Seattle, Washington
Quality Assurance	National Institute of Standards and Technology, Chemical Science and Technology Laboratory, Gaithersburg Maryland
Specimen Banking	National Institute of Standards and Technology, National Biomonitoring Specimen Bank, Gaithersburg, Maryland

Program Planning and Review

An important part of the Program management is consultation and coordination of these efforts with recognized scientists in the fields of research pertinent to the MMHSRP. A mechanism for achieving this coordination is the formation of scientific working groups to provide input during program planning. The Program has two such groups: the National Marine Mammal Tissue Bank Team of Scientists and the Task Group on Unusual Marine Mammal Mortalities.

The National Marine Mammal Tissue Bank Team of Scientists was formed early in the development of the Program (1989) to insure peer review of all Program activities. The Team meets on an annual basis to review program activities of the previous year and to discuss proposed work for the coming year. The Team consists of scientists in marine mammal biological research and management, marine mammal physiology, toxicology, chemical contaminant research, ecology, analytical chemistry, and environmental specimen banking (Table 3).

The Task Group on Unusual Marine Mammal Mortalities, which was set up in 1991, is consulted when an unusual mortality event is suspected. An effort was made to include in the Task Group scientists from a number of different specialties including: epidemiology, veterinary pathology, life history, toxicology, and detection and treatment of diseases in marine mammals (Table 4). Specialists for both pinnipeds and cetaceans and individuals familiar with strandings are included. The Task Group has established a series of criteria for determining whether an unusual mortality is occurring. They provide suggestions and guidance during the course of investigations and review the results of studies.

Tissue Access Policy

Because of the costs associated with the collection and archival of specimens in the NMMTB and the increasing value of these specimens after years of storage, an access policy has been established that addresses the disposition of the banked tissues and the procedures, justification, and review process required for access to this resource. The "NMMTB Access Policy" is provided in Appendix I, pages 33-35.

Information and Data Management

A computer-based Information and Data Management System will be established for the MMHSRP. Before the passage of Title III, the following were identified as requiring such a system to be established and maintained by the NMFS Office of Protected Resources in support of the Tissue Bank: sample tracking, analytical data tracking, supplemental information tracking, report bibliography, and specimen banks database.

Sample tracking would be facilitated through a continuously updated database which indicates the location and fate of each sample collected by the Program for archival. This database would also track those samples collected from the same animals for other research purposes.

Analytical data tracking would include the identification and descriptions of analyses that have been conducted on samples collected by the Program and would provide information that would enable one to access this data. (e.g., identified reports, publications and journal articles, or individuals and organizations having this data, if unpublished).

Table 3.--Members of the National Marine Mammal Tissue Bank Team of Scientists.

Member	Affiliation
Howard W. Braham	National Marine Mammal Laboratory National Marine Fisheries Service Seattle, WA
John Calambokidis	Cascadia Research Collective Olympia, WA
Greg Early	New England Aquarium Central Wharf Boston, MA
Sylvia Galloway	Southeast Region National Marine Fisheries Service Charleston, SC
Joseph R. Geraci	Ontario Veterinary College University of Guelph Guelph, Ontario, Canada
Romona Haebler	USEPA Narrangansett, RI
Robert J. Hofman	Marine Mammal Commission Washington, DC
Linda Lowenstine	School of Veterinary Medicine University of California - Davis Davis, CA
James Mead	National Museum of Natural History Smithsonian Institution Washington, DC
Daniel K. Odell	Sea World of Florida Orlando, FL
Usha Varanasi, Chair	Environmental Conservation Division Northwest Fisheries Science Center National Marine Fisheries Service Seattle, WA
Stephen A. Wise	Chemical Science & Technology Lab. National Institute of Standards & Technology Gaithersburg, MD

Table 4.—Members of the Task Group on Unusual Marine Mammal Mortalities.

Member	Affiliation
Gregory D. Bossart	Miami Seaquarium, Miami, FL
Leslie Dierauf	Washington, DC
Joseph R. Geraci, Chair	Ontario Veterinary College, University of Guelph Guelph, Ontario, Canada
Romona Haebler	USEPA, Narrangansett, RI
Robert J. Hofman	Marine Mammal Commission, Washington, DC
Jeff Horwath[1]	USFWS, Arlington, VA
Thomas Lipscomb	Armed Forces Institute of Pathology, Walter Reed Army Medical Center Washington, DC
James Mead	National Museum of Natural History Smithsonian Institution, Washington, D.C.
Thomas O'Shea	National Ecology Research Center, USFWS Fort Collins, CO
John Reif	Department of Environmental Health Colorado State University, Fort Collins, CO
Thomas Roffe	National Wildlife Health Research, Center USFWS, Madison, WI
Linda Schlater	National Veterinary Services Labs, Ames, IA
Randall Wells	Mote Marine Laboratory, Sarasota, FL
Dean Wilkinson,[1] Executive Secretary	NMFS, Office of Protected Resources Silver Spring, MD
Thomas Williams	Carmel, CA
Kate Wynne	University of Alaska Sea Grant Program Marine Advisory Program, Fairbanks, AK
Nina M. Young	Center for Marine Conservation, Washington, DC

[1] Nonmember agency contact.

Supplemental information tracking would be facilitated through a database that identified all supplemental information collected on the individual animals during sampling, including necropsy reports and other information obtained by stranding networks or researchers.

Report bibliography would consist of abstracts of all reports generated by the MMHSRP and other research conducted on the animals sampled by the Program.

Specimen banks database would contain information on all applicable holdings of tissue banks other than the NMMTB. This information would be useful to investigators interested in acquiring access to such samples, many of which do not require the stringent archival procedures used by the NMMTB.

With the passage of Title III, the information and database requirements have been expanded to include reference data on the health of marine mammals and populations of marine mammals, and data on species of marine mammals that are subject to unusual mortality events. Without additional funding for the Program, the Information and Data Management System can not be expanded at this time.

International Coordination and Cooperation

Although the MMHSRP is concerned with marine mammals of U.S. coastal waters, it is recognized that most, if not all, of the species of concern are distributed throughout the world's oceans and many of the populations that occur in the U.S. coastal waters migrate through the waters of several nations. Incidents of unusual mass standings, die-offs, and occasions of pollutant incidents are not occurring only within one national or even regional boundary, but is a world-wide phenomenon. It is, therefore, important that the Program actively pursue international coordination and cooperation in the various avenues of monitoring and research conducted by the Program.

The Program is presently and will continue to be involved in the International Biomonitoring Specimen Banking Symposia and the International Committee on Environmental Specimen Banking. This committee is involved in planning, organizing, and sponsoring the biannual symposia and is attempting to standardize technical requirements for environmental specimen banks.

The Program is also pursuing avenues of cooperative studies and information exchange with the International Arctic Community through mechanisms established by the Arctic Research Policy Act (ARPA) of 1984 and the International Arctic Science Committee, as well as cooperative exchanges of samples and data set up by the AMMTAP with the Department of Fisheries and Oceans Canada. The Arctic is the recipient of many kinds of airborne contaminants from the lower latitudes. The community of Arctic nations, which includes the United States, has many shared natural resources, specific populations of marine mammals, and common sociological and economic considerations, including a unique subsistence lifestyle that relies on certain species of marine mammals as a supplemental food source. It is therefore mandatory that, if the MMHSRP includes the Arctic, collaboration and cooperation with the work of other Arctic nations be an integral part of the Program.

The Program will be pursuing means of cooperation and coordination with researchers in the Antarctic through the National Science Foundation's Polar Programs. Another avenue of international cooperation is through the Program's quality assurance (QA) activities. Investigators from Canada and Germany are already involved in this Program component. The expansion of the QA component will continue with the intent that eventually most of the marine mammal researchers of the world involved in chemical contaminant analyses will be participants in the MMHSRP QA activities.

The discussion presented above indicates only a few avenues for building international connections. Other avenues will undoubtedly appear as the Program progresses.

Program Reports

In addition to special technical reports that will be produced by the program (e.g., Handbook on Marine Mammal Strandings), the following reports will be produced by the Program on an annual basis: 1) Annual Program Report, 2) Tissue Bank Report, 3) Stranding Network Report, 4) Contaminant Monitoring Report, and 5) Quality Assurance Report.

The Annual Program Report will be produced by NMFS, Office of Protected Resources. This report will contain a summary of the results of other program components, descriptions of planned projects, a description of any interagency and international cooperation or collaboration, a discussion of outreach activities, an updated bibliography of reports and publications generated by the program, an updated database report, and a presentation on budget expenditures.

The Tissue Bank Report will be produced by NIST. It will provide a description of all significant activities of the Tissue Bank and will include an inventory of archived specimens and the results of any chemical analysis that were performed for the monitoring of sample integrity.

The Stranding Network Report will provide a general overview of the activities of each of the Regional Stranding Networks as related to enhanced network capabilities, other Program components (e.g., monitoring and tissue banking), and response to any unusual mortality events. This report will be produced by the National Stranding Network Coordinator with input from the Regional Stranding Network Coordinators.

The Contaminant Monitoring Report will be produced by NMFS, NWFSC, ECD. This will be a technical report providing analytical results of the Contaminant Monitoring Component along with appropriate interpretation of the data.

The Quality Assurance Report will be produced by NIST, with input from ECD. It will be a technical report providing the results of interlaboratory comparisons, SRM characterizations, etc.

Outreach

It is the policy of the MMHSRP to encourage and support the publication of data resulting from the program in scientific peer-reviewed publications. However, with the interest of the general public in the environment and particularly in marine mammals, it is apparent that a variety of means other than journal publications and annual program reports are required for disseminating information resulting from the Program to a broader audience. The interested parties will probably include the general public, industry, educational organizations, and various public interest groups, in addition to government agencies and scientists. This will require the development of an "outreach strategy" for the program which will probably result in the production and distribution of several types of information media, including newsletters, educational videotapes, general information pamphlets, special technical reports, and popular articles for magazines and lay journals. The details of this "outreach strategy" is yet to be developed.

Program Milestones

Presented below are major milestones in the development of the MMHSRP. Additional details on specific projects being conducted by the program components will be provided in the Annual Program Report.

Specimen Bank

- Establish the NMMTB 5/89
- Establish the NMMTB Team of Scientists 12/89
- Initiate Pilot Program in NE 9/90
- Initiate NMMTB Program in NE 10/91
- Initiate Pilot Program on West Coast 5/92
- Initiate NMMTB Program on West Coast 5/93
- Initiate Pilot Program in SE .....
- Initiate NMMTB Program in SE .....

Stranding Network

- Initiate Stranding Network enhancement procedures 10/90
- Establish the Task Group on Unusual Marine Mammal Mortalities 3/91

Monitoring

- Initiate the Contaminant Monitoring in the NE 3/91
- Initiate the Contaminant Monitoring in the SE 3/91
- Initiate the Contaminant Monitoring on the West Coast 3/92

Quality Assurance

- Initiate interlaboratory comparison exercises 3/91
- Initiate Blubber SRM Development 10/91
- Initiate Liver SRM Development 10/92

Management

- Initiate the development of a database management system 5/92
- Initiate the development of an "outreach strategy" 5/92

Additional Research Requirements

Not all of the analytical tools, research approaches, or basic biological and physiological information are readily available for evaluating the significance of anthropogenic contaminant loads in marine mammals and for understanding the effects of human activity on the health of marine mammals and their environment. Many questions can only be addressed through long-term experimental research. For example, analysis of wildlife tissues or other sentinel organisms can provide a long-term picture of the state of contamination integrated over area and time, its increase or decrease, and the arrival of new anthropogenic contaminants. However, the determination of biological significance of the contaminant concentrations relative to the health of the individual animals and the population requires the kind of data used to construct toxicokinetic and ecotoxicological models (Clark et al., 1987). Rates of uptake, metabolism, and clearance must be understood so that the status of the organism with respect to equilibrium or steady state can be determined. Feeding habits, migration or mobility, and changes in physiological compartments (e.g., lipid) can influence concentrations in tissues; therefore, natural history, physiology, and ecology must also be understood.

Much of this basic research is outside the scope of the MMHSRP; however, two avenues of research and development which have been identified as being within the scope of the Program and are being considered for future addition are discussed below.

Creation of a marine mammal blood serum bank. Within the last fifteen years a number of epizootics have occurred that have been caused by disease agents. In 1979-80, an unprecedented number of harbor seals stranded on Cape Cod. An influenza A virus was determined to be responsible (Geraci et al., 1982). In 1984, an epizootic caused by leptospirosis occurred in the west coast California sea lion population (Dierauf et al., 1985). In 1988, over 17,000 pinnipeds in Europe died as a result of phocine distemper virus (Osterhaus et al., 1990). Most recently a distemper virus has been implicated in the deaths of large numbers of striped dolphins in the Mediterranean. In order to determine appropriate actions, it is important to know what diseases are endemic in a population and whether a disease is being introduced to an immunologically naive population. As an additional element in assessing the status of marine mammal health, it is, therefore, necessary to monitor diseases affecting populations.

A record of diseases may help in isolating specific pathogens. Disease antibodies can be detected in blood serum. In much the same way as retrospective analyses can be valuable in contaminant analysis, examination of historical blood serum can provide information on whether a disease agent has been recently introduced or is endemic in a population. As resources become available, the MMHSRP will set up blood serum banks that can be used for current analysis of diseases in marine mammal populations and for retrospective analysis. Both live animals and freshly dead animals can be used for the collection of blood serum samples. Blood serum can be frozen for later analysis.

Development of biotoxin methodologies. There is evidence that biotoxins created by algal blooms can affect marine mammals (Gilmartin, 1987; Geraci, 1989; Geraci et al., 1989; and O'Shea et al., 1991). Stomach contents from dead animals and analysis of prey have been the most commonly used methods for detecting biotoxins. As a long-term goal, the Program should include developing more direct methods for determining the presence and impact of biotoxins on marine mammals.

Application of biomarker techniques to marine mammal studies. Determining the tissue concentrations and body burdens of selected contaminants in marine mammal tissues does not lead directly to a full understanding of the toxicological significance of the chemicals detected. The developing field of biomarker measurements in wildlife toxicology shows future promise in identifying physiological responses that will provide information on exposure to a range of toxic

compounds and to integrate toxicologic interactions from mixtures of chemical contaminants (Peakall, 1992). Currently the application of biomarkers to marine mammals is in the very early stages of development, and no biomarkers of contaminant exposure nor physiological response have been adequately validated for use with marine mammals. Further, the utility of biomarkers in evaluating mass mortality events is only beginning to be assessed. Thus the Program should encourage further development and evaluation of biomarkers to complement the techniques currently used in monitoring chemical contaminant exposure and response in marine mammals.

Acknowledgments

Numerous individuals have been directly involved in the development of the Marine Mammal Health and Stranding Response Program and have provided review comments on this report. They include the National Marine Mammal Tissue Bank Team of Scientists; Usha Varanasi and her staff at the National Marine Fisheries Service (NMFS), Northwest Fisheries Science Center, Environmental Conservation Division; Steve Wise and Barbara Koster, National Institute of Standards and Technology; Gloria Thompson, NMFS Headquarters; Bob Hofman, Marine Mammal Commission; and W. L. Hobart, NMFS Scientific Publications Office. A special acknowledgment is given to Nancy Foster, NMFS Deputy Assistant Administrator, who provided the original concept that has developed into the program described in this document.

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Appendix I, National Marine Mammal Tissue Bank Protocols

Sampling Materials

The following materials are used for collecting marine mammal tissue samples. These materials, except where noted, will be provided by the NBSB:

Teflon sheeting,
Dry shippers (LN_2 cooled) with shipping containers,
LN_2 in container for freezing samples on site,
Dewar and lid,
Lab coats (disposable),
Insulated gloves, safety glasses, and tongs for handling the LN_2 and frozen samples,
Balance for weighing samples (supplied by the facility performing the necropsy),
Surgical scissors, forceps (supplied by the facility perfoming the necropsy),
Labels for exterior of sample jars,
Tape for securing exterior jar labels,
Lid labels,
Shipping labels,
Data recording forms (an example is shown on pages 30-32).

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

4 - pairs non-talced vinyl gloves,
1 - 14x1s Teflon FEP (Fluorinated ethylene propylene) bag or sheet to provide a clean working surface,
1 - 12x12" Teflon FEP bag for transporting sample from the field to the processing facility,
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).

Sample Collection

General

Stage I, tissue removal, may occur under conditions of limited control.

Stage II, tissue processing, will occur 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.

Stage I, Tissue Removal

1. Basic Considerations for all Sampling

   a. The size of the tissue sample removed from an animal should be sufficient to provide two subsamples of 150-200 g each for archival.

   b. The anatomical location of the tissue removal is specified in order to maintain consistency and comparability between the same tissue types.

   c. Sterile, cleaned, non-talced vinyl gloves are used by all personnel involved in sample removal and handling. Caution must be taken throughout the procedures to reduce the risk of chemical contamination of the sample.

   d. No animal will be considered a candidate for sampling, if tissues cannot be collected within the specified time after death and if handling of the animal has not followed protocol criteria or can not be documented.

2. Procedures

   a. Record information on location, time taken, and other pertinent data on the individual animal on the data recording form.

   b. Take and record all measurements required on the data recording form.

   c. With the animal lying on its side, confirm gender.

   d. Using a stainless steel instrument, make an incision through the skin and blubber on the dorsal side, anterior to the dorsal fin and posterior to the blow hole and measure blubber thickness.

3. The tissue samples are removed as soon as possible after opening the body cavity. Opening the body cavity and initial cutting of the skin to expose adipose and muscle tissue may be performed with high quality stainless steel dissection tools previously rinsed in high purity water.

   a. Adipose Tissue (Blubber)
   The anatomical site of blubber removal will depend on the distribution of fat layers on the animal.

      An incision is made anterior to the dorsal fin and posterior to the blow hole with a stainless steel implement. Two more cuts are made perpendicular to the first incision near the fin and blow hole respectively on the left side of the animal. A three-side flap of skin is cut. Using the titanium knife and grasping a corner of the blubber with hemostatic forceps, separate a section of blubber from the muscle layer which is below the blubber. After the blubber is separated from muscle, separate the blubber from the skin in the same manner. A total of 300 g of blubber are required for storage. Place the blubber in a clean Teflon bag for immediate transport to the processing area.

   b. Liver
   Note the general appearance of the liver before removal, including any unusual coloration, texture, shape, etc.

      Preferably using a stainless steel instrument, remove the entire liver and place it in a clean Teflon bag. If the entire liver can be removed, use a titanium knife to remove a

150-200 g section from both sides of the medial indentation at the distal end of the liver and place in a clean Teflon bag for immediate transport to the processing area.

4. Following the removal of the NBSB samples, any additional samples and measurements are taken and the information is included on the NBSB Sample Data Form.

Stage II, Tissue Processing

1. Tissue processing should take place within a laboratory facility under the cleanest conditions available. 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.

3. Liver

   a. Tare the weight of the pre-cleaned Teflon jars, record that weight, and label the jar for the sample.

   b. Remove the sample from the Teflon bag. Holding the sample over a sink, 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. Allow the specimen to drain for several minutes. Place the rinsed specimen on a clean Teflon sheet.

   c. If the whole liver is transported to the processing facility, remove a 150-200 g portion of the distal end of both sides of the medial indentation using a titanium knife. Dissect each 150-200 g portion into two equal parts and place one section in the A jar and the other section in the B jar. Repeat the procedure for the remaining 150-200 g section removed from the other distal end. Each sample must fit in a Teflon jar with a volume of 180 mL (49 mm diameter and 120 mm length).

   d. If the liver sample is 300-400 g, use the titanium knife to 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).

   e. After the sample has been dissected and placed in the Teflon jar, weigh the jar and record with the tared weight.

   f. When the work on the sample has been completed, 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 dry. Rinse the knife with ethanol and air dry.

   g. Continue to Stage III, Tissue Packaging and Shipping.

4. Blubber

   a. Tare the weight of the pre-cleaned Teflon jar, record that and label the jar for the sample.

   b. Remove the specimen from the Teflon bag; 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 or skin attached to the blubber and to trim edges cut with the stainless steel implements. Also, any tissue that came in contact with a contaminant, i.e., animal hair, hemostats, etc., should be excised and discarded. Excise a 300-400 g section from the specimen.

d. Using a clean 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).

e. After the sample has been dissected and placed in the Teflon jar, weigh the jar and record the tared weight.

f. After the sample has been dissected and placed in the Teflon jar 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 dry. Rinse the knife with ethanol and air dry.

g. Continue to Stage III, Tissue Packaging and Shipping.

Stage III, Tissue Packaging and Shipping.

1. Record the weights on the data recording form and sample labels. Affix the sample labels to the jars with wide tape and place the lid labels in the recessed slot on the jar lid.

2. Freeze each sample by immersing in the LN_2 for 10 minutes. If LN_2 is not immediately available, freeze sample in freezer at -70 °C or lower.

3. The LN_2 shipper should be filled with liquid nitrogen for at least 6 hours to fully prepare it for shipping. Pour off the excess LN_2 and place the frozen samples in the shipper (10-12 jars per shipper).

4. Once the shipper is full, transport it to the NIST; do not store the samples 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 Form.

6. Place a copy of the completed forms in the shipper.

7. Samples should be shipped within 48 hours or as soon as possible after sample collection using 24 hour express package service to:

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

   Shipping expenses will be borne by NIST. 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 and NIST.

8. The Specimen Bank personnel should be notified by telephone as soon as possible after the specimens are shipped: Barbara Koster (301) 975-6291, or Steve Wise (301) 975-3112.

Sample Archival

1. Samples are received at NIST and transported to the NBSB facility. The shippers are unpacked and samples are inspected for any packaging problems and for unsuitable temperatures. Data Recording Forms and samples are compared to insure that they correspond and that all information has been included. These samples are stored in a temporary LN_2 freezer and are logged into the temporary storage log book. They remain in temporary storage until assigned an NBSB number and permanent LN_2 freezer space.

2. When the samples are moved into the permanent freezer location, a storage form, which contains storage location information, is completed, and the information is entered into the inventory form on the NBSB personal computer. The samples are placed in cylindrical cardboard tubes (6.0 cm diameter x 63.5 cm length); each tube will hold up to five samples. These samples will remain stored in the LN_2 freezer at about -150 °C until they are requested for analysis.

3. Information describing the animal is recorded and maintained, both in hard copy and in a computer database, as part of the documentation for each sample in the Tissue Bank.

4. The duplicate samples of each tissue (Samples A and B) are stored in different LN_2 freezers to provide additional security. Sample A is intended for long-term storage while sample B is available for any analyses as required.

5. As part of the specimen banking activities, NIST determines the concentrations of selected organic and inorganic constituents in approximately 10-20% of the archived specimens in order to (1) evaluate the stability of the specimens during long-term storage, (2) compare with data obtained from other laboratories analyzing similar samples, which were collected at the same time from the same site as part of the monitoring component (i.e., quality assurance), and (3) compare with data from samples to be collected in the future for monitoring long-term trends in pollution. An important aspect of these analyses is that they provide both inorganic and organic baseline data on the same specimens. Additional samples may be analyzed by other laboratories as part of collaborative efforts with the NMMTB. Samples to be analyzed are homogenized using Teflon instruments and a cryogenic grinding procedure designed to minimize sample contamination and reduce the likelihood of changes in sample composition due to thawing and refreezing (Zeisler et al., 1983; Wise et al., 1989).

Literature Cited

Wise, S. A., B. J. Koster, R. M. Parris, M. M. Schantz, S. R. Stone, and R. Zeisler. 1989. Experiences in environmental specimen banking. Int. J. Environ. Anal. Chem. 37:91-106.

Zeisler, R., S. H. Harrison, and S. A. Wise (Editors). 1983. The Pilot National Environmental Specimen Bank -- analysis of human liver specimens. U.S. Dep. Commer., Nat. Bur. Standards, Gaithersburg, Md, NBS Spec. Publ. 656, 135 p.

Data Recording Form

NATIONAL BIOMONITORING SPECIMEN BANK

Sampling Data

National Marine Mammal Tissue Bank

Animal ID Number _____ Species _____

Sample Source _____

Collection Site ID _____ Lat. _____ Long. _____

Time of Death: day mo yr hr Method of Collection _____

Intermediate Storage (temp/remarks) _____

Necropsy Location _____

Sample Type: ☐ Liver ☐ Kidney ☐ Muscle ☐ Blubber ☐ Other _____

Time of Collection: day mo yr hr Collected by _____

Intermediate Storage (temp/remarks) _____

Time of Preparation: day mo yr hr

Processor _____

Time of LN_2 Freezing

Time Shipped From Site:

Shipper _____

Time Received at Archive:

Receiver _____

Protocol: ☐ Standard ☐ Modified (Please note modification below)

Remarks:

Animal ID Number _____

Condition: ☐ Alive ☐ Freshly dead Other (explain) _____

Sex: ☐ Male ☐ Female Total length [ ] Total weight [ ]

Baleen/Tooth counts (erupted or total) UL/LL [ ] UR/LR [ ]

Specify Unit of Measurements

Snout to melon	[ ]	Snout to center of anus	[ ]
Snout to blow hole	[ ]	Snout to center of genital aperture	[ ]
Snout to center of eye	[ ]	Snout to ant. insertion of flipper	[ ]
Snout to ant. insertion of fin	[ ]	Flipper length	[ ]
Snout to fin tip	[ ]	Flipper width	[ ]
Snout to fluke notch	[ ]	Fluke width	[ ]
Snout to caudal end of ventral grooves	[ ]	Fin height	[ ]

Girth: Axillary [ ] Max (location) [ ] Anal [ ]

Blubber thickness (location)

dorsal [ ] lateral [ ] ventral [ ]

Parasites (location)

external _____

internal _____

Stomach contents _____

_____

Reproductive condition: Preg./Lactating/Fetus length _____

Gonad wt. L/R left [ ] right [ ]

Animal ID Number _____

Method of Collection _____

Age _____ Method of Age Estimation _____

General Comments:

General Appearance of Individual:

General Appearance of Organs:

Histological Samples:

Individual/Organization _____

Final Destination _____

Tissues Sampled _____

Sample Weights:	A		B			A		B	
Liver		g.		g.	Blubber		g.		g.
Kidney		g.		g.	Other		g.		g.

Prepared by: _____
Name (print)

_____
Signature

A copy of this form should be shipped with samples to:

National Institute of Standards and Technology
Bldg 235, Rm B118
Gaithersburg, MD 20899

Attn: Barbara Koster
(301) 975-6291

Appendix II, National Marine Mammal Tissue Bank Access Policy

The goal of the National Marine Mammal Tissue Bank (NMMTB) is to maintain quality controlled marine mammal tissues to permit retrospective analyses for the purpose of, determining environmental trends, and conducting analyses using new and innovative analytical techniques.

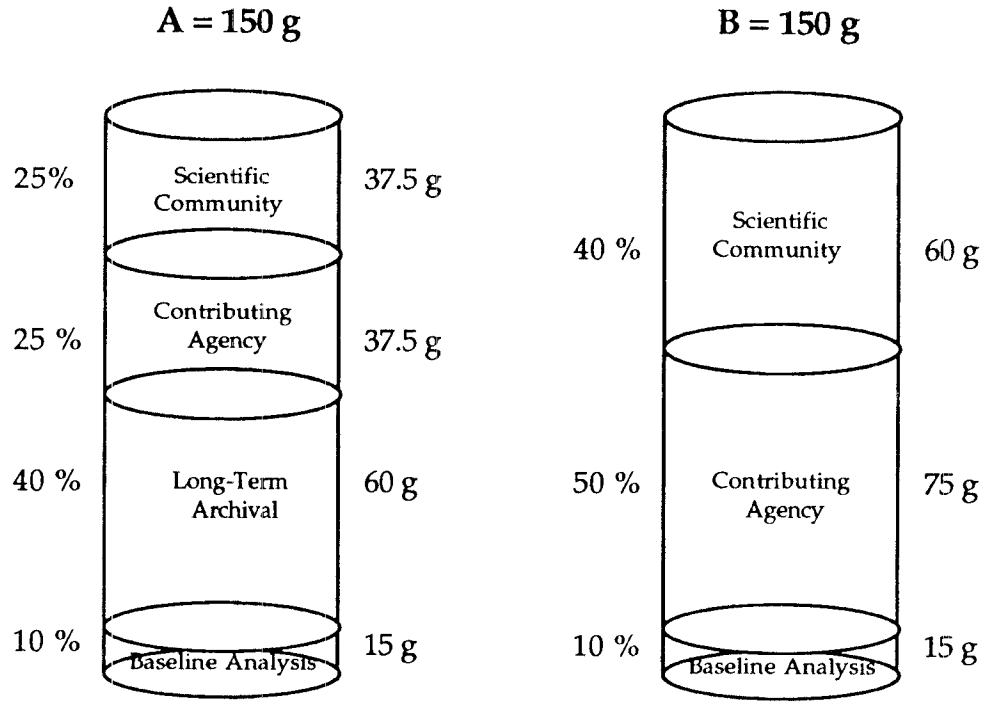
The National Marine Fisheries Service (NMFS), Office of Protected Resources (PR) has the responsibility for managing the NMMTB. NMMTB tissues are banked in the National Institute of Standards and Technology (NIST) National Biomonitoring Specimen Bank (NBSB) located in Gaithersburg, Maryland.

Duplicate samples (denoted A and B) of ~150 g for each tissue specimen are banked in the NMMTB. Subsamples of the "B" samples can be homogenized and aliquoted into approximately 20 subsamples of 6-8 g each which are then available for scientific research. The "A" samples will remain as bulk samples and will only be homogenized after all portions from the "B" sample are depleted and sufficient justification exists to homogenize the remaining material. Thus, 50% of each specimen is available to the scientific community for research and scientific evaluations consistent with the goals of the NMMTB program and 50% is intended for long-term storage as a more permanent archive for posterity.

The homogenized subsamples of "B" samples are divided into three categories. Category 1 constitutes 40% of the homogenized material and is available to the scientific community for research that is consistent with the goals of the NMMTB. Formal requests for access to these tissues must be made as described below. Category 2 consists of 50% of the homogenized material and is reserved for use by the contributing agencies. A procedure for access to these samples will be established by the contributing agencies and the NMMTB. Category 3 is the remaining 10% of the homogenized material which is reserved to perform baseline analyses of the specimens to monitor storage stability, to compare with analyses from real-time monitoring programs and to conduct research investigations within the NMMTB program.

If the "A" sample is eventually homogenized, the subsamples are divided into the following four categories. Category 1 consists of 25% of the material and is available to the scientific community as described above. Category 2 consists of 25% of the material and is reserved for use by the contributing agencies. Category 3 consists of 10% of the material for use within the NMMTB program as described above. Category 4 is the remaining 40% which is intended as a permanent archive that will not be utilized unless high priority needs exist; the determination of this need will be made by an advisory committee to the NMMTB. Combining the "A" and "B" samples, the total percentage sample allocations are as follows: Category 1 (32.5%), Category 2 (37.5%), Category 3 (10%), and Category 4 = (20%). For clarification, the relative amounts of each category are illustrated in the following figure.

Formal requests for banked tissues in Category 1 must be submitted to the Director of the NMFS Office of Protected Resources. The request will be reviewed by an informal review committee consisting of no less than three individuals, who are members of the NMMTB's Team of Scientists. The review committee will be convened by the Director and shall have representation from both the government and academic/private sector. At least one member of the review committee shall be an expert in the field that is related to the proposed research activity. The Director will make the final decision based on the advice provided by the review committee.



Requests for samples from the NMMTB must include a clear and concise statement of the proposed work and be consistent with the goals of the NMMTB. The following specific information should be included in the request for samples:

1. Name of principal investigator and affiliated research or academic organization,
2. Specific tissue sample and quantity desired,
3. Explanation of proposed research to be conducted,
4. Justification for use of banked tissue,
5. Research facility where analyses will be conducted,
6. Analytical quality control procedures to be used,
7. Estimated completion of research, and schedule/date of subsequent reports,
8. Agreement that all results/findings be reported to the NMMTB,
9. Agreement that credit and acknowledgement will be given to NOAA/NMFS and the NMMTB for use of banked tissues.

Of particular importance in determining whether a sample request will be granted is the justification that samples from the NMMTB are required to accomplish the research and that suitable samples to accomplish the goals of the proposed research could not be obtained from other sources. The NMMTB is not intended to be used as a readily accessible source of marine mammal tissue for any researcher needing specimens, but only for research requiring banked samples from the past.

An informal review process will also be set-up for banked tissues withdrawn from Category 2; however, the final decision on Category 2 samples will be made by the contributing agency. Use of Category 3 tissues will be determined by NMFS and NIST. Category 4 tissues cannot be withdrawn until all tissues from the other categories have been depleted. When Category 4 tissues are withdrawn, the same review process will be followed as described for Category 1 tissues with the final determination made by the Director.

Formal requests shall be submitted to the following address:

Director,
Office of Protected Resources (F/PR)
National Marine Fisheries Service, NOAA
U.S. Department of Commerce
1335 East-West Highway
Silver Spring, Md 20910.

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NOAA
U.S. DEPARTMENT OF COMMERCE