Marine Mammals and Persistent Ocean Contaminants:

Proceedings of the Marine Mammal Commission Workshop Keystone, Colorado, 12-15 October 1998

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TABLE OF CONTENTS

List of Tables:

List of Figures:

EXECUTIVE SUMMARY

The Marine Mammal Commission convened a workshop in Keystone, Colorado, 12–15 October 1998, to review what is known, and what needs to be learned, about the effects of persistent ocean contaminants on marine mammals. Concern about the possible effects of anthropogenic compounds and trace elements in marine mammals has increased in recent years for two main reasons. The first is that disease outbreaks, involving marine mammals with high concentrations of organochlorines in tissues, appear to have occurred with increasing frequency. The second is that experimental and other evidence has shown that certain contaminants often found in the tissues of marine mammals have deleterious effects on reproduction and the immune system. Prompted by these and other contaminant-related concerns, the Marine Mammal Commission, with the co-sponsorship of the U.S. Geological Survey, the National Marine Fisheries Service, the Environmental Protection Agency, and the National Fish and Wildlife Foundation, organized this international workshop, involving scientists from seven countries. Expertise of workshop participants included environmental toxicology, environmental chemistry, marine mammal health and husbandry, pathology and disease, physiology, immunotoxicology, marine mammal population dynamics and ecology, experimental design, environmental risk assessment, and wildlife epidemiology.

The workshop objectives were to (1) review and summarize the state of knowledge about the types and levels of potentially harmful persistent contaminants found in marine mammals, and about the known and potential effects of these substances on marine mammal health and population dynamics; (2) identify and rank in importance the critical uncertainties concerning the presence, levels, sources, fates, and effects of organochlorines, toxic elements, and other persistent contaminants on marine mammals; (3) outline research and monitoring programs needed to resolve the critical uncertainties as quickly as possible; and (4) assess how ongoing and planned research and monitoring programs should be restructured or expanded. Much of the focus of the workshop was on the persistent ocean contaminants classified as organochlorines. These include many compounds that originated as pesticides or industrial chemicals. Although some countries no longer use or produce most of these compounds, they continue to be manufactured and used in many parts of the world, and they are redistributed to the global marine ecosystem by a variety of transport mechanisms. Marine mammals, many of which exist at the top of complex ocean food webs, continue to be sinks for organochlorines. Only limited consideration was given at the workshop to potentially toxic trace elements and other persistent substances in the marine environment. This was primarily because there is much less evidence suggesting direct harm to marine mammals from these substances. It was acknowledged that marine mammals may also be regularly exposed to other contaminants, such as non-persistent polyaromatic hydrocarbons from petroleum and industrial sources, but these contaminants were not a focal subject of consideration.

The workshop consisted of a series of plenary addresses, each followed by a panel discussion, and deliberations and report preparation by four working groups. These working groups were

organized to address priority concerns in the following topic areas: immunotoxicology, pathology, and disease; endocrinology and reproduction; risk assessment; and likely future trends. The workshop report includes a summary of priority uncertainties and a list of 20 principal conclusions and recommendations derived from the working group reports (Section II), detailed reports prepared by the working groups (Section III), extended abstracts of the plenary presentations (Section IV), and a small series of appendices (Section V).

Reproductive problems observed during the 1960s and 1970s in female California sea lions off southern California, female harbor seals in the Wadden Sea, and female ringed and gray seals in the Baltic Sea were associated with high body burdens of organochlorines, particularly PCBs and metabolites of DDT. A study of captive harbor seals in The Netherlands, published in 1986, demonstrated an association between impaired reproduction and exposure to organochlorines in the seals' diet. Other studies in the 1980s and 1990s suggested that organochlorines affect endocrine and immune function in some marine mammals. Beluga whales downstream from heavily polluted portions of the St. Lawrence River–Great Lakes watershed and seals in the Baltic Sea have various lesions strongly suggestive of contaminant effects. Die-offs of seals and small cetaceans in Europe and North America from disease (primarily morbilliviruses) during the late 1980s and 1990s evoked much public concern about the role of contaminants. Although investigations of the links between disease outbreaks and contaminants based on studies of carcasses were inconclusive, they led to experimental studies with harbor seals in Europe showing organochlorine-linked effects on immunocompetence. Additional studies reviewed at this workshop added weight to the argument that organochlorines have immunosuppressive effects on marine mammals.

The workshop concluded that there is good reason to be concerned that survival and reproduction in certain marine mammal populations may have been affected, and are being affected, by persistent contaminants, particularly organochlorines. The potential effects of contaminants may include morbidity and mortality from acute toxicity (although mortality has not yet specifically been shown in marine mammals), disruption of endocrine cycles and developmental processes causing reproductive failures or birth defects, suppression of immune system function, and metabolic disorders resulting in cancer or genetic abnormalities. Onset and severity of potential effects may vary according to exposure level, the animal species, age, sex, and general condition, and the presence of other contaminants (e.g., synergism, antagonism, detoxification by enzyme induction, etc.). Concern was also expressed about the effects of eutrophication of coastal waters by excessive inputs of nitrogen and phosphorus, which could diminish the capacity of coastal fish and invertebrate communities to support marine mammal populations, and which might lead to increased frequency and scale of toxic algal blooms that are known to kill them. However, there remains great uncertainty about specific effects of contaminants in marine mammals, to what extent such effects may occur in marine mammals in the wild, and what impact such effects are having on marine mammal population dynamics. Seven general areas of uncertainty were defined by workshop participants as having the highest priority for resolution. The closing of these knowledge gaps will make science better able to

guide policy, management, and regulatory decisions related to contaminant impacts on marine mammals. These are uncertainties about (1) the pathologic effects of persistent ocean contaminants on marine mammals; (2) the relationships between exposure to environmental contaminants and immunotoxicity or health effects specific to marine mammals; (3) the role of environmental contaminants in marine mammal reproductive failure; (4) the potential impacts of endocrine-disrupting contaminants on marine mammals; (5) predicting the risk to individual marine mammals and to marine mammal populations associated with exposure to persistent contaminants; (6) future trends with currently known contaminants; and (7) future trends with less widely recognized contaminants.

For most of the past 40 years the study of marine mammals and contaminants has emphasized determination of chemical residues in tissues. Contaminants have now been documented in many thousands of individuals. However, much less emphasis has been placed on designing and executing studies that allow interpretation of the significance of this contamination to marine mammal health and population dynamics. A consistent theme during the workshop was the need for multidisciplinary studies that integrate physiological, behavioral, reproductive, clinical, pathologic, and toxicological data to evaluate the relationships of immune status, health, reproduction, and survival of individuals to population- and ecosystem-level trends. Such studies should be conducted on both wild and captive populations. Long-term research and monitoring programs are especially valuable and need a stable funding base. Understanding the subcellular mechanisms by which contaminants affect marine mammals can only be achieved through *in vitro* studies using marine mammal cell lines or through experimentation. Invasive experiments can use laboratory animals as surrogates for marine mammals, although variation in response among species means that results must be treated with caution. Therefore, establishment of dose-response relationships and response thresholds may require well-designed, nonlethal experimentation with marine mammals. To a considerable extent, model species that have been well studied and are readily available in captivity (e.g., California sea lions, harbor seals, bottlenose dolphins, and beluga whales) can be used to represent other related species in future field studies or research on captive marine mammals, although extrapolation must be done judiciously. Because most marine mammals are exposed to multiple contaminants in nature, experiments on cell lines, surrogate species, or captive marine mammals should include exposures to complex mixtures in addition to single chemicals. Biochemical and physiological indices of exposure or effects, often called “biomarkers,” can be useful monitoring tools. More biomarkers need to be developed and validated for marine mammals. The workshop emphasized the potential for major problems in the future with well-known contaminants, substances not yet identified by current analyses, and many potential “new” contaminants that are being developed or are already in production. Additional findings and more detailed recommendations are made in each of the working group reports, extended abstracts, and appendices to this report.

Section I. BACKGROUND AND OBJECTIVES

Introduction

All marine mammals alive today have been exposed to chemical compounds and trace elements introduced to aquatic systems by human activities. Many of these substances enter the aquatic environment directly as a result of runoff, leakage, dumping, or atmospheric transport. They are also dispersed and distributed in the environment via food webs. As high-trophic level consumers, most marine mammals experience the effects of biomagnification. They can acquire relatively large burdens of persistent pollutants by ingesting contaminated prey, and can also be exposed to airborne contaminants while breathing. The physiological processes involved in storage, metabolism, and elimination of contaminants in marine mammals are poorly understood, as are the effects of exposure. Also, there is great uncertainty about the mechanisms and pathways of contaminant flux in marine and freshwater environments.

The global marine ecosystem is the ultimate repository for most well-known persistent contaminants, such as the organochlorines. It is well established that many of these contaminants have found their way into the tissues of marine mammals during the past 50 years, and with expanding world industrialization there is a likelihood that anthropogenic chemical contamination of the oceans will increase in amount and complexity. Concern about the conservation and protection of marine mammals inevitably extends to consideration of the role of contaminants. How and to what extent does exposure to contaminants affect the health, reproduction, and survival of individual marine mammals? What are the implications of such effects for marine mammal populations? Do contaminants play a role in causing population declines or slowing population recoveries?

The possible negative impacts of contaminants on marine mammal health and population trends have received increasing attention over the past three decades, and especially during the last few years. Among the issues that have been raised are (1) the apparently increasing incidence of disease outbreaks, sometimes associated with seemingly high levels of contaminant exposure; and (2) the experimental and other evidence that certain contaminants often found in marine mammal tissues have deleterious effects on survival and reproduction in other species. Marine mammal management authorities have traditionally been preoccupied with the problems of large-scale directed killing and bycatch of marine mammals in fisheries. While substantial progress has been made in addressing these problems (at least in North America, Europe, and Australasia), there has been a tremendous growth in information documenting the presence of contaminants in marine mammal tissues. This has led to mounting concerns and uncertainties about their effects in these species. Thus it is appropriate for the impacts of environmental contaminants to become an additional focus of scientific, management, and regulatory efforts directed toward marine mammals.

Recognizing the issue's importance, and the fact that numerous organizations are giving increased attention to contaminant-related research and monitoring, the Marine Mammal Commission, with the cosponsorship of the U.S. Geological Survey, the Environmental Protection Agency, the National Marine Fisheries Service (National Oceanic and Atmospheric Administration, Department of Commerce), and the National Fish and Wildlife Foundation, organized and convened the Workshop on Marine Mammals and Persistent Ocean Contaminants. The workshop, held in Keystone, Colorado, 12–15 October 1998, was attended by 54 scientists from seven countries (Appendix A). Their expertise spanned a wide range of disciplines: environmental toxicology, environmental chemistry, pathology and disease, physiology, immunotoxicology, and marine mammal health, husbandry, population dynamics and ecology. Most participants had expertise on contaminants in marine mammals; some were experts in epidemiology, human and laboratory-animal toxicology, and environmental risk assessment.

The workshop was organized by a steering

committee chaired by Thomas J. O'Shea of the U.S. Geological Survey, Midcontinent Ecological Science Center, Fort Collins, Colorado. Other members of the committee were Gregory D. Bossart, University of Miami; Michel Fournier, University of Quebec at Montreal; Joseph R. Geraci, National Aquarium in Baltimore; L. Earl Gray, Environmental Protection Agency, Research Triangle Park, North Carolina; Robert J. Hofman, Marine Mammal Commission; Robert Kavlock, Environmental Protection Agency, Research Triangle Park; Alison Kirk Long, Marine Mammal Commission; John S. Reif, Colorado State University; and Teri K. Rowles, National Marine Fisheries Service.

Various aspects of the topic of marine mammals and contaminants have also been considered by other workshops and symposia held under different auspices over the past decade. Chemical pollutants and cetaceans were the focus of a workshop held in Bergen, Norway in March 1995, convened by the Scientific Committee of the International Whaling Commission (Reijnders et al. 1999*)*. In April 1995 a workshop in Lerwick, Shetland, U.K., was sponsored by the North Atlantic Marine Mammal Commission, and included a focus on the effects on human health from eating contaminated marine mammal products (Sanderson and Gabrielson 1996). A symposium devoted to the topic of marine mammals and chemical contaminants was also convened in Kamogawa, Japan, in February 1993 (Tatsukawa et al. 1994). Most recently, a workshop was held in 1998 in Sidney, British Columbia, on environmental contaminants and marine mammal health, with an emphasis on the Pacific coast of Canada and the United States (Ross and De Guise 1998). Broader information pertaining to Arctic contamination that includes information on Arctic marine mammals has been compiled by the Arctic Monitoring and Assessment Program (AMAP) and is available online at http://www.grida.no/amap.

The Marine Mammals

There is no unambiguous definition of "marine mammal." However, the term has acquired a customary meaning, at least within the United States, as a result of the Marine Mammal Protection Act of 1972. The Act defines marine mammals as all members of the orders Cetacea (whales, dolphins, and porpoises) and Sirenia (manatees and the dugong); all members of the three Carnivora families Phocidae (true or earless seals), Otariidae (fur seals and sea lions), and Odobenidae (the walrus); two members of the Carnivora family Mustelidae (the sea otter and marine otter); and one member of the Carnivora family Ursidae (the polar bear). From a global perspective, this arbitrarily defined group of animals lives in a large variety of habitats and pursues diverse strategies for feeding, reproduction, and other vital activities. Thus, they are exposed to many kinds and levels of contaminants. Feeding strategies, in particular, influence the nature and degree of exposure to contaminants. Carnivorous species that feed at relatively high trophic levels tend to have higher body burdens of certain contaminants. Thus, for example, the planktivorous baleen whales tend to have lower concentrations of organochlorines in their tissues than the more piscivorous toothed whales (O'Shea and Brownell 1994). In general, coastal populations of dolphins, seals, and sea lions tend to have higher contaminant levels than the more oceanic populations, presumably because their food webs are more exposed to concentrated terrestrial run-off. Several species, such as manatees, gray whales, and walruses, are benthic feeders. By feeding in or near bottom sediments, they risk exposure to types and concentrations of contaminants that may differ from those to which the more pelagic-feeding species are exposed.

Although the title of this workshop refers to persistent *ocean* contaminants, it is important to bear in mind that some marine mammals (e.g., river dolphins, manatees, freshwater seals) inhabit river and lake systems deep within continental land masses and far removed from marine environments. The workshop's emphasis on the marine environment was not meant to exclude consideration of the

freshwater species and populations of "marine" mammals.

Workshop Objectives

The workshop had four primary objectives:

(1) To summarize what is known about the types and levels of potentially harmful persistent contaminants that have been found in marine mammals throughout the world, and the known and potential effects of these contaminants on marine mammal health and population dynamics;

(2) To identify and rank by relative importance the critical gaps in knowledge concerning the presence, levels, sources, fates, and effects of organochlorines, toxic elements, and other persistent contaminants in marine mammals;

(3) To determine and describe the types of research and monitoring programs, including new and innovative approaches, that would be required to resolve the most critical uncertainties as quickly as possible; and

(4) To evaluate how certain ongoing and planned research and monitoring programs could be restructured or augmented to better meet critical information needs.

Workshop Agenda and Procedures

The workshop agenda is given in Appendix B. The first evening and most of the second day were devoted to the first objective of summarizing the knowledge about the types and levels of potentially harmful persistent contaminants that have been found in marine mammals throughout the world, and their known and potential effects on marine mammal health and population dynamics. This was accomplished during seven plenary addresses, each followed by a short question period and a half-hour panel discussion. Extended abstracts of the seven presentations are given in Section IV of this report, and one of the full papers appears as Appendix C.

This is the report by T. Rowles and L. Thorsteinson, which provides an overview of federal and international programs on contaminants and marine mammals. Two additional documents prepared at the workshop are a part of this report. Appendix D is a summary prepared by Anders Bergman that provides an overview of historical and recent work on contaminants in seals of the Baltic Sea, one of the longest known cases of heavy contamination in marine mammals. Appendix E is a statement prepared during and after the workshop by Robert Risebrough giving his perspective on the value of controlled experiments in understanding the effects of contaminants in marine mammals.

Participants were divided into four working groups charged with meeting objectives 2–4. Following the verbal presentations at the workshop, a brief plenary session was held to explain the terms of reference and working procedures for these four working groups. The subjects to be addressed by the working groups were

- Immunotoxicology, pathology, and disease;
- Endocrinology and reproduction;
- Risk assessment; and
- Future trends.

The terms of reference and composition of the working groups had been determined in advance by the workshop steering committee, based on consultations by e-mail with all participants. Approximately two months before the workshop, participants were asked to list up to six of what they considered the most pressing unanswered scientific questions (critical uncertainties) regarding marine mammals and contaminants. They were also asked to indicate their preferences for working group assignments and to provide lists of five or more critical uncertainties and key issues specifically related to each of the subjects to be addressed by the working groups. The steering committee edited and organized the participant-generated lists of critical uncertainties and assigned them to the appropriate working group. A briefing book sent to participants prior to the workshop contained, for each working group, a list of members, a list of the main topics to be addressed (ranging in number from six to ten),

and a list of key issues and critical uncertainties under each topic. Participants were urged to communicate with each other about these issues and uncertainties prior to arriving at the workshop.

Working groups met briefly during the evening of Day 2 to begin their tasks of supplementing, consolidating, and ranking issues and critical uncertainties. Explicit instructions were provided concerning written statements to be produced by the groups. Each working group was asked to identify the highest-priority issues and uncertainties in its area of concern and to prepare written statements on up to five of these. All of Day 3 was devoted to working group meetings. A final plenary session was held in the morning of Day 4. During this session, working group leaders or rapporteurs summarized their group's findings and fielded questions from all participants. After a brief discussion in plenary, the working groups reconvened to review and revise their reports. Although the initial expectation was that each group would break into subgroups to develop and prepare statements on particular subtopics and critical uncertainties, not all groups functioned in this way.

The working group reports constitute the main body of this report. They are presented in full in Section III. The group and subgroup reports typically consist of sections that present the Issue Title, Issue Description and Rationale, Means to Resolve the Critical Uncertainties Surrounding the Issue, and Suggestions Regarding Existing Monitoring and Research Programs. This format reflects the guidelines provided by the steering committee to the working groups, with the intention of establishing consistency in the way issues and critical uncertainties were defined and addressed. However, during deliberations some groups or subgroups modified this reporting format to suit unique aspects of their particular topic or approach.

The working group reports were edited following the workshop by the leaders and rapporteurs, then circulated to the respective group members for review. The final submissions were subjected to further editing by the report editors, and the draft workshop report was then sent to all participants for final review.

Acknowledgments

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Section II. PRINCIPAL WORKSHOP FINDINGS AND CONCLUSIONS

MAJOR UNCERTAINTIES

Prior to the workshop, participants generated a list of more than 400 scientific issues and uncertainties on the topic of environmental contaminants and marine mammals. These were assembled in the briefing book and used to guide decisions about what to include in a list of the most important knowledge gaps. The working group reports (immunotoxicology, pathology and disease; endocrinology and reproduction; risk assessment; and future trends) examine major gaps in knowledge as defined by the expert participants, and discuss measures that may be required to fill those gaps. The high-priority uncertainties are summarized here as well.

Pathologic Effects of Persistent Ocean Contaminants on Marine Mammals

Knowledge about contaminant-induced pathologic effects and associated diseases in marine mammals is limited even though very high levels of certain contaminants have been documented repeatedly in marine mammal tissues. More needs to be known about the relationships among systemic and target-organ exposure, tissue burden and distribution of contaminants, body condition, and clinical and pathologic effects. Failure to gain an understanding of the pathologic effects of contaminants on marine mammals will render scientists unable to inform and advise policy makers, and marine mammal health may deteriorate as ocean contamination continues.

Relationships between Exposure to Environmental Contaminants and Immunotoxicity or Other Health Effects

In recent years, there have been large-scale die-offs of marine mammals caused by disease. Many of the animals that died during these disease outbreaks had high levels of organochlorine contaminants in their tissues. Although many contaminants in the marine environment, including organochlorines, heavy metals, and polycyclic aromatic hydrocarbons (PAHs), are well characterized as immunotoxicants in laboratory animals, immunotoxic effects on marine mammals have been demonstrated only to a limited extent. If the effects of these contaminants on marine mammals remain uncertain, it will be difficult or impossible to assess the role of contaminants in future die-offs and in affecting population trends. In addition, we will remain ignorant about the possible chronic effects of contaminants on marine mammal immune function.

The Role of Environmental Contaminants in Reproductive Failure

Field observations on breeding colonies of pinnipeds, pathology observed in carcasses, and experiments with captive seals all have shown associations between reproductive failure of females and exposure to certain organochlorines or mixtures of organochlorines. These generally take the form of preimplantation disorders (thought to be due to effects on endocrine physiology), spontaneous abortions, or premature pupping. However, the specific compounds or mixtures that may be responsible for the observed disorders, the physiological mechanisms leading to the effects, the interactions with or roles of disease agents, and the population-level significance and extent of occurrence of these phenomena in most marine mammal populations remain uncertain. If these gaps in knowledge are not addressed, the causes of population declines, including those involving endangered or threatened populations and strategic stocks (in the sense of the U.S. Marine Mammal Protection Act), are less likely to be determined or recognized. The result will be that management or regulatory measures are not taken in a timely manner.

Potential for Impacts of Endocrine-Disrupting Contaminants

Exposure to endocrine-disrupting chemicals during gestation, lactation, and adulthood is known to alter reproductive function and development in laboratory and domestic animals, humans, and free-ranging wildlife species. Cause-and-effect relationships between endocrine disruptors and altered reproductive function or development in marine mammals have not been established. However, laboratory studies and research on other wildlife, and the documented high tissue exposure to some of these compounds in some pinnipeds and cetaceans, suggest that effects may occur in marine mammals. Resolving this uncertainty will improve understanding of the possible effects on marine mammal populations of chronic exposure to pollution.

Predicting the Risk to Individuals and Populations Associated with Exposure to Persistent Contaminants

There is much uncertainty in estimating the risk of exposure to contaminants for individuals or populations of marine mammals. Formal risk assessment procedures have not been widely applied in contexts involving marine mammals. However, for endangered or severely depleted populations, the death of even a single individual could negatively influence the population's survival prospects. The uncertainty in estimating risk can be reduced by using an iterative approach to risk assessment and risk management. Discussion between the risk assessors and risk managers must occur as information is developed. This will require case-specific information on contaminant exposure and effects, and risk characterization based on the weight of evidence. Without a formal, integrated process for evaluating contaminant risks, managers and regulators will lack a coherent basis for decision-making, and this will ensure substantial scientific and public controversy.

Future Trends with Currently Known Contaminants

Persistent ocean contaminants to which marine mammals are known to be exposed include several bioaccumulative organochlorines and their metabolites, particularly certain PCBs and DDTs. Although concentrations of some of these contaminants have declined in some marine mammal populations since certain countries placed major restrictions on their production and use, their future global distribution and flux remain uncertain. This is particularly true because some organochlorines continue to be produced, or are stockpiled and in use in many parts of the world. In addition, the effects of chronic, low-level exposure to some of these chemicals are uncertain. Thus, potentially harmful exposure to organochlorines will continue for many years, and in some regions is likely to increase. Information on projected usage patterns and environmental fates of organochlorines needs to be expanded, synthesized, and analyzed for various regions. Uncertainties about effects also need to be reduced to allow such projections. Otherwise it will be difficult for policy makers and regulators to assess likely future impacts on marine mammals.

Future Trends with Less Widely Recognized Contaminants

It is uncertain which of the many new chemical contamination problems on the horizon will have serious impacts on marine mammals. Such impacts are difficult to anticipate or prevent in the absence of basic information on production, use, exposure, and effects. There are an estimated 2,400 lipophilic and persistent chemicals with potential to become problems in the environment. There are many additional chemical substances that are less persistent but that have potential for chronic effects on marine and coastal ecosystems. Among these are the polycyclic aromatic hydrocarbons, non-organo-halogen biocides, polymers and low molecular weight monomers, and pharmaceuticals. Eutrophication of coastal waters from anthropogenic inputs of nitrogen and phosphorous compounds will continue to cause major ecosystem changes, with

uncertain effects on marine mammals. An increase in algal blooms and biotoxin poisoning of marine mammals appears to be one alarming consequence.

PRINCIPAL CONCLUSIONS AND RECOMMENDATIONS

The summary list of principal conclusions and recommendations (Table 1) described below is based on common threads and major elements in the working group reports. These conclusions and recommendations were developed by the report editors and circulated to each workshop participant for review and comment with a draft of the full report. A broad range of opinions can be expected on the relative importance of some of these topics, and they are therefore not listed in any particular order of priority. The full working group reports (Section III) provide detailed explanations and greater specificity.

(1) Integration of Multiple Approaches

A greatly improved understanding is needed of the linkages between specific chemical exposures (both type and amount) and endpoints of concern (e.g., impaired health, immunosuppression, and reproductive disorders). Integration of laboratory, captive animal, and field studies was a consistent theme of workshop deliberations. No single approach is likely to be adequate for resolving the critical uncertainties that arise in relation to contaminants and marine mammals. Thus, there is a need for multidisciplinary studies that integrate physiological, behavioral, reproductive, clinical, pathological, and toxicological data, with the ultimate goal of linking immune status, health, reproduction, and survival of individuals to trends observed or predicted at the population and ecosystem level. Dose-response relationships are critical, and quantification of these relationships should be a goal of all studies of individuals (see No. 17). Surrogate species can be particularly useful in studies of mechanisms of action (see Nos. 9 and 11).

(2) Stable Support for Critical Long-Term Programs with Increased Emphasis on Wider Collaborations

Long-term monitoring and research programs provide consistent, accumulative information that is essential for understanding trends and impacts of contaminants in marine mammals. Established ongoing programs should be viewed as long-term investments that are necessary for the effective assessment and management of marine mammal populations. They should remain a high priority of sponsoring agencies and organizations and be given firm support. New efforts and enhancements to existing programs should also be implemented with assurances for long-term, stable support. Examples of ongoing programs appear in the report by Rowles and Thorsteinson (Appendix C). In addition, the study of contaminant effects is well-established in the fields of medicine and environmental toxicology. Marine mammal scientists should be encouraged to collaborate with programs in these fields. In the United States, such links could be established via programs formally co-sponsored by the National Institutes of Health, the Environmental Protection Agency, the Marine Mammal Commission, or the National Marine Fisheries Service. Numerous other avenues exist for developing collaborations, assuming that adequate support is made available by the relevant agencies involved in marine mammal conservation. Marine mammal science is an almost uniquely international discipline. Collaboration between international programs is greatly encouraged.

(3) Long-Term Interdisciplinary Studies of Local Populations

Long-term interdisciplinary studies provide opportunities to measure contaminant exposure, monitor the health and immune responses of individuals, and relate findings to population-level trends, all in the context of knowing a great deal about the animals' life history, distribution, abundance, population dynamics, and demography. Long-term studies of local populations hold out the possibility of obtaining insights on contaminant effects, grounded in observations from nature. In this context, the population of bottlenose dolphins centered in Sarasota Bay, Florida is probably the best studied of all cetacean populations in the world. Most individuals in the population have been caught, marked, sampled, and released, many of them repeatedly, since 1970. Although the Sarasota Bay situation may be unique, there are similarly important opportunities with at least a few other wild populations. For example, similar studies of bottlenose dolphins are underway in Scotland, and long-term studies are ongoing with the northern right whale population off eastern North America, the beluga population in the St. Lawrence River, the killer whale population in inshore waters of Washington and British Columbia, Florida manatees, California sea otters, and the California sea lion population that breeds on San Miguel Island, California. Other populations are subject to studies that provide a great deal of information about feeding ecology and other important subjects — for example, studies of the "bachelor" group of Pacific walruses that haul out on Round Island in Bristol Bay, Alaska. It is important that these and similar programs continue to be supported and that long-term tracking of the health, contaminant, and disease status of individuals be incorporated into data-collection protocols. Selection of study populations should not be limited to those that are depleted or threatened or that have experienced a recent die-off. Any long-term longitudinal study offers opportunities to assess the effects of chronic pollution, and it is valuable to investigate effects during periods of population decline, stability, and recovery.

(4) Compilation and Dissemination of Information

Existing systems for compiling, interpreting, and disseminating data on the production, use, physical and chemical properties, toxicology, and ecological effects of persistent contaminants should be improved. It is currently estimated that there are roughly 2400 lipophilic and persistent chemicals, of which at least 390 are clearly toxic and bioaccumulative. In order to prevent long-term pollution from these largely unknown chemicals, basic information about them needs to be made widely available. This will require enhanced international cooperation, preferably within the existing framework of chemical contaminant programs, such as the Existing Chemicals Program of the Organization for Economic Cooperation and Development, the Program on Persistent Organic Pollutants of the United Nations Environment Programme, and the International Program on Chemical Safety. The Marine Mammal Commission should facilitate a dialogue among U.S. government agencies and relevant international bodies with the goal of ensuring that a greater amount of basic information on contaminants is available. Such information is crucial for anticipating future problems associated with chemical contaminants in the marine environment. Contaminant categories of particular concern in relation to marine mammals include organometallic compounds and persistent, toxic, bioaccumulative compounds not on the standard persistent organic pollutants (POPs) list but that have been used or produced in large quantities, such as polychlorinated naphthalenes and polychlorinated diphenyl ethers. Other contaminants of concern that could present a risk to marine mammals include non-persistent chemicals with high bioaccumulation potential and heavy or widespread use, chemicals that contribute to coastal eutrophication, polymers and low molecular weight monomers, and a variety of chemicals that may cause endocrine disruption. (See also No. 5.)

(5) Monitoring Environmental Loads and Ongoing Inputs of Persistent Contaminants

In spite of encouraging evidence that levels of some compounds (e.g., PCBs, DDT) have declined in some areas (e.g., the North American Arctic, western Europe), production and inputs to the environment continue in areas such as the former Soviet Union (PCBs, perhaps until very recently) and many tropical countries (DDT and other organochlorine pesticides). This situation creates an ongoing need for global-scale monitoring. Monitoring contaminants in tissues of marine mammals is of direct importance for issues related specifically to this group, but more sensitive detection of contaminant trends in the marine environment in general requires monitoring of other ecosystem components. Attention needs to be paid not only to analyses of water and biological samples, but also to identifying emission sources, transport mechanisms, and transport pathways, and to calculating environmental loads. (See also No. 4.)

(6) Universal Protocols

Tissues are sampled and biomarkers measured from biopsies of living marine mammals and from the fresh carcasses of stranded, bycaught, or hunted animals. Universal protocols should be adopted for sample collection and storage, laboratory analytical procedures, and data reporting. Sample collection should include specified basic data and supporting documentation. Tissue specimens should be archived under consistent conditions in dependable, long-term programs. As analytical techniques are developed, data comparability between and among laboratories should be assured on a global scale. Laboratories should adopt a performance-based, quality-assurance approach that incorporates standard reference materials (SRMs) and inter-laboratory comparisons. Existing SRMs should be analyzed, and, if necessary, new SRMs developed for emerging chemicals. Quantitative information should be generated for as many compounds as possible in order to provide robust data sets for future use. Protocols should stipulate quantitation of a minimum number of compounds of persistent contaminants in order to enhance the comparability of data sets for purposes of risk assessment and trend analysis. Existing analytical techniques should be used to the fullest to identify all anthropogenic chemicals in tissues, and thus expand the number of existing and new chemicals known to accumulate in, and potentially pose threats to, marine mammals.

(7) Use Formal Risk Assessment Procedures to Evaluate Threats

Formal risk assessment procedures should be adapted and used to evaluate threats of contaminants to specific marine mammal populations. The Ecological Risk Assessment approach established by the U.S. Environmental Protection Agency couples risk assessment to risk management in a process that can be iterative. This process begins with problem formulation: contaminant sources, fates, and pathways are described; contaminants of concern and their modes of action are defined; and receptors of concern are identified. A risk assessment would describe specific hypotheses to be tested and measurements that can be made to evaluate endpoints of concern in the analysis phase. Risk characterization, which follows the analysis phase, uses information on exposure and effects to evaluate the risk, generally using a "weight of evidence" approach. This approach balances the information on effects, usually obtained from mechanistic laboratory studies, against the results of captive-animal or field studies. Uncertainty about aspects of risk assessment should be clearly discussed. Discussion should include the ecological significance of the risk, how effects on individuals have been extrapolated to effects on populations, and how risks from contamination have been compared to risks from other stressors. The ideal outcome of risk characterization is identification of threshold concentrations, above which risk becomes significant and risk management is required.

(8) Use of Rehabilitated and Captive Marine Mammals and Associated Databases

Marine mammals held in captivity that are fully

rehabilitated or on display are an under-used resource for the study of contaminant impacts on physiological processes and health. These animals are likely to be exposed to contaminants in routine daily rations of marine products, at least at low concentrations, and are likely to have contaminants in their bodies. Clinical measurements of blood chemistry and hematology, and detailed information on health, reproduction, and survival are routinely recorded for these animals. Provided that care is taken in the design of sampling to avoid confounding effects of prior injury, disease, or response to captivity, use of this resource may provide basic insight about the variability in and relationships between contaminants and basic health and physiological processes, biomarkers, reproduction, and survival. Such insight could prove critical to interpretation of contaminant impacts on wild marine mammal populations.

(9) Use of Surrogate Animal Models

The extensive literature on mammalian toxicology provides many examples in which studies of surrogate species have been used in place of humans. A similar approach can be useful for marine mammals. Studies of surrogate species can provide insights about mechanism of action, the comparative risks presented by different chemicals, and dose-response relationships. Experimental control makes it possible to eliminate confounding factors and reduce variability within treatment groups, thus providing the statistical power necessary to detect effects. The greater availability of species-specific reagents, and the ability to carry out invasive studies, represent additional advantages of using surrogate models. The selection of an appropriate surrogate model depends on the question being posed and the endpoint being assessed. The usefulness of information generated from studies of surrogate models will depend upon certain assumptions and extrapolations which require critical evaluation at all stages of the research. (See also No. 1.)

(10) Controlled Experimental Studies to Address Critical Questions[1]

The need for controlled experiments to answer critical questions about the effects of contaminants on marine mammals was generally acknowledged by all working groups at this workshop. Some of the critical questions regarding impacts of even the most commonly observed persistent contaminants in marine mammals have been asked for more than 25 years, without clear answers. For example, premature pupping of wild California sea lions in the late 1960s and early 1970s was associated with high concentrations of DDE in tissues, but other confounding factors were also observed. A cause-and-effect relationship between DDE and premature pupping has never been established. Today, we

[1]Editors' note: The recommendation that non-lethal experimentation on captive marine mammals be employed to help resolve critical uncertainties was made in every working group report. This recommendation was discussed during plenary sessions with few objections raised from the floor. During review of the full draft report, two participants voiced personal opinions that the use of surrogate species and a "weight of evidence" approach would suffice in place of captive experiments under any circumstance. One participant questioned the feasibility of captive experimentation. Recognizing that there are differing views about the appropriateness of controlled experimentation with either captive or free-ranging marine mammals, agencies with management and regulatory responsibility should establish procedures for deciding when experimentation is warranted. This will help to minimize public controversy, and to prevent environmentally costly delays,. Such procedures would identify all stakeholders (most of whom will undoubtedly share concern about the impacts of contaminants on marine mammals) and ensure that their views are appropriately represented in the decision-making process. The procedure would also create a mechanism or mechanisms for ensuring that experiments are humane and optimally designed. In the experiments on captive harbor seals that were carried out in The Netherlands, for example, the "contaminated" source was market fish, and observed reproductive effects were reversible once the seals were placed back on a less contaminated diet.

recognize that DDE is likely present, at least in trace amounts, in virtually every marine mammal in the world. Since premature pupping in the California sea lion was first observed, it has been learned that elsewhere DDE co-occurs at high levels with other contaminants in some populations of marine mammals. Various reproductive lesions and susceptibility to diseases have also been observed in these populations. Yet we still do not know definitively what role this ubiquitous contaminant actually plays in marine mammal reproductive disorders or diseases.

One reason for this chronic uncertainty is that it has been difficult to obtain funding, facilities, or authorization to carry out controlled experiments on captive marine mammals. Without the highly convincing kind of evidence that only experimentation can provide, the uncertainty and debate regarding cause-and-effect relationships and necessary mitigation are bound to continue for decades. Meanwhile, the health of individual animals and the persistence of species and populations may be in jeopardy. Risebrough's personal account (Appendix E) illustrates how uncertainty as to whether DDE was responsible for eggshell thinning in birds was resolved in part by simple experiments in captivity. Experiments contributed to a ban on the use of the parent compound, DDT. Had such measures not been implemented at the time, a likely outcome would have been the extinction of many North American bird populations and species.

As emphasized in the report of the Future Trends Working Group (Section III of this volume), marine mammals will face a widening array of "new" chemical compounds in the immediate future. Many of these contaminants could adversely affect marine mammals directly, or they could affect other components of the food webs on which marine mammals depend. Most workshop participants agreed that a two-pronged strategy should be followed in confronting this reality. First, procedures similar to those currently being developed by the U.S. Environmental Protection Agency should be developed and applied globally to assess the effects of "new" chemicals on the survival and productivity of representative ecosystem components. Such assessments should be made *before* these chemicals are mass produced. Second, when there is uncertainty about a chemical's effects, and it is determined that a delay in resolving that uncertainty could jeopardize the survival of a marine mammal species or population, it may be necessary to conduct experiments with captive marine mammals. Such experiments would involve non-lethal exposures to doses of contaminants similar to those experienced by some wild populations. The studies would need to be well justified and well designed. Design would have to include power analysis to ensure that the results would be conclusive.

(11) Understanding Processes Linking Exposure to Effects

Understanding the linkages between contaminants and the health, immune system, or reproductive fitness of individual marine mammals at the cellular or molecular level is most likely to come from laboratory studies. Cell culture and *in vitro* techniques can contribute by providing species-specific data on topics such as toxic equivalency. In so far as it is possible, toxic equivalencies should be developed and validated using the cell lines of the marine mammal species of interest. Severe Combined Immune Deficient (SCID) mice and other laboratory mammals can be used for the detailed, invasive types of experimentation needed to improve understanding of the biochemical processes linking exposure and effects. Data from such studies can be employed to strengthen (or weaken) arguments involving the extrapolation of *in vitro* results to whole organisms. Semi-field trials and epidemiological studies of wild populations (using biopsies of free-ranging animals, blood drawn from animals that are captured and released, and specimens salvaged from strandings, bycatch, and subsistence hunts) can be used to validate the inferences based on laboratory evidence or extrapolation from model or surrogate species. A "weight of evidence" argument can be established on the basis of these different types of studies. A difficulty that will arise in evaluating the evidence for some marine mammal populations is that, under protection or well-regulated exploitation, they are in the process of recovering and experiencing phases of rapid population

growth. This can mask or confound the evidence for contaminant-related effects on population dynamics and abundance, whereas such effects may be more readily discernible in depleted populations.

(12) Destructive Sampling of Tissues for Biomarkers and Contaminants

The usefulness of "biomarkers" has been widely discussed, but their application and validation for marine mammals remain at an early stage of development. Biomarkers can be used to assess chemical exposure and with further development should, in the future, be capable of predicting effects in marine mammals. Biomarker studies should be included with other types of contaminant-related research on marine mammals. The results of such studies need to be closely linked to information on contaminant burdens and exposures, and on physiological and life history traits. Some widespread contaminants and metabolites have low potential for bioaccumulation and are short-lived, but their ingestion or inhalation by marine mammals may be a cause for concern. For assessing exposure to such compounds, biomarkers may be very useful. Understanding the mechanisms of biomarker production is critical, but at least three key distinctions should be made: (1) biomarkers that simply demonstrate exposure vs. those that show or predict effect; (2) biomarkers of persistent vs. ephemeral contaminants; and (3) biomarkers for single vs. multiple-chemical stressors. Biomarkers for marine mammals should be sensitive, rapid, inexpensive, and field-adaptable. Similarly, non-destructive sampling is often the preferred approach for collecting contaminant exposure data from marine mammals. However, samples that can be collected non-destructively are often not from the target site for the contaminants of concern. This situation is not unique to marine mammals. The same dilemma can arise in studies with humans, and the relevant literature on humans and other mammals should be directly applicable. The primary issue of concern is the validation of a measurement in a tissue, such as blood, as a reliable surrogate for a target site in an internal organ. Validation studies will be necessary to increase the value of non-destructive samples as surrogates for target sites in marine mammals.

(13) Expansion of Sampling and Monitoring Programs to Include Histopathology, Immunotoxicity, and Life History Information

Some contaminants to which marine mammals are exposed, including organochlorines and trace metals, are known to affect the immune systems of other species. It is uncertain to what extent they may also affect the immune systems of marine mammals. There is sufficient reason for concern, however, and long-term programs for sampling marine mammals should include protocols for obtaining samples and data to support investigations of histopathology and immunotoxicology. In all cases, it is important to emphasize the need to collect associated life history information. The latter is essential for proper interpretation of findings.

(14) Selecting Model Species of Marine Mammals

It is unrealistic to expect that adequate studies will be conducted on most marine mammal species. Therefore, model species must be identified and employed in relevant studies. It was generally agreed that the most appropriate marine mammal species to use as study subjects are those for which considerable information is already available about population dynamics, life history, and physiology; that are in captivity or otherwise easily accessible; and that are already involved in related ongoing studies. Other factors to consider are the feasibility of obtaining high-quality samples in large enough numbers to ascertain sources of variation; whether the species occurs across a gradient of habitats (highly polluted vs. relatively pristine); and conservation status (endangered, threatened, depleted, etc.). Among the cetacean species that come the closest to meeting these requirements are the bottlenose dolphin, harbor porpoise, and beluga whale. The 1995 International Whaling Commission workshop on contaminants and cetaceans also identified these as suitable species for study. Captive studies of baleen whales are out of the question,

but given the considerations listed above, the bowhead whale is probably the most suitable candidate for a model baleen whale in the United States. This is due to the availability of specimens from the subsistence hunt by Alaska natives and the relatively solid background of life history and other information on the species. On a global scale, the minke whale may serve as an even more useful model, because of regional variations in ichthyophagy and availability of specimens from hunting. The harbor seal and gray seal are the best candidates among phocid seals, whereas the California sea lion and northern sea lion are the prime candidates among otariids. The polar bear can be studied directly. Although sirenians were not generally seen as a high-priority group in the context of typical organochlorine contaminants, the Florida manatee is obviously the best model sirenian because of the well-organized stranding program, number of captive individuals, and ongoing life history studies. Because sirenians feed near the bottom in coastal and inland waterways and are herbivores, their exposure to contaminants may include less widely recognized chemicals that are more prevalent in such ecological contexts. The mink is a widely used experimental animal, and it can be used as a surrogate for the closely related marine mustelids (sea otter and marine otter). Even among mustelids, however, mink are known to be exceptionally sensitive to certain contaminants.

(15) Selecting Model Contaminants

The 1995 workshop on cetaceans and contaminants sponsored by the International Whaling Commission identified three categories of chemicals according to how regularly they had been monitored in cetaceans. It was suggested that the criteria for selecting model compounds for priority monitoring and study should include levels of production, potency of bioaccumulation, and toxicity. The present workshop emphasized the need to place highest priority on chemicals with known adverse effects and/or that marine mammals are known to be exposed to. Based on current knowledge, PCBs, DDT and metabolites, other organochlorines, butyltins, and a few trace elements are the most obvious candidates. The mutagenic and genotoxic effects of PAHs warrant study in exposed populations.

(16) Complex Mixtures

All marine mammals have body burdens of many different contaminants, in variable amounts, stored or circulating in different organ systems. Although in standard toxicological studies effects are generally related to a single chemical, marine mammals in the wild are exposed to complex mixtures rather than to single chemicals. Effects from exposure to multiple contaminants may be synergistic or antagonistic. For this reason, experimental exposures should include complex mixtures that mimic as closely as possible the types of exposure experienced by animals in nature. However, exposures to complex mixtures should not be conducted until dose/response relationships for the individual chemicals involved are understood. Care should be taken to avoid conclusions based solely on *in vitro* exposures, which may be more abrupt and thus exaggerated in their effect, compared with the gradual and perhaps lower-dose exposure experienced by the whole organism in nature. However, it should also be considered that some single substances may need to be studied in isolation in order to assist managers and policy-makers in decisions about production, use, and fate of specific chemicals.

(17) Dose-Response Relationships

Ideally, the effects of contaminants should be understood well enough to predict responses from specific doses, including likely “safe” or no-effect levels of exposure. Analyses of the dose-response relationship must be pursued on a species-by-species, contaminant-by-contaminant basis. Experiments and sampling designs should require that exposure be controlled or at least measured, and that potentially confounding factors (e.g., age, sex, reproductive status, nutrition, etc.) be taken into account as completely as possible when investigating dose-response relationships.

(18) Endocrine Disruption

In recent years, endocrine disruption has become

recognized as a potentially widespread, insidious conservation problem. Although evidence that hormonal or developmental dysfunction in marine mammals is linked to contaminant exposure has been slow to accumulate, some common contaminants of marine mammal tissues (such as several of the organochlorines) are known to affect the endocrine systems of other species. A precautionary attitude requires, at a minimum, that the potential for endocrine disruption in marine mammals be regarded as a serious possibility and be subject to aggressive research and evaluation. A "weight of evidence" approach should be used in judging whether measures should be taken to reduce exposure out of concern about this danger. Systematic appraisal of a number of morphological and other endpoints of endocrine disruption should be incorporated in routine marine mammal stranding and health evaluations (see the report of the Working Group on Endocrinology and Reproduction in Section III). Trans-generational effects in long-lived species like marine mammals may be particularly difficult to discern without incorporating such appraisals in ongoing studies.

(19) Understanding Blubber Physiology and Estimating Total Body Burdens of Lipophilic Contaminants

Physiological condition affects the distribution of lipophilic contaminants in the bodies of marine mammals. Further research on lipid dynamics is required to improve understanding of the processes determining this distribution (i.e., how lipid dynamics and physiological demands affect circulating levels of contaminants that ultimately reach target sites of action). Most published reports refer only to concentrations of contaminants in blubber. Concentrations in muscle or other tissues are rarely mentioned. The ability to estimate total body burden is important for determination of exposure factors in risk assessment. Estimating the total body burden of contaminants requires not only information on distribution, but also estimates of the total amount of blubber, muscle, and other tissues.

(20) Statistical Power in Experimental Designs and Sampling Designs

Regardless of whether a contaminant study is to be conducted in the laboratory or the field, it is essential that hypotheses be clearly formulated and that a statistical model be developed to determine the appropriate sample size before the experimental or sampling protocol begins. It is of no benefit to study a sample that is too small for statistically valid results. Nor is there any benefit in sampling more animals than is necessary. In the laboratory, standard statistical methods of determining significance and power can be applied. In the field, it might prove necessary to adapt the mathematical models of epidemiology to understand the impacts of contaminants on whole groups of animals. This would involve stratification by age, sex, reproductive condition, season, location, and other factors.

Table 1. Principal Workshop Recommendations

- Increase Integration of Multidisciplinary Laboratory, Captive Animal, and Field Studies.
- Provide Stable Support for Critical Long-Term Programs and Increase Emphasis on Wider Collaborations in the Environmental Health Sciences.
- Include Components on Contaminants and Health in Ongoing Long-term Population and Life History Studies of Marine Mammals.
- Enhance International Cooperation and Improve Systems for Compiling, Interpreting, and Disseminating Data on Persistent Contaminants in Marine Ecosystems.
- Monitor Environmental Loads and Ongoing Inputs of Persistent Contaminants to Marine Ecosystems on a Global Scale.
- Follow Universal Protocols in Contaminant Sample Collection and Storage, Laboratory Analytical Procedures, and Data Reporting and Maximize the Number of Contaminants Identified
- Use Formal Risk Assessment Procedures to Evaluate Threats of Contaminants to Marine Mammal Populations.
- Use Marine Mammals that are in Captivity for Display, Research, or Rehabilitation, and their Associated Databases, for Insights about Contaminants and Health, Physiology, Reproduction, and Survival.
- Use Experiments with Surrogate Animal Models to Provide Insights about Mechanisms of Action, Comparative Risks Presented by Different Chemicals, and Dose-Response Relationships.
- Conduct Controlled Experimental Studies on Captive Marine Mammals to Address Critical Questions.
- Increase Understanding of Processes Linking Exposure to Effects at Cellular or Molecular Levels.
- Continue to Develop Biomarkers and Validate Results of Non-Destructive Contaminant Sampling in Exposure Studies.
- Expand Sampling and Monitoring Programs to Include Histopathology, Immunotoxicity, and Life History Information.
- Select Model Species of Marine Mammals for Intensive Contaminant Studies on the Basis of Availability, Feasibility, and Background Knowledge of Biology and Habitat Use.
- Select Model Contaminants for Study Based on Levels of Production, Potential for Bioaccumulation, Toxicity, and Suspected or Known Adverse Effects on Marine Mammals.
- Study Effects using Complex Mixtures that Closely Mimic the Types of Exposure Experienced by Marine Mammals in the Wild.
- Determine Dose-Response Relationships.
- Evaluate the Potential for Endocrine Disruption, and Develop Protocols to Assess Morphological and Other Endpoints of Endocrine Disruption as Components of Ongoing Studies.
- Improve Understanding of Blubber Physiology and Estimate Total Body Burdens of Lipophilic Contaminants in Marine Mammals.
- Apply Rigorous Statistical Experimental and Sampling Designs in Field and Laboratory Studies.

Section III. WORKING GROUP REPORTS

Working Group on Immunotoxicology, Pathology, and Disease

Working Group Members: Beckmen, Carvan, De Guise (Rapporteur), Fisher, Fournier, Gulland, Haebler, Hall, Holladay, Lipscomb (Leader), Moore, O'Hara, and Thomas.

The topics addressed by this working group fell into two areas: pathology/disease and immunotoxicology. Accordingly, two subgroups were established, and each of them prepared a separate report addressing high-priority issues. The overview statement from the entire working group is presented below, followed by the subgroup reports.

Overview and General Recommendations

The range of issues discussed in one or both subgroups included the following.

(1) The need to better understand the linkages between specific chemical exposure and immunosuppressive endpoints. The strength of reported linkages varies substantially. Knowledge of dose-response relationships would help to establish cause and effect.

(2) The need for multidisciplinary studies on selected well-known populations in order to integrate toxicological, physiological, behavioral, reproductive, clinical, pathologic, and population data. Such studies will improve understanding of the role of exposure to contaminants in clinical, pathologic, and immunologic effects.

(3) The need to determine the predictive power of molecular and cellular indices of chemical exposure and effect in immunologic and metabolic studies of persistent toxic contaminants. *In vitro* and *in vivo* mechanistic studies are needed. Data to allow species-specific understanding of toxic equivalency should be developed using cell culture and *in vitro* techniques.

(4) The need for tightly coupled laboratory, captive-animal, and field studies linking immunology, health, and disease.

(5) The need to link findings in individual animals with assessments of chemical impacts at the population level.

(6) The need to expand long-term monitoring and sample archival programs to include histopathologic and immunotoxicological samples and data, including life history information required for proper interpretation.

In summary, the potential impacts of persistent ocean contaminants on marine mammal immune function and health are of critical concern. Molecular, cellular, organismal, and population-level processes need to be better understood. Relevant data are best gathered and interpreted using carefully designed, multidisciplinary studies that link laboratory, captive, and field studies.

Pathology and Disease Subgroup Report

Subgroup Members: Beckmen (Co-Rapporteur), Carvan, Gulland, Lipscomb (Leader), and Moore (Co-Rapporteur)

Issue Title: Current Understanding and Future Directions of Research on the Pathologic Effects of Persistent Ocean Contaminants on Marine Mammals

Issue Description and Rationale:

Why Is This Issue Important? Some populations of marine mammals are declining for reasons that are poorly understood. Environmental contaminants have been suggested as potentially important factors in this regard. Knowledge of direct contaminant-induced pathologic effects and associated diseases is limited in spite of the repeated documentation of very high levels of a variety of xenobiotic chemicals in tissues of many marine mammal species. The effects of some of these chemicals are relatively well understood in other animals, and they are likely to be similar in marine mammals. Lesions can indicate acute and chronic effects of contaminants that are persistent either through bioaccumulation or repeated exposure. Contaminant-induced lesions have been shown to affect fecundity and survival of individual animals of other species and, therefore, may also affect the population dynamics of marine mammals. Detection of unusual lesions can provide an early warning of the effects of unrecognized contaminants. In addition, such lesions may be detected in individuals before a change could be detected by routine stock assessments. Pathology is, therefore, a critical tool for the conservation of marine mammal populations.

What Is Generally Known about the Issue? High levels of contaminants have been reported in a wide range of marine mammal species. These levels vary between and within species, spatially, temporally, and stochastically. The trophic level of a population and the extent to which it uses habitat in or near areas of high-density human activity are important factors influencing contaminant burden. There are no pristine areas unaffected by anthropogenic contamination. Contaminants have been associated with lesions in marine mammals, but whether or not the contaminants caused the lesions is uncertain. Some examples are uterine stenosis and occlusions in gray seals and ringed seals (Bergman and Olsson 1985); adrenal hyperplasia in gray seals (Bergman and Olsson 1985) and beluga whales (Lair et al. 1997); a high incidence of neoplasia in beluga whales (De Guise et al 1994b); intersex conditions in polar bears (Wiig et al. 1998) and beluga whales (De Guise et al 1994a); skull lesions in gray and harbor seals (Bergman and Olsson 1985, Stede and Stede 1990, Bergman et al. 1992, Olsson et al. 1992); and skin/integumentary lesions in gray, ringed, and northern elephant seals and sperm whales (Bergman and Olsson 1985, Beckmen et al. 1997, Jauniaux et al. 1998).

What, in General, Needs to be Determined to Resolve the Critical Uncertainties? Convincing evidence is needed demonstrating a cause/effect relationship between contaminant exposure to the development of specific lesions in free-ranging marine mammals, assuming that such relationships exist. This requires that other potential etiologies be fully considered and that accurate determinations of exposure be made. The interrelationships need to be determined among systemic and target-organ exposure, tissue burden and distribution, body condition, and clinical and pathologic effects. The validity of toxico-kinetic and pathologic extrapolations from laboratory animals to marine mammals must be further investigated.

What Are the Likely Consequences If These Issues Are Not Addressed? Failure to gain an understanding of the effects of contaminants on individuals and populations will render scientists unable to inform and advise policy makers and managers. This will have negative impacts on marine mammal health and population status as contamination of the oceans continues. Ultimately, loss of genetic diversity within species and loss of species could occur.

Means to Resolve the Critical Uncertainties Surrounding the Issues

In order to determine whether or not particular lesions are induced by one or more contaminants, studies are needed that are long-term, multidisciplinary, and well documented. Such studies need to examine all likely etiologies systematically. It is essential that studies have adequate controls and sufficient sample sizes to address variables of concern, such as age and reproductive status. Groups of study subjects with documented high levels of the contaminants in question should be examined in parallel with comparable groups that have low levels of these contaminants. Model species should be selected to take advantage of current knowledge, accessibility, and ongoing studies. The success of this approach will rely on obtaining as wide a dynamic range of contaminant concentrations as possible (orders of magnitude if possible). Because persistent contaminant concentrations tend to be relatively uniform on a regional basis, collection of data on an inter-regional or even global scale will probably be required.

The health status of living animals should be monitored using physical examination, biopsy, and blood-sample analysis during capture/release and rehabilitation. Carcasses in good postmortem condition from the same populations should receive complete necropsies with full histopathologic and appropriate toxicological assessment, along with additional tests as indicated by the pathologic findings. All of the available data for an individual animal must be considered to reach a diagnosis. Findings from many individuals can then be collated to assess significant trends. In this way, lesions can be correlated with contaminant levels and considered in the context of other variables, thereby facilitating correct interpretation. Furthermore, these long-term, multidisciplinary studies may facilitate recognition of changes that reflect biological effects of previously unrecognized contaminants.

Additional issues that can be addressed using this approach include the following:

- Determining relationships among contaminant exposure, immunosuppression, and outbreaks of infectious disease.
- Potential recognition of chemical-specific lesions.
- Determination of relationships among levels of contaminants in blubber, blood, and target organs, and correlation of these levels with clinico-pathologic findings.
- Development and validation of practical indices of exposure or effect (commonly referred to as “biomarkers”).
- Validation of extrapolating from studies of laboratory animals to marine mammals or from studies of one marine mammal species to others.

The last item on the above list requires further discussion. Some of the most persistent oceanic contaminants are the halogenated aromatic hydrocarbons (HAHs), which include PCBs, PCDDs, and PCDFs (see Appendix F). Hazard and risk assessments of complex HAH mixtures have used a toxic equivalency factor (TEF) approach, in which the toxic equivalents (TEQs) of any mixture are equal to the sum of the concentrations of individual congeners (i) times their potencies (TEF_i) relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, TEF = 1.0). TEFs have been used to estimate risk associated with many toxicological outcomes, including immunotoxicity, hepatotoxicity, and carcinogenicity (Andersen and Barton 1998, Vos et al 1997–98).

The toxicity of HAH congeners is species-specific, and so necessarily are TEFs, because toxicity depends upon receptor-ligand interactions. Several studies have demonstrated correlations between TEFs derived by *in vivo* and *in vitro* methods (Safe 1998a), which have been used to develop toxico-kinetic models (Kreuzer et al 1997) and estimate risk even at low levels of exposure (Andersen and Barton 1998). Given the species-dependent sensitivity to HAH, species-specific TEFs for marine mammals have to be developed. This can be done by using *in vitro* tools, such as chemically responsive cell lines and recombinant proteins. These can also contribute to the determination and quantification of complex interactions

such as antagonism and synergism. Such tools would allow the analysis of metabolic enzyme activity, binding of xenobiotics to cellular receptors, and gene and protein structure.

Differences in metabolic enzyme activity account for 90 to 95% of the interspecies variability in response to xenobiotics. The remaining 5 to 10% is related to the binding of chemicals to cellular receptors which control enzyme levels (Nebert and Weber 1990). TEF/TEQ data are currently being generated for estrogenic compounds (Safe 1998b), and this approach could be extended to other chemical classes that act through specific receptor-ligand interactions.

Suggestions Regarding Existing Monitoring and Research Programs

(1) Include Additional Data and Tissue Samples When Marine Mammal Contaminant Samples Are Archived. The National Marine Mammal Tissue Bank, working in cooperation with the Arctic Marine Monitoring and Trends Assessment Program, has stringent collection criteria and quality control that are excellent for contaminant analyses. However, other complementary data and samples need to be collected in conjunction with the archived frozen samples. These should include life history information, fixed tissues for histopathology, frozen serum, and frozen whole blood.

(2) Provide Strong, Long-Term Support to Stranding Programs. The structure of the Marine Mammal Health and Stranding Response Program is solid, but the program needs increased central and regional support. Scientific study of stranded animals during their humane rehabilitation, as exemplified by operations at the Marine Mammal Center in Sausalito, CA, can provide important information that is otherwise difficult to obtain. Programs that attempt this kind of study should be encouraged and supported.

(3) Encourage and Expand Long-Term Interdisciplinary Research That Includes Life History and Health Studies. Long-term interdisciplinary studies to monitor the health of free-ranging populations provide crucial information. Programs such as the Sarasota Dolphin Research Project in Florida, involving the capture and release of live animals and detailed examinations of dead animals, should be encouraged and expanded to other regions. In addition, repeated sampling of catalogued individuals (i.e., animals that are naturally or artificially marked so that they can be re-identified) in wild populations can be extremely informative. Such sampling needs to be integrated with long-term ecological and demographic studies of those populations. For example, comprehensive studies using individual identification have made it possible to document reduced reproductive success in California sea lions on San Miguel Island and in North Atlantic right whales. To determine the underlying causes of such recruitment failures, the ongoing studies of these populations will need to be augmented by multidisciplinary investigations of health and disease.

(4) Fully Use Information from Hunts and Bycatch. Subsistence hunts and fishery bycatch should be fully used as sources of high-quality samples.

Immunotoxicology Subgroup Report

Subgroup Members: De Guise (Rapporteur), Fisher, Fournier, Hall, Hollady, O'Hara, and Thomas

Issue Title: Relationships among Exposure to Environmental Contaminants, Immunotoxicity, and Individual and Population Health Effects in Marine Mammals

Issue Description and Rationale:

Why Is This Issue Important? In recent years, large-scale epizootics have affected several populations of phocid seals (Geraci et al. 1982, Osterhaus et al. 1988) and dolphins (Domingo et al. 1990, 1992, Lipscomb et al. 1994, 1996, Schulman et al. 1997). Many of the animals that died during these disease outbreaks had high levels of environmental contaminants in tissues (Hall et al. 1992, Aguilar and Borrell 1994). Viral infections were strongly implicated as the primary causes of these epizootics. However, experimental animals chronically exposed to PCBs are known to have increased susceptibility to viral infections. A wide variety of viruses were used in the experiments, including duck hepatitis virus, a picornavirus (Friend and Trainer 1970), murine leukemia virus, a retrovirus (Koller 1977), and herpes simplex virus (Imanishi et al. 1980). Accordingly, an immunosuppressive role of organohalogens has been suggested to explain the severity of the cetacean and pinniped epizootics (Eis 1989).

Contaminant-induced immunosuppression has also been suggested as an underlying cause of abnormal conditions observed in two other wild marine mammal populations: the high incidence, severity, and diversity of lesions, often associated with opportunistic and mildly pathogenic bacteria, found in dead beluga whales from the St. Lawrence River (Martineau et al. 1988, De Guise et al. 1995) and the prevalence of infectious disease as a mortality factor in southern sea otters (Kannan et al. 1998). Although many contaminants in the marine environment, including organochlorines, heavy metals, and polycyclic aromatic hydrocarbons (PAHs), are well characterized as immunotoxicants in laboratory rodents, immunotoxic effects on marine mammals exposed to these agents have been demonstrated to only a limited extent.

What Is Generally Known about the Issue? Direct determination of the effects of environmental contaminants on the immune systems of free-ranging marine mammals is difficult largely because of logistical and ethical considerations. Approaches to evaluating the effects have ranged from semi-field and field studies to *in vitro* exposures and animal models (Table 2).

Immune cells of beluga whales from native hunts in the Canadian sub-Arctic were exposed *in vitro* to heavy metals and organochlorines. The proliferation of lymphocytes was significantly reduced in cells exposed to concentrations of 10^{-5} M of $HgCl_2$ and $CdCl_2$ (but not in cells exposed to lower concentrations) when compared to unexposed control cells (De Guise et al. 1996). These concentrations are within the ranges of mercury and cadmium found in tissues of free-ranging beluga whales (De Guise et al. 1996). Similarly, the proliferation of lymphocytes exposed to 20 ppm or more of PCB 138 and 50 ppm or more of *p,p'*-DDT, but not to PCB 153, 180, or 169, or to *p,p'*-DDE, was significantly reduced when compared to unexposed control cells (De Guise et al. 1998). Interestingly, some PCB congener concentrations which had no effect on splenocyte proliferation when tested individually (5 ppm) were found to reduce proliferation significantly when three of them were mixed (De Guise et al. 1998). It is difficult to explain why the addition of PCB 169, a coplanar congener that is usually considered particularly immunotoxic, would have reduced the toxicity of the mixture on beluga whale cells. These data suggest that some organochlorine compounds, at concentrations within the range of those measured in the blubber of free-ranging St. Lawrence beluga whales (Muir et al. 1990), have a synergistic effect on beluga splenocytes. Overall, the results of these *in vitro* assays demonstrate the susceptibility of beluga cells to toxic compounds at levels comparable to those found in some tissues of wild animals. The synergistic effects of *in vitro*

exposure to mixtures of organochlorines, at levels at which individual compounds have no effect on immune function, raise questions regarding the potential danger of environmentally relevant complex mixtures.

Animal models have been developed to simulate exposure to environmentally relevant complex mixtures and to evaluate the health effects of such exposure. In one model, rats were fed highly contaminated blubber from St. Lawrence beluga whales and compared with control rats fed blubber from much less contaminated Hudson Bay belugas (De Guise et al. unpublished data). The rats that ate highly contaminated blubber exhibited a slight reduction of plaque-forming cells (PFC) in response to sheep red blood cells (De Guise et al. unpublished data). In another model, rats were fed oil extracted from highly contaminated Baltic Sea fish and compared with control rats fed oil from much less contaminated Atlantic Ocean fish (Ross et al. 1997). The rats that were fed highly contaminated fish oil had impaired cellular immune responses, as evidenced by decreased mitogen-induced lymphocyte proliferation in spleen and thymus, lower CD4:CD8 lymphocyte ratios in thymus, and lower viral infection-associated increase in natural killer (NK) cell activity (Ross et al. 1997). These experimental models are useful in assessing possible cause-effect relationships of exposure to environmentally relevant complex mixtures of contaminants on the immune system.

The *in vivo* effects of exposure to environmentally relevant concentrations of pollutants were demonstrated experimentally in a study of harbor seals. The treatment seals were fed contaminated Baltic Sea fish (e.g., daily intake of 1460 ：g PCBs, and 497 ：g DDTs) and the control seals were fed fish from the Atlantic Ocean that were less contaminated (e.g., daily intake of 260 ：g PCBs and 102 ：g DDTs) (De Swart et al. 1994, 1996, Ross et al. 1996a,b). Results indicated impaired NK cell activity, T-lymphocyte function, and delayed-type hypersensitivity in the animals fed Baltic fish (De Swart et al. 1995, Ross et al. 1995, 1996b). This experimental design is very useful for investigating the effects of exposure to environmental contaminants through food consumption. However, the perinatal transfer of contaminants (through the placenta and milk) that occurs in nature and that may affect the highly sensitive, developing immune systems of fetuses and neonates is not addressed in such experiments.

Field studies, however difficult, are important for evaluating the effects of chronic exposure to environmental contaminants. In a study of free-ranging bottlenose dolphins in Florida, regression analysis found a correlation (r^2>0.75) between decreased mitogen-induced lymphocyte proliferation and increased blood concentrations of several organochlorines (including pentachlorinated and hexachlorinated PCBs, p, p'-DDT, o, p'-DDE and

Table 2. Crude summary of current knowledge on immunotoxicology in laboratory and other animals and in marine mammals

	Other species	Marine mammals
Presence of contaminants	+++	+++
Immune effects of exposure	+++	+
Consequences on individual	+++	?
Population consequences	?	?

Key: +++ = abundant and reliable; + = limited; ? = too scarce to formulate an opinion

p, p'-DDE) in five individuals (Lahvis et al. 1995). In a study of northern fur seal pups captured in Alaska, a correlation was found between the mitogen-induced proliferation of blood lymphocytes, along with other immunoassays and health parameters, and whole blood levels of organochlorines.

There was an inverse correlation between lymphocyte proliferation and the tissue concentrations of nine PCB congeners (Beckmen, unpublished data). This study also found that pups of primiparous dams, the class with the highest levels of organochlorines in blood, had significantly lower antibody production in response to vaccination with tetanus toxoid when compared to pups of old dams with lower organochlorine concentrations in blood (Beckmen, unpublished data). Taken together, these data strongly suggest that environmental contaminants which are present in high concentrations in tissues of marine mammals, and which are known to have immunotoxic effects on laboratory animals, also have immunotoxic effects on some marine mammal species in the wild.

What, in General, Needs to Be Determined to Resolve the Critical Uncertainties? The following considerations provide a basis for concern about the potential immunotoxic effects of contaminants on marine mammals: (1) the evidence of immunotoxicity of environmental contaminants in rodents and other species; (2) the presence of high concentrations of these contaminants in some marine mammals; (3) the *in vitro* effects of these contaminants on marine mammal immune system cells; and (4) the demonstrated immunotoxic potential of environmentally relevant mixtures of contaminants in animal models and in a small number of controlled *in vivo* exposures in pinnipeds.

There is, nonetheless, insufficient evidence to establish a cause-and-effect relationship between exposure to environmental contaminants and injury to the immune system that might increase susceptibility to disease. Moreover, the significance to populations of lowered disease resistance in individuals is uncertain.

What Are the Likely Consequences If These Issues Are Not Addressed? If these issues are not addressed, it will remain difficult or impossible to assess the role of contaminants in epizootics and in trends in marine mammal populations. The chronic effects of contaminants on marine mammal immune function will continue to be uncertain. As a consequence, our ability to recommend appropriate regulatory measures and management policies will be hampered.

Means to Resolve the Critical Uncertainties Surrounding the Issues

In order to address the uncertainties identified above, we recommend a combination of field studies and carefully designed, controlled studies of captive marine mammals to determine the effects on the immune system of selected contaminants of critical concern. Confounding factors such as sex, age, nutrition, genetics, body condition, life cycle, reproductive status (e.g., gestation, parity), blubber condition (e.g., depth, lipid content), current disease, and previous exposure to pathogens should be addressed in all studies. Sampling animals in the field only allows the measurement of current immune function (i.e., the *in vitro* capacity of immune cells to perform their immediate function). In contrast, controlled studies of captive animals allow evaluation of the organism's potential to mount an immune response, in other words, antigen processing, presentation, and collaboration between cells resulting in an appropriate, specific response. Multidisciplinary and multi-laboratory approaches are highly recommended. The preferred approach would include evaluations of at least the following aspects:

(1) Effects of Contaminants on Immune Function in Marine Mammals. The following relevant, logistically manageable species are recommended for use in captive-animal studies: harbor seal and gray seal (to compare differences in susceptibility) and polar bear. Studies involving cetaceans would be costly and technically difficult, but they may be needed. *In vitro* studies should be performed in a specific context and designed to answer specific questions. They cannot replace *in vivo* studies.

In conjunction with captive-animal experiments,

studies using well-characterized surrogate animal models such as rats and mice should be used to better define the links between exposure and response. Rodent-based TEFs should be used cautiously because they may differ among species, and immune effects may not parallel enzyme induction, as is indicated by the relative *in vitro* potency of the PCB congeners in the beluga studies discussed above. Species-specific TEFs should be developed and validated for marine mammals using cell lines. This would allow better comparison of experimental results to the extensive literature on laboratory animals. Also, surrogate animal models, such as mink (for sea otters) and marine mammal immune cell-reconstituted Severe Combined Immune Deficient (SCID) mice, would allow more invasive, detailed, and mechanistic studies. Cellular and molecular mechanisms of immunotoxicity of organochlorines in marine mammals are unknown. An appropriate way to investigate them would be to examine mechanisms in animal models, and to evaluate similarities and differences in these mechanisms in marine mammals. The effects of relevant mixtures of contaminants also should be addressed. For all experimental procedures, Good Laboratory Practices Act protocols should be followed.

In order to improve understanding of immunology and other health-related parameters, as well as to establish reference data, efforts should be directed toward obtaining information from captive marine mammals. Public display facilities and research-oriented institutions such as the Alaska Sea Life Center could be used to study a variety of species, including the beluga whale, harbor porpoise, bottlenose dolphin, killer whale, harbor seal, gray seal, northern sea lion, polar bear, and sea otter.

Field studies should be carried out to assess potential immunotoxic effects on free-ranging marine mammals. The recommended criteria for choosing species for field studies include (1) selection of populations in nature that exhibit a gradient of exposure to contaminants and (2) the ability to sample a sufficient number of the animals to ensure statistical power. The species listed in Table 3 are recommended, based on documented exposure to organochlorines, metals (mercury and cadmium), and organotins. This is not a list of species of concern, but rather a list of species that can be used to investigate specific issues.

Table 3. Some recommended species for field studies

	Documented exposure to:		
Species	**Organochlorines**	**Metals**	**Organotins**
Beluga whale	Yes	Yes	Yes
Bottlenose dolphin	Yes	Yes	Yes
River dolphins	Yes	Yes	
Harbor seal	Yes	Yes	Yes
Gray seal	Yes	Yes	
Northern fur seal	Yes	Yes	Yes
Sea otter	Yes	Yes	Yes
Polar bear	Yes	Yes	
Other coastal species	Yes	Yes	Yes

Although West Indian manatees and northern elephant seals do not necessarily meet the criteria, they may provide opportunities to address particular issues, such as those related to exposure to biotoxins or effects of eutrophication.

In designing studies, it is important to strive to incorporate (1) immune-function and immune-response assays that are as complete as possible; (2) sample sizes that ensure statistical power; and (3) exposures that mimic environmental mixtures. To investigate the relationships between organochlorines and immune function, measurements of organochlorines in blubber and blood should be employed as well as estimates of total body burdens.

(2) Consequences of Contaminant-Induced Immune Impairment on the Health of Individual Animals. In view of results with several other species, it is reasonable to suspect that reduced immune functions in marine mammals will decrease the animals' resistance to challenge by pathogens. Nevertheless, such decreased resistance has never been directly demonstrated for marine mammals. Presenting a pathogenic challenge to one or more marine mammals in a study context would raise serious ethical and logistical problems. The possible need for such an experiment was discussed in the subgroup, but no agreement was reached. In a practical sense, any decision is likely to depend on the weight of evidence, level of confidence, and statistical power required in specific circumstances. The use of marine mammal immune cell-reconstituted SCID mice could provide an alternative model for testing the susceptibility of contaminant-exposed animals to pathogenic challenge.

(3) Consequences of Contaminant-Induced Impairment of Individual Animal Health to the Population as a Whole. It would be useful to generate baseline data at the population level. This is most likely accomplished by long-term monitoring of some populations, with the goal to establish normal values while accounting for seasonal variations, differences according to location, age, sex, and life stage, and other potentially confounding factors. This would make it possible to detect changes in immune function in the population, or at least in a portion of the population. When possible, the population-monitoring tools should be Tier I (screening) assays, including but not limited to lympho-proliferation assays, immuno-phenotyping, neutrophil phagocytosis and respiratory burst, and NK cell activity. When feasible, it would be highly desirable to follow cohorts through time.

Toxin-mediated immune suppression may cause acute and chronic disease processes, including epizootics, that have effects at the population level. However, many other factors can influence immune functions, such as food resources, climate, hormonal status, and disease processes; these factors must also be assessed. Natural history and population dynamics must also be integrated into such studies. This requires well-designed protocols. In particular, contingency plans should be designed to take advantage of sampling opportunities during unusual strandings or mortality events. Long-term field studies should be designed to determine variables affecting immune responsiveness.

Suggestions Regarding Existing Monitoring and Research Programs

Some institutional programs exist for monitoring mortality and contaminant burdens and for archiving tissue. One objective of the NMFS Marine Mammal Health and Stranding Response Program is to investigate biological linkages between chemical contaminants in the marine environment and specific population health indices or parameters in key populations. In addition, the National Institute of Standards and Technology maintains the Marine Mammal Tissue Bank, which ideally includes life-history information related to the tissues that are being optimally maintained and archived for future studies. The NMFS Marine Mammal Stranding Networks continue to investigate and study stranded marine mammals in the United States. As noted in Appendix C, these include investigators supported by the U.S. Department of the Interior who sample sea otters, manatees, walruses, and polar bears (species for which the Department of the Interior has lead agency responsibility under the U.S. Marine Mammal Protection Act). In addition, many investigators throughout the world have tissue collections, contaminant data, and stranding data. The content and methods for maintenance of these other collections vary.

Currently, there are no monitoring programs relevant to immunotoxicological assessment. We

therefore recommend that the Marine Mammal Health and Stranding Response Program and other future monitoring programs include the key species identified above as particularly relevant to immunotoxicology. In this regard, we support the ongoing efforts to initiate the archiving of histological samples and serum. Further, we recommend that methods be developed for the cryo-preservation of lymphocytes for analysis by current and future techniques. We recommend that a standardized suite of analytical procedures be adopted for measurement of immune function in monitoring programs.

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Working Group on Endocrinology and Reproduction

Working Group Members: Addison, Béland, Bergman, DeLong, Gray, Mattlin (Rapporteur), Reddy, Reijnders, Reynolds (Leader), Rolland, Rowles.

Overview and General Recommendations

To address the subject of effects on reproduction, the group concentrated on early development and premature pupping. A multi-faceted approach (i.e., a multidisciplinary approach, using a variety of tools and perspectives) to assessing the potential and success of marine mammal reproduction is essential, and it must involve *in vitro* studies as well as studies on captive, "semi-captive," and free-ranging animals. The studies advocated by this group would have optimal value when combined with assessments of life history parameters, reproductive histories of individual animals, demography, immune function, pathology, and environmental conditions (noise, productivity, trophic relationships, etc).

Long-term longitudinal studies of free-ranging animals permit scientists to evaluate vital rates (e.g., natality, age-specific survival and reproduction). These rates provide a context for understanding population-level changes that may be associated with environmental changes. In addition, longitudinal studies permit scientists to assess the effects of chronic pollution (not just acute events) during periods of population decline, stability, and recovery. Finally, such studies permit scientists to relate behavior and reproductive success to contaminant levels.

Recommendations of the working group include the following:

- Develop international programs and collaborations to promote comparability;
- Conduct controlled experiments with captive, semi-captive, and free-ranging animals, while also remaining alert to opportunities for gaining insight into aspects of species biology in other ways;
- Use non-marine mammal surrogates in a sensible manner. Inasmuch as cellular and molecular homologies exist across a wide range of taxa, rodent models can provide insight into mechanisms and lead to identification of appropriate biomarkers for marine mammal studies. However, such models are not necessarily appropriate for questions at the organismic or population levels. In other words, the choice of a surrogate depends on the question being asked;
- Establish priorities for chemicals and species to be tested. It is impractical to investigate endocrinological and reproductive effects for all chemical contaminants and for all marine mammal species. Initially, model species should be selected based on criteria such as ease of captive maintenance, status of knowledge about physiology, breadth of distribution (including whether the species occurs in a variety of habitats along a pollution gradient), feasibility of obtaining quality samples, and status of stocks (e.g., endangered, threatened, or strategic). Among the species that emerge as logical models are the harbor seal, California sea lion, northern sea lion, gray seal, bottlenose dolphin, beluga, harbor porpoise, polar bear, walrus, and sea otter. Priorities for chemicals should start with those that have demonstrated adverse impacts or a high likelihood of exposure and bioaccumulation;
- Evaluate the appropriate tissues for exposures and contaminant levels, and develop an understanding of toxicokinetics of transplacental transfer; and
- Ensure adequate funding. New partnerships should be forged to bring as many resources as possible to bear on the critical issues.

Issue Title: Understanding the Effects of Persistent (and Non-Persistent) Chemi-

cal Contaminants on the Reproductive Potential and Reproductive Success of Marine Mammals.

Likely Consequences If This Issue Is Not Addressed. Unless and until substantial progress is made in resolving this issue, we will be faced with the following general consequences:

(1) Problems will not be recognized in a timely manner for investigation and mitigation;

(2) Causes of population declines, including those involving endangered or threatened populations and strategic stocks (in the sense of the U.S. Marine Mammal Protection Act), are less likely to be determined;

(3) The effects of new pollutants will not be detected until serious impacts or population declines have occurred;

(4) Without data from more common species of marine mammals, no good models or validated techniques will be available for application to threatened, endangered, or strategic stocks that are not accessible for study; and

(5) Opportunistic approaches to address relationships between environmental changes and population status will remain less focused. In any event, such approaches can be costly and generally provide less definitive results.

Means To Resolve the Critical Uncertainties Surrounding the Issue. Three general approaches to achieving the necessary understanding were identified. The goals with all of these approaches are to elucidate mechanisms and effects, and then to use the insights gained to facilitate mitigation and conservation.

General Approach 1: Disorders in the Estrous Cycle before the Implantation Stage

Description and Rationale. Reproductive failure in the early stages of the reproductive cycle may have demographic consequences for any marine mammal population. Comparative studies onharbor seal populations showed that pup production in the population in the Dutch part of the Wadden Sea was low compared to that of other populations in the Danish and Schleswig Holstein part of the Wadden Sea (Reijnders 1980). Average pup production per female in the Dutch population had declined by approximately 30%. Toxicology studies revealed that, of all the organochlorines analyzed, PCB levels were significantly higher (by 5 to 7 times) in the population with the lower reproduction rate.

What Is Known. Because experimental studies had provided evidence for negative effects of PCBs on reproduction in other mammals, it was hypothesized that PCBs were responsible for the observed low reproduction in the Dutch Wadden Sea harbor seal population. To test this hypothesis, an experimental feeding study was carried out with two groups of harbor seals, each group receiving a diet with a different pollutant load. Pup production was significantly lower in the group receiving the more contaminated diet. Moreover, it was evident that the reproductive cycle had been interrupted at approximately the time of implantation, accompanied by temporarily lower levels of estradiol-17ß. This phenomenon has not been investigated in other marine mammal species.

Uncertainties About Pre-Implantation Disorders and How to Resolve Them. Uncertainties about pre-implantation disorders can be grouped under three headings: (1) identification of which chemical agents, if any, are responsible for the disorders; (2) mechanisms leading to the observed effect; and (3) occurrence and significance of pre-implantation disorders in other harbor seal populations and in other marine mammal species.

The experimental study (Reijnders 1986, Boon et al. 1987) concluded that it was highly likely that the observed effect was due to PCBs (although there always remains the possibility that some of the many other persistent contaminants present played a role). The uncertainties are related to the question, "Which congeners and/or PCB metabolites are the responsible compounds?" It was suggested in this study that these were the CYP 1A-inducer PCBs (dioxin-like PCB congeners and Ah receptor agonists). Three mechanisms were suggested:

(1) enhanced steroid breakdown due to PCB-related

P450 enzyme induction;

(2) receptor interference at the target tissue or carrier proteins due to PCB metabolites, particularly the binding of PCB methyl sulfones (PCB-MSFs) to uteroglobin; and

(3) interference with hormone synthesis and metabolic activity of the P450 enzyme system.

Subsequent *in vitro* experiments suggested that the first mechanism (i.e., increased hydroxylation of estradiol leading to lower estradiol levels) was the most plausible of the three possibilities. The uncertainty regarding the roles of the other two mechanisms noted above might be resolved by investigating whether the PCB-MSFs binding to uteroglobin found in experimental laboratory mammals (e.g., rabbits) also occurs in harbor seals, and whether aromatization of testosterone (e.g., in ovarian tissue) is inhibited. Additionally, seal uterine tissue could be tested for its hormone-binding capacity.

It is not known whether this problem occurs in other harbor seal populations or in other marine mammal species. In order to resolve this uncertainty, it will be necessary to assess whether other marine mammal species are equally sensitive. The first step would be to investigate hormonal cycles (i.e., progesterone and estradiol) in other species, either by analyzing archived plasma or serum samples, or by carrying out studies with captive animals. These investigations would need to be followed either by feeding studies using model species in captivity (e.g., ringed seal, or an otariid and a small cetacean) or by field studies of populations that are exposed to differing amounts of pollution. When choosing one or more populations for field studies, the pollution levels in the presumed prey should be such that the daily intake by the wild marine mammals is at least similar to that of the two groups involved in the experiment, and if possible should also include marine mammals from areas with even higher levels in prey. Differences in reproduction rates of the populations, and exposure levels as reflected in blood and blubber contamination, should also be clearly established.

General Approach 2: Premature Births in Otariids

Description and Rationale. Premature births have been recorded in California sea lions (DeLong et al. 1973), northern (Steller) sea lions (Pitcher and Calkins 1981), and northern fur seals (Gilmartin et al. 1976). Unfortunately, data on contaminant levels were available only in the studies involving California sea lions. Observed rates of prematurity in California sea lions were as high as 20% in the early 1970s (DeLong et al. 1973). High rates of premature births decrease the potential for population growth, no matter what the species or the location. It is therefore important to develop ways of evaluating the role of new (and old) chemical pollutants in influencing the rate of premature births. The production and use of DDT continue in some parts of Asia, and global PCB levels are not expected to decline dramatically, particularly in view of recent production and use in the former Soviet Union and continued environmental inputs and cycling (see Future Trends Working Group Report). Long-range transport and equilibration of contaminants could lead to elevated levels of organochlorines in Southern Hemisphere otariid populations and other marine mammals, possibly leading to reproductive problems similar to those experienced in California sea lions. An understanding of how, or if, organochlorines have influenced premature births in California sea lions would provide a valuable basis from which to begin investigating premature births in other otariids.

What Is Known. An association has been documented between high organochlorine levels in postparturient female California sea lions and miscarriages or premature pupping during the last two trimesters of pregnancy. The majority of premature pups are born alive during the third trimester, but all die within several hours of birth. No significant differences have been found in serum estrogen and progesterone levels between less-than-full-term and full-term parturient females sampled within 6 hours postpartum. The association of organochlorines with prematurity is confounded by the presence of diseases capable of inducing abortions: serological evidence of leptospirosis and calicivirus has been found in both groups. Further-

more, the frequency of prematurity is higher in El Niño years, indicating that the nutritional status of the females also influences the probability of prematurity.

Uncertainties and How to Resolve Them. The greatest uncertainty is whether or not the premature births are induced — directly or indirectly — by the levels of organochlorines in the females. Too many confounding variables (including age, nutrition, and diseases such as leptospirosis and San Miguel sea lion virus infection) are at play to allow a clear cause-and-effect relationship to be inferred based solely on the presence of contaminants and prematurity (Addison 1989). This is a multi-factorial situation that will require differentiating among all potential contributing causes, and should include consideration of factors related to serology, age, nutrition, disease, and genetics as well as contaminant exposure.

Assuming that any effect of organochlorines is mediated through the endocrine system, the first requirement is to establish the normal hormonal control of reproduction. This would require regular analysis of circulatory hormones known (from other animal studies) to be involved in the reproductive cycle. The most useful measurement is probably the ratio of progesterone to estradiol (Reijnders 1990) because the former declines during pregnancy and the latter tends to increase just before birth. Even though the California sea lion has a life history that allows collection of samples year-round from potential representatives of both classes of females (i.e., those that give birth prematurely and those that reach full term), this kind of study would best be done with animals in a captive breeding colony in a controlled environment (including light regimes similar to that experienced by the wild population). The next step would be to validate the measurements in captive animals by analysis of wild animals at critical stages during the cycle. (The behavior of wild sea lions is such that it becomes difficult to resample the same individual more than three or four times. Furthermore, prematurity is difficult to predict, so a relatively large number of samples would have to be taken to ensure that a significant number of females giving birth to premature pups is available for comparison with ones giving birth to full-term pups.)

A second set of "biochemical lesions" which should be investigated is the possible interference of organochlorines with aromatase, as well as ovarian hydroxylation of estradiol (Reijnders, in press; see also Section IV). This would require that the normal cycle of aromatase as well as ovarian hydroxylation of estradiol activity be established in captive animals, with eventual validation in field samples. This would probably be more demanding than the above approach using the progesterone:estradiol ratio, because aromatase and hydroxylation measurements *in vivo* require injection of a suitable substrate, followed by sampling of blood and analysis of product after appropriate intervals. It would be possible, but difficult, to carry out such studies in the field.

The California sea lion is the best model pinniped species for investigating the causes and frequency of premature pupping. It is not threatened as is the northern sea lion. Also, it is well represented in captivity, and there are several captive breeding colonies (Asper et al. 1990). Because of their natural history, adult female California sea lions are accessible on land year-round. This creates the opportunity to make observations and collect samples from individuals that ultimately experience premature and full-term births. Although the current model is applicable only, or primarily, to premature birth in pinnipeds, there is a developing data set from the U.S. Navy's population of bottlenose dolphins which could eventually allow development of a model for evaluating reproductive failure in small odontocetes (Aguilar and Borrell 1994, Reddy et al. 1998a,b, Reddy and Ridgway 1995, Ridgway and Reddy 1995, St. Aubin et al. 1996).

General Approach 3: Indices of Reproductive and Developmental Functional Endocrine Toxicology in Marine Mammals

Description and Rationale. Exposure to endocrine-disrupting chemicals (EDCs) during gestation, lactation, and adulthood is known to alter

reproductive function in laboratory and domestic animals, humans, and free-ranging wildlife species (Colborn and Clement 1992). Although contaminant exposure levels are often extremely high, few cause-and-effect relationships have been established for marine mammals. The approach described below is cost-effective and takes advantage of existing resources and infrastructure.

What Is Known. Background information on this topic can be categorized in three areas: (1) homolo gy of endocrine function among mammals; (2) sex determination and differentiation; and (3) results of controlled experiments with rodents.

(1) *Homology of endocrine function among mammals: from rats to marine mammals*. Our recommendations (below) include not only studies on captive, "semi-captive," and free-ranging marine mammals, but also surrogate mammalian species. Rodent studies are recommended specifically to address physiological, biochemical, cellular, and molecular *mechanisms of action* of contaminants on reproductive function. At the cellular and molecular level, endocrine function is highly conserved. The steroid and thyroid hormones of all mammals are identical. The hormones act by binding to hormonal receptors in the individual cells. These hormone-receptor complexes alter gene expression through binding DNA at specific response elements on specific genes. At this level, endocrine function is highly homologous among mammals. However, although the androgens induce male development and function and maintain the reproductive tract in males of all species, and estrogens and progestins play similar roles in some aspects of female reproductive function in all mammals, some *effects* may be quite species-specific. In addition, all mammals may not metabolize or excrete EDCs in an identical fashion. Hence, we propose that rodents be used to identify *mechanisms of action* of contaminants, as these mechanisms are likely to be conserved in marine mammals. *Effects* studies in rodents are also useful, but we must be aware of the limitations of extrapolating from the laboratory to the field.

With respect to the EDCs, it is essential to consider the unique sensitivity of the developing reproductive system. *In utero* or perinatal exposure to a contaminant can irreversibly alter reproductive development in mammals in a manner that cannot be anticipated from the effects seen in adults. Chemicals that alter sex differentiation in rodents may have similar effects in marine mammals, as many of the basic mechanisms underlying the developmental process are similar (Gray 1992, Gray et al. 1998, Schardein 1993). For example, in most mammals exposure to the synthetic estrogen diethylstilbestrol (DES), androgens, or progestins results in morphological and pathological alterations of reproductive function. DES treatment causes cancer, infertility, and serious morphological abnormalities in all species examined.

(2) *Sex determination and differentiation*. Sexual differentiation, the development of the male or female phenotype from an indifferent state, entails a complex series of events. Genetic sex is determined at fertilization, and this governs the expression of the "male factor" and the subsequent differentiation of gonadal sex. At this stage of embryonic development, both sexes have bisexual potential. Following gonadal sex differentiation, testicul ar secretions induce the further differentiation of the sexual phenotype. The morphological and physiological development diverges, resulting in the formation of the male and female phenotypes. The development of phenotypic sex differentiation includes persistence of either the Wolffian (male) or Müllerian (female) duct system, differentiation of the external genitalia, and sex differentiation of the central nervous system (neurobehavior and neurological characterization of the central nervous system through hormonal control). Other organ systems, like the central nervous system and liver, are sexually "imprinted" as well. The male phenotype arises due to the action of testicular secretions — testosterone and Müllerian inhibiting substance. In the absence of these secretions, the female phenotype is expressed (whether or not an ovary is present). In the human embryo, the onset of testosterone synthesis by the testis occurs 65 days after fertilization. Testosterone induces the differentiation of the Wolffian duct system into the epididymi s, vas deferens, and seminal vesicles, while its

metabolite, dihydrotestosterone (DHT), induces development of the prostate and male external genitalia. In the central nervous system, testosterone is metabolized both to estradiol and DHT, and it has been suggested for some species that all three hormones play a role in masculinization of the central nervous system. In the rat, mouse, and hamster, the aromatization of testosterone to estradi ol is responsible, in part, for central nervous system sex differentiation, while in certain other mammals (e.g., the rhesus monkey) the androgenic (DHT) pathway is essential.

Aberrations of the chemical and genetic forces that regulate sex differentiation in mammals are relatively well understood because they are generally not lethal. Abnormal sexual development can be induced by drugs, chromosomal non-disjunction, and single gene mutations. There is an extensive database on sexual differentiation in rodents, and it is clear that the fundamental mechanisms of sexual differentiation are the same in all mammals, although the timing of certain anatomical developments varies. During sexual differentiation, there are a number of critical periods when the reproductive system is uniquely susceptible to chemically induced perturbations. At these times, an inappropriate chemical signal can result in irreversible lesions, whereas similarly exposed young adults may only be transiently affected. It is also of concern that some of the abnormalities may not be expressed during fetal and neonatal life and only become apparent after puberty.

(3) *Results of controlled experiments with rodents*. The biological effects seen in rodents exposed to contaminants in controlled experimental settings can provide guidance as to the types of effects that might be seen in marine mammals exposed to the same chemicals in the environment. Table 4 lists the biochemical and whole-animal effects seen in rodents exposed to organochlorines and other synthetic chemicals, some of which have potential to affect marine mammals. Table 5 lists effects seen in male and female offspring that have been exposed to various contaminants of concern during development, a uniquely sensitive time for exposure to chemicals. With regard to Tables 4 and 5, it is important to recognize that the indicated effects are dose-dependent and that in many cases, it is not known to what extent marine mammals encounter these chemicals in amounts likely to cause many of the effects listed.

Fetuses are particularly sensitive to TCDD, PCBs, estrogens, and anti-androgens (such as *p, p'*-DDE and DDT and phthalates). Effects can occur in fetuses at dose levels that are orders of magnitude below those that affect adult reproductive function. In female rodents, treatment during perinatal life with hormones or chemicals that disrupt hormone function (e.g., estrogens, anti-estrogens, diethylstilbestrol (DES), androgens, phytoestrogens, and estrogenic pesticides) can induce abnormal sex behavior, persistent vaginal cornification (PVC), and infertility via the Delayed Anovulatory Syndrome (DAS) (Tables 4 and 5). In male rodents, potent estrogens like estradiol and RU-2858 anti-estrogens, anti-androgens, and progestins alter morphological sex differentiation and cause infertility. Environmental contaminants that are weakly estrogenic, like the estrogenic pesticides, appear to antagonize the action of testosterone on phenotypic sex differentiation. Several chemicals like the phthalates, vinclozolin,

(Table 4)

procymidone, and linuron antagonize the action of male sex hormones during differentiation and result in malformations of the reproductive tract (e.g., hypospadias, undescended testes, agenesis of sex accessory glands, and reduced anogenital distance) (Tables 4 and 5). Sex differentiation in both male and female mammals is altered by perinatal exposure to contaminants or treatments that lack or do not display estrogenic or anti-androgenic activity (Table 5). Such treatments include PCBs, TCDD, the soil fumigant and nematicide dibromochloropropane (DBCP) and fenarimol (an aromatase inhibitor), some neuroactive drugs, and the herbicide nitrofen.

Means to Resolve the Critical Uncertainties. The following elements need to be included in a strategy for resolving uncertainties about endocrine disruption in marine mammals: establishment of baselines on structure and function of reproductive and other endocrine organs; establishment of linkages between contaminant loads and changes in structure or function of endocrine organs; and separation of the effects of contaminants on reproductive and other endocrine functions from the effects of confounding variables such as diet and disease. These elements can best be achieved by (1) defining endpoints of endocrine disruption that can be examined and tested in marine mammals; (2) development of new assays; (3) expanding sampling of target tissues for maternal/fetal pharmacokinetics of contaminants; (4) taking advantage of opportunities afforded by existing marine mammal research programs; and (5) developing criteria for selecting animals for more stringent sampling.

Table 5. Effects seen in laboratory rodents and other organisms exposed *in utero* or perinatally to organochlorines and other chemicals of concern for marine mammal conservation. ***(Effects may be dose-dependent and, for some contaminants, it is not known to what extent marine mammals encounter these chemicals in amounts likely to cause the effects listed.)***

Chemical	Male Offspring Effects	Female Offspring Effects
In utero TCDD	Epididyal agenesis Reduced epididymal and ejaculated sperm Delayed puberty Small reductions in sex organs Testis affected at higher dosages Infertility	Ovarian atrophy Female tract and ovarian tumors Malformed external genitalia Shortened reproductive life span Lower implantation rate (hamster) Low survival of pups (hamster - F2)
In utero PCBs - AH R agonist	Same Greater effect on testis - smaller and low sperm	Same
In utero p,p DDE	Shortened anogenital distance at birth Reduced sex accessory gland size Hypospadias Retained nipples	None yet detected
Perinatal xeno-estrogens	Slight reduction in epi-sperm and testis size (5%) Sex organ size No known malformations	Pseudoprecocious puberty Shortened reproductive life span with loss of estrous cyclicity
Perinatal PCBs	Hypothyroidism leading to: Enlarged testis Hearing loss Central nervous system problems	Same central nervous system problems as male Same reproductive effects as prenatal PCBs

(1) *Defining endpoints of endocrine disruption that can be examined and tested in marine mam-*

mals. Carefully selected endpoints from the comprehensive list in Table 6 should be used to study the effects of EDCs on marine mammals. Studies should include controlled, longitudinal experiments using captive or semi-captive marine mammals, as well as strategic sampling of animals from wild populations (stranded, bycaught, or taken in hunts). Some endpoints can only be affected by *in utero* exposure, as developmental life stages are extremely sensitive to EDCs. Other endpoints are appropriate for both developmental and adult exposures. Elsewhere (under "Criteria for Selecting Animals for More Stringent Sampling," item 5 below), several key species are identified for extensive additional investigations. These species were selected because we have considerable information on their reproductive biology and demographics, and therefore subtle endocrine alterations could be detected and evaluated. For these species, we highly recommend that additional endpoints be used, depending on the hypothesis and study design. Extensive use of the endpoints listed in Table 6 may be warranted on a case-by-case basis, dictated by the nature of the problem.

The endpoints described here include morphological, histological, biochemical, gravimetri c, morphometric, endocrine, and behavioral assessments. Those specifically altered by *in utero* exposure, but that are assessed after birth or during adulthood, are indicated by an asterisk. Multiple endpoints measured on a target organ are more valuable than a single endpoint examined in isolation. This is because a comprehensive evaluation provides a "weight of evidence," indicating that an observed effect is not spurious and is very likely the result of contaminant exposure. Moreover, as two endpoints never provide exactly the same information, examination of multiple endpoints strengthens our understanding of the effects of a specific contaminant on the reproductive system. Note that several of the endpoints listed in Table 6 need to be standardized and validated for use with the marine mammal under investigation. Comprehensive necropsy of animals and an added emphasis on collection of fetal contaminant data would help reduce uncertainty about the effects of EDCs during the developmental life stages of marine mammals.

(2) *Development of new assays*. The following *in vitro* cellular and molecular assays need to be developed for selected species of marine mammals: (1) isolation and cloning of steroid and marine mammal nuclear receptors and an appropriate luciferase reporter construct; and (2) identification of cellular and molecular biomarkers indicative of exposure to EDCs during development, with emphasis on abnormal gene expression in reproductive tissues. (Some existing assays used with non-marine mammals may be suitable, especially for estrogens, androgens, and thyroid hormones.)

(3) *Maternal/fetal pharmacokinetics: recommendations for expanded sampling of target tissues for contaminants*. Most tissue-contaminant analyses in marine mammals have measured levels of organochlorines in blubber, and to a much lesser extent blood and milk. Because chemical exposures during development are of particular concern, there is a need to learn more about the toxicokinetics of the transfer of contaminants between maternal and fetal tissues. This could be studied experimentally in selected species, allowing predictions of exposure in various fetal target tissues during gestation.

Expanded sampling of fetal and neonatal tissues from bycaught, stranded, or hunted marine mammals would improve knowledge of gestational exposure. Of particular value would be paired sampling of mothers and their offspring (e.g., when pregnant females are found stranded, or killed in bycatch or hunts). Additional tissues to collect for contaminant analysis in adults (in addition to blood, blubber, and milk) include liver, brain, gonadal, kidney, and placental tissues. In fetuses and neonates, recommended tissues for contaminant analysis include brain, liver, blood, muscle, blubber, and gonadal tissues. The contaminant levels from various target tissues would be very useful to guide the dose ranges used for mechanistic studies with

surrogate species (such as laboratory rats) in a controlled experimental setting.

(4) *Opportunities afforded by existing marine mammal research programs*. The infrastructure and trained personnel exist for necropsy and live-capture-release collections through the Marine Mammal Health and Stranding Response Program (MMHSRP). The MMHSRP coordinates a national stranding program that uses volunteers to monitor mortality events, collect baseline data from strandings, and collect specimens from stranded animals for scientific investigations. In addition, the MMHSRP coordinates a biomonitoring program that collects specimens for health and contaminant monitoring or investigations through hunting, fishery bycatch, unusual mortality events, and mass strandings. The program also provides training for specimen collection to support forensic investigations and stranding response. The program has relied on partnerships with non-government and other government agencies and on a network of trained personnel and volunteers who follow well-established protocols for specimen collection. This network provides access to a large number and a great diversity of samples.

In addition to the MMHSRP, there are archived specimens and ongoing programs at oceanaria and other facilities. These can supplement the tissues, fluids, and biological data available from the MMHSRP and thus enhance support for the research needs outlined above. Marine mammal cell lines have been developed from several species and from several organs, including kidney, epidermis, liver, and lymph gland. Further development of cell lines is underway.

(5) *Criteria for selecting animals for more stringent sampling*. The following criteria should be taken into account in selecting animals for more stringent sampling: (1) focus on an area where trained personnel are readily available; (2) examine animals with a range of health conditions; (3) examine animals with a range of ages and sexes; (4) examine animals that occupy a gradient of habitats (i.e., from relatively pristine to highly polluted); (5) consider the feasibility of obtaining quality samples, in terms of both freshness and quantity; and (6) choose species for which there is adequate information on reproductive physiology.

Based on these criteria, we propose the following as index or model species: (1) Phocidae, harbor seal; (2) Otariidae, sea lions (northern and California); (3) Odontoceti, harbor porpoise, bottlenose dolphin, and beluga whale; (4) Mysticeti, bowhead whale and minke whale; (5) Sirenia, Florida manatee. Additional potential exists for using sea otters, walruses, and polar bears.

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Working Group on Risk Assessment

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Introduction and Overview

Marine mammals are exposed to a complex array of environmental contaminants, many of which are persistent, fat-soluble, and accumulate through the food web. Understanding the risks that these chemicals pose to marine mammals is not easy. It requires a systematic approach involving extrapolation from model species and laboratory experiments. Risk assessment is considered here in the context of the formal evaluation process established by the U.S. Environmental Protection Agency (EPA). In the EPA's Ecological Risk Assessment Framework, the risk assessment is a necessary step preceding risk management. A key component of the framework is an iterative approach between risk assessment and risk management, which ensures discussion between the risk assessors and risk managers as information is developed (Figure 1). This process begins with problem formulation: contaminant sources, fates, and pathways are described; contaminants of concern and their modes of action are discussed; and receptors of concern are identified. One outcome of problem formulation is a conceptual model that, in this instance, describes the ways in which marine mammals or their tissues might be exposed to and affected by contaminants. A second outcome is identification and assessment of endpoints that are explicit expressions of attributes that should be evaluated and protected. Typically, a risk assessment describes specific hypotheses to be tested and measurements that can be made in the analysis phase to evaluate the assessment endpoints of concern.

Exposure assessments are conducted to evaluate contact with particular contaminants of concern. Contact often comes about through ingestion, and exposure is evaluated through measurements of body burden. Information for exposure assessment can be derived from available databases or through directed sampling. Databases for descriptions of exposure are expanding internationally. The EPA and the National Toxicology Program have created a vast database on chemical production and use. They are in the process of setting priorities for testing and screening for endocrine disruption the tens of thousands of chemicals presently used in the United States. Production volume, use, persistence, and environmental and human exposure are being considered, along with toxicological information that is already available about a small fraction of chemicals in use. In related efforts, the EPA is collaborating with the Organization for Economic Cooperation and Development (OECD) and the Japanese Environment Agency to develop common methodologies and to apportion screening and testing responsibilities.

Effects assessments can involve direct measurements of effects on the animals of interest or the modeling of effects using measurements obtained from surrogate species. As indicated in Figure 2, the interplay of controlled laboratory or captive studies and field correlative studies can be crucial to providing the requisite information on effects. Laboratory studies might include *in vitro* experiments, or exposure trials using single chemicals or complex mixtures in laboratory animals. The selection of a surrogate model or set of models for marine mammals will depend on the nature of the question being posed (e.g., whether it relates to reproduction, immunotoxicology, pharmacology, or the potential for endocrine system disruption). Researchers should consider in advance how strong the inferences about a marine mammal species will be when based on comparisons (extrapolations) from observed processes or effects in the model species. Laboratory rodents have been used extensively in studies of human toxicology and pharmacology. Work with surrogate models can generate mechanistic information that is badly needed re-

(Figure 1)

(Figure 2)

garding toxicity, and can complement controlled studies of captive marine mammals or more ecologically relevant field studies of marine mammals.

Risk characterization uses the information from exposure and effects assessment to evaluate the risk, generally using a "weight of evidence" approach. This approach balances the information on effects, obtained mainly or entirely from the mechanistic understanding provided by laboratory studies (often associated with high levels of confidence), against the results of captive-animal or field studies which have greater ecological relevance but are often associated with lower levels of confidence (Fig. 3). Uncertainty in all assessment components should be clearly discussed so that the uncertainty in conclusions about risk is understood. The ecological significance of the risk should also be discussed, including how effects on individuals have been extrapolated to effects on populations and how risks from contamination have been compared to risks from other stressors. The ideal outcome of risk characterization is identification of threshold concentrations, above which risk becomes significant and risk management is required.

The working group examined the risk assessment approach relative to marine mammals and persistent ocean contaminants. The following four elements of this approach were evaluated by separate subgroups: (1) risk characterization; (2) important factors that determine exposure; (3) measuring adverse effects in marine mammals; and (4) surrogate approaches to assessing the effects of contaminants on marine mammals. A fifth subgroup examined a case study and performed the initial stages of a risk assessment on the California sea lion. (The purpose of the latter exercise was to use the risk assessment framework to explore a case example with a fair amount of background information readily available, not necessarily because of any current critical population problems.) The results of these efforts are presented on the following pages. Below is a list of overall recommendations from the full working group.

Overall Recommendations

(1) Integration of Field and Laboratory Studies. Field studies and controlled-exposure experiments are valuable tools for determining cause-and-effect relationships between environmental contaminants and adverse effects. In the case of marine mammals, both approaches have limitations, and it is unreasonable to expect that one approach alone will be sufficient to determine whether cause-and-effect links exist. Progress in this area will be made most rapidly and cost effectively by integrating the two approaches.

The major difficulties of conducting field studies and controlled-exposure experiments with marine mammals are (1) marine mammals are exposed in the wild to complex contaminant mixtures, (2) controlled-exposure studies with marine mammals in captivity are logistically difficult, prohibitively expensive, and have ethical aspects that must be carefully considered, and (3) by themselves, only very extensive field or laboratory studies could prove a cause-and-effect relationship.

We recommend an integrated approach, as outlined in Figure 4. A similar approach has proven successful in studies of the effects of contaminants on wildlife in the Great Lakes region (Giesy et al. 1994). Extensive field studies are conducted to provide evidence of adverse effects in the species of concern. These studies must involve some analytical work to provide correlative evidence of the involvement of specific chemicals or chemical classes. Extracts containing the chemicals of concern are used to expose a bank of likely surrogate species. The surrogate species chosen will include easily manipulated laboratory species, phylogenetically allied species, or even *in vitro* cell cultures. The key to selecting appropriate surrogate species will be the observation of the same or homologous effects as observed in the wild population. The effects of interest could range from biochemical responses to complex biological responses such as immunotoxicity.

Figure 3. Extrapolation in risk assessment: the "weight of evidence." (Adapted from Ross 1999)

Once a suitable surrogate model species has been chosen, the following procedures can be used to identify the chemical agents responsible for the (observed effect. The causative chemical agents are identified by a series of fractionation procedures which are monitored using the surrogate model species. Successive fractionation procedures provide a mixture containing fewer and fewer chemicals, which is eventually amenable to analytical characterization of all components. At the end of this process, the agents which caused the adverse effect in the surrogate species have been identified. To confirm the cause-and-effect relationship, the chemical agent or mixture can be reconstituted from pure chemicals. This agent or mixture should then cause the same adverse effect in the surrogate species. If required, the same reconstituted mixture can be applied to the initial target species of concern to confirm the cause-and-effect relationship inferred from the original field studies.

Advantages of this approach include abilities to (1) handle complex mixtures and identify causative agents; (2) select the most appropriate surrogate model species for the field species and endpoint of concern; (3) make a "weight of evidence" decision at various stages; (4) eliminate the final target-species testing if adequate weight of evidence is provided; and (5) cope with various chemical classes based on the methods and tissues used to prepare the crude extract.
(Figure 4)

(2) Standardization of Protocols for Sampling and Analyses. To insure comparability of data from a variety of studies, a basic set of standardized protocols should be developed for sampling and analyses. These protocols should be applied to targeted sampling projects, and they should be expanded with the goal of achieving international acceptance and application. It is recommended that an explicit list of samples be developed to allow optimal comparability across studies, but this list should be limited to those protocols which are feasible under field conditions, and are not unduly burdensome. Reporting procedures should be standardized and refer to total body burdens of chemicals (taking into account variable blubber thickness) rather than simply chemical concentrations. Reports should present data from individual animals, not summary statistics alone.

(3) Development of Biomarkers to Aid in Validation of Risk Assessment. The current suite of biomarkers for contaminant exposure must be systematically examined and applied whenever useful to marine mammals. However, we urge research into the development of sensitive, rapid, inexpensive, and field-adaptable biomarkers. Many procedures currently necessary for biomarker or contaminant samples are difficult to conduct in field conditions. Furthermore, the assay costs can be restrictive. The net result is that the correct samples may not be taken and when they are, may never be analyzed. If biomarkers were available for tissue or blood samples that could be easily obtained, stored, transported, and assayed, this would significantly increase our ability to assess contaminant impacts in the field.

(4) Study Designs That Allow for Increased Confidence and Reduced Uncertainties. Study designs should take into consideration the required statistical power, should present clear hypotheses, should take into account life history and environmental variables (e.g., age, sex, season, etc.) and should include controls whenever possible.

(5) Use of a "Weight of Evidence" Approach in Reaching Decisions for Risk Management. Risk characterization incorporates data from well-designed field studies, laboratory analyses, surrogate animal models, *in vitro* experiments, biomarker studies, and other sources. The weight of evidence should be considered in reaching risk-management decisions. In the absence of conclusive data, a precautionary approach is recommended.

(6) Additional Information on Dose-Response Relationships. We endorse the EPA risk assessment approach. Based on it, we encourage the design and implementation of studies that quantify relationships between contaminants and marine mammals, either directly or through laboratory studies of models or surrogates. There is a need to identify critical uncertainties, leading to specific research programs for characterizing the risk posed by persistent ocean pollutants.

Risk Characterization Subgroup Report

Subgroup Members: Aguirre, DeMaster, Harwood (Co-Rapporteur), Matta (Co-Rapporteur), Reif

Issue Title: Risk Characterization

Issue Description and Rationale:

Risk characterization considers estimates of exposure along with information about responses to contaminants to provide an indication of the expected effect(s) of the contaminants on an individual or a population. This risk-evaluation process is a fundamental component of the EPA ecological risk-assessment framework. To the best of our knowledge, it has not been applied to any marine mammal population.

Although this report is specifically concerned with identifying the types of information required for risk characterization, it also discusses actions required to resolve some of the issues that can complicate the application of a risk-assessment approach to ecological problems. These include the role of episodic or catastrophic events, the effects of other environmental stressors, and issues of morbidity and chronic effects as they affect estimation of the risk from toxic chemicals.

Means to Resolve the Critical Uncertainties Surrounding the Issue

Risk Integration for Endangered and Depleted Species. The probability that an individual will be affected by a particular contaminant can be estimated from a knowledge of the exposure to which each individual is subjected and the response associated with each level of exposure. In other words, we need to know the statistical distribution of exposures and the precise form of the dose-response curve. The ways by which this information can be obtained are described elsewhere in this working group's report.

For populations of endangered species, the death of even a single individual can influence the survival prospects of the population. Therefore, it is sufficient to estimate a feasible range for the number of individuals which may be affected by the contaminant in question, and to decide whether this potential loss is acceptable. As part of the process, it must be recognized that the dynamics of small populations have unique features which are a direct consequence of their small size. These features are a result of the population's susceptibility to stochastic effects, inbreeding depression, etc. Population viability analysis is an approach specifically designed to incorporate these factors into an evaluation of a population's potential to persist over a given period of time.

Parameterizing the effects of contaminants in models of depleted populations requires information on the induced changes in the age-specific rates of survival and reproduction. Recently developed mark-recapture techniques, using longitudinal data sets, are remarkably good at producing acceptably precise estimates of survival. These techniques can be modified to incorporate information on co-variates to allow for the evaluation of survival, conditional on a specific value for a given co-variate. Contaminant levels can be such co-variates. A similar approach could be used to estimate the impacts of contaminants on reproduction. The key to such analyses is the availability of long-term data on marked or individually identifiable animals.

Unfortunately, efforts to parameterize the effects of contaminants in population models for marine mammals based solely on current trends in abundance are likely to be unsuccessful. This is because of the relatively long times needed to estimate current trends in abundance for marine mammal populations. Further, given the almost complete absence of quantifiable information on the mechanisms that regulate the growth of marine mammal populations, attempts to attribute changes in the population growth rate to contaminant effects are likely to be confounded by density-dependent responses. Because of these difficulties, it will usually be impossible to accurately predict the level of effect due to a contaminant that would cause a specified change in the size of a marine mammal population over a particular time interval.

The Role of Episodic or Catastrophic Events. It

is often suggested that the negative effects of contaminant exposure are likely to be most obvious when a population is under stress during episodic or catastrophic events such as epizootics, blooms of toxic algae, or El Niño events. Such events can provide particularly good opportunities for detecting the role of contaminants. A comparison between the distribution of contaminant burdens in animals which die during the event and the distribution before the event can provide some information on the form of the dose-response curve. Thus, it is important to have good baseline information on populations believed to be at risk from such events, and to have in place a protocol and logistic support for adequate sample collection during the event. Although the impact of such events on a population may appear severe, the long-term impact depends on the interval between them.

Other Environmental Stressors. Even if a risk assessment indicates that a population of marine mammals is likely to be negatively affected by exposure to contaminants, the effect may be relatively small in comparison to the effects of other stressors (e.g., environmental change, disturbance, or fishery interactions). Indeed, changes over time in these other stressors may make it particularly difficult to detect the effects of contaminants against a background of high variability in survival and fecundity. It is therefore important to document the effects of these stressors on survival and fecundity. Networks, such as the Marine Mammal Health and Stranding Response Program, that attempt to determine the cause(s) of death of animals found dead, are an important component of this process.

Chronic and Sub-Lethal Effects. Chronic and sub-lethal effects attributed to contaminants will not normally have an immediate effect on the dynamics of an affected population. However, they can provide an important indication that more acute effects may occur at higher levels of exposure. Provided that there is agreement on what constitutes an unacceptable level of occurrence for such effects, and that there is enough information to derive a dose-response relationship, these effects can be used as endpoints for risk assessment.

Suggestions Regarding Existing Monitoring and Research Programs

Programs that integrate laboratory and field studies in order to determine exposure and response to contaminants, as recommended elsewhere in this report, need to be implemented before risk assessment can be carried out in a substantive way on most marine mammal populations. Programs to monitor survival and fecundity rates, as well as population size, for populations which may be affected by contaminants need to be established as soon as possible.

Exposure Subgroup Report

Subgroup Members: Grose, Harwood (Rapporteur), Jones, Stein

Issue Title: Important Factors That Determine Exposure

Issue Description and Rationale:

Characterizing exposure is fundamental to any risk assessment. However, available data on the concentrations of contaminants in specific marine mammal tissues cannot be used to estimate exposure directly. The challenge in measuring the exposure of marine mammals to specific contaminants is that most approaches used in conventional risk assessment are inapplicable.

The main routes of exposure for marine mammals are directly through food, by transmission from mothers to their offspring in gestation and lactation, by absorption through the skin, and by inhalation. Exposure can also be indirect in the sense that contaminants bioaccumulate in certain tissues (such as blubber), and are subsequently remobilized in response to metabolic demand. The most important route of exposure varies among contaminants and species. The timing of exposure, in terms of both the annual cycle and the species' life history, may be critically important in determining effect. All of these factors influence the degree to which individuals are exposed to contaminants on a daily basis.

Organic contaminants which are resistant to metabolism and are hydrophobic (such as dioxins, the organochlorine pesticides, and PCBs) will continue to bioaccumulate in the tissues of marine mammals. They are therefore a continuing cause for concern. PAHs and many industrially produced chemicals (e.g., insecticides, herbicides, fungicides, fire retardants, plasticizers, low molecular-weight polymers, and other unidentified high-volume compounds) are now ubiquitous in the marine environment. Some of them are metabolized by many organisms, especially vertebrates. There is less potential for bioaccumulation of these compounds. However, some of them or their metabolites are highly toxic, and their ingestion or inhalation by marine mammals would be a cause for concern. For such compounds, it may be very appropriate to measure biological markers of exposure. There is considerable uncertainty about the occurrence, pathways, and fate of many of these contaminants in the marine environment. This means that there is also uncertainty about the ways in which marine mammals may be exposed to them.

Marine mammals may be exposed to metals in both their inorganic and organic forms. By no means all of the exposure to these compounds is of anthropogenic origin. This is particularly true for cadmium, selenium, and mercury. In general, the organic forms of metals are more toxic, have higher potential to be more bio-available, and tend to accumulate more than the inorganic forms. The pathways by which these compounds reach marine mammals, and their toxicity, depend to a great extent on speciation and transformation within the animal.

Marine mammals are exposed to complex mixtures of all of the above contaminants. The combined effect can be assessed if the constituents of a mixture have the same mode of action at the physiological or biochemical level (e.g., estrogenici ty, or dioxin-like Ah receptor binding). Where this is the case, it should be possible to apply the toxic equivalency factor (TEF) approach developed for assessing the potential effects of dioxin-like compounds. The combined effect can then by estimated by summing the toxic contribution for each contaminant. However, if compounds in a mixture have more than one mode of action, then the potential for synergism or antagonism must be considered.

The ways in which contaminants are distributed in the marine environment depend on how they enter the system (e.g., from a point source or atmospherically), and on how they are transported through the system and deposited by physical and biological processes. Temporal and spatial overlap between contaminant distribution and the distribution of each marine mammal species governs the extent of exposure. Thus, better information on

sources and paths of distribution is essential if past and future levels of exposure are to be assessed. Another factor that introduces uncertainty into risk assessments is the variability in sample collection, analytical techniques, and the ways in which data are presented and summarized.

Means to Resolve the Critical Uncertainties Surrounding the Issue

(1) Use Models Derived from Other Species. The mechanisms by which metals and non-accumulating organic compounds are taken up and distributed by marine mammals are expected to follow the same principles as observed in other mammals. Thus, models derived from other species should be directly applicable to marine mammals, although validation of this assumption will be necessary in at least some contexts.

A unique physiological characteristic of marine mammals is the presence of a large blubber layer. The dynamics of lipids in marine mammals markedly affect the distribution of hydrophobic organic contaminants. However, lipid dynamics have little influence on the distribution of inorganic forms of metals. The distribution of metals is primarily determined by mechanisms that operate in organs such as the liver and kidney. Therefore, disposition and biotransformation of metals in other mammalian species should be directly applicable to marine mammals. Species-specific differences may exist, however, so that comparative physiological and toxicological studies may be necessary to assure the extent of direct application to marine mammals.

(2) Estimate Population Exposure. Average population exposure to accumulating organic compounds can be estimated from the distribution of total burden of each contaminant per animal in specific age and sex categories.

An expression for the expected total burden of a particular contaminant in the depot tissues, such as blubber, of an individual can be derived. It involves the average exposure to that contaminant, the statistical distribution of the contaminant in prey, and the rate at which the contaminant is metabolized or excreted. This expression can be used to describe the expected distribution of contaminant burdens in a particular section of the population (e.g., all sub-adults, all adult males, all mature females). The expected distribution can then be fitted to observed distributions in order to estimate the average exposure for the population. Unfortunately, most published reports only document the concentration of contaminants in blubber (often on a lipid weight basis). An estimate of the total quantity of blubber carried by the animal is required if total body burden is to be estimated. This additional information is available for some populations (e.g., harbor seals and gray seals in the North Sea). Although muscle tissue is typically much lower in lipids than blubber, it constitutes a major proportion of body mass, and therefore in some instances contaminant burdens in the lipid fraction of muscle may be necessary to enter into estimations of total body burdens.

(3) Increase Knowledge about Lipid Dynamics and Blubber Physiology. Physiological condition affects the distribution of accumulating organic compounds within an animal's body. Further research on lipid dynamics is required if the processes determining this distribution are to be understood (i.e., how do lipid dynamics and physiological demands affect circulating levels of lipid and contaminants?). The lipid stores in a marine mammal's blubber represent a reservoir for the storage of lipophilic contaminants. Depletion of this lipid reservoir due to fasting, starvation, or lactation can lead to release of contaminants into the blood stream and delivery of these contaminants to sites of action. As with the metal contaminants mentioned above, it is assumed that adverse contaminant effects at sites of action can be predicted from contaminant concentrations in the blood. Therefore, studies which relate adverse effects to blood contaminant concentrations in other mammal species will, in general, be predictive of adverse effects in marine mammals. Exceptions may arise when there are class- or species-specific differences in particular cellular receptors (e.g., Ah-receptor), resulting in variations in species sensitivity to individual contaminants. In these situations, *in vitro* studies can

help determine the extent of variation between species.

The dynamic between accumulation from food sources and storage, and release from blubber reserves represents a buffering system for contaminant concentrations in the blood. As such, it is a critical factor affecting circulating levels of contaminants. Information already available on contaminant levels in blubber would have greater value if we had a better understanding of how blubber dynamics affect blood levels of bioaccumulative organic contaminants. The relationship between blubber and blood contaminant concentrations is not adequately understood. Information about this relationship is best obtained by parallel analysis of blubber and blood samples from animals whose "condition" is assessed by measuring blubber thickness and lipid content. Suitable subjects for such studies would include animals sampled in catch-and-release programs, animals taken as bycatch in fisheries, animals being rehabilitated after stranding, and animals held captive under controlled experimentation.

(4) Determine Mechanisms of Action and Target Sites. Interactions of contaminants at target sites will determine their biologically effective dose. The nature of these interactions is unlikely to be unique to marine mammals. Therefore, information from studies of other mammals (including humans) should be directly applicable.

For complex mixtures of chemicals operating through the same mode of action, an additive TEF mechanism has been extensively validated. As the same mechanisms are expected to operate in marine mammals, this approach can be used for these chemical mixtures. Some validation of TEF values using *in vitro* techniques will be required. The possibility exists of interactions between toxic processes (e.g., similar to drug interactions). The nature and consequences of these interactions need to be assessed with model species in laboratory tests before their impacts on marine mammals can be considered.

The measurement of concentrations of a contaminant in tissues will not always provide sufficient information to assess the dose that would be toxicologically significant, referred to as the biologically effective dose. For example, some metabolites (e.g., DDT methylsulphones, hydroxy-PCBs) of bioaccumulative compounds are considered toxic. Moreover, for non-bioaccumulating contaminants, measurement of the interaction of the contaminant or its metabolite with a target site or receptor (e.g., DNA, acetylcholinesterase) is necessary to determine the toxicologically relevant dose.

In marine mammals, nondestructive sampling is often the preferred approach for collecting exposure data, particularly in free-ranging animals. However, samples that can be collected non-destructively are often not from the target site for many of the contaminants of concern. This situation is not unique to marine mammals. Studies with humans have the same considerations, and the literature on relevant studies should be directly applicable. The primary issue of concern is the validation of a measurement in a tissue, such as blood, as a reliable surrogate for a target site in an internal organ. Validation studies in marine mammals are likely necessary to increase the value of non-destructive samples as surrogates for target sites/organs in marine mammals.

(5) Augment Sampling with Information from Multiple Sources of Animals. The most useful information on exposure is likely to come from free-ranging or rehabilitated animals. This will include samples from by-caught and hunted animals. Samples from stranded animals will be most useful for investigating exposure associated with episodic events or for augmenting studies based primarily on free-ranging animals.

Detailed information on circulating levels of contaminants is required if exposure is to be determined reliably. Such information is best obtained from free-ranging animals using biopsy sampling or similar techniques. However, there are limits to the type and quantity of material that can be collected in this way, and results from this kind of sampling program need to be augmented with samples from animals which are still alive or have only recently died. The best sources of such samples are rehabili-

tated, by-caught, and hunted animals. Samples from stranded animals can be informative, particularly when episodic events result in large-scale mortality. However, it should be recognized that the origin of stranded animals is often difficult to determine, and that tissue samples from such animals can be in poor condition.

(6) Develop an Internationally Accepted Protocol for Sampling and Analysis in Relation to Exposure. This should ensure standardization of basic data collection and, wherever possible, the collection of a full set of complementary information on age, sex, "condition," body composition, stomach contents, and environmental conditions. A number of models for this protocol already exist (e.g., HARMS, IWC recommendations). It is desirable that this protocol be organized in a hierarchical fashion, with samples assigned to high, medium, and low priorities. In addition, the protocol should contain a Quality Assurance (QA)/Quality Control (QC) component to ensure comparability of analytical results. This component should include analysis of blind control samples, exchange of samples between participating laboratories, and, where possible, banking of samples for future analysis. It would be desirable to provide access to the data sets produced through the application of this protocol, either directly or in spreadsheet format, over the Internet.

(7) Develop Information on Dietary Uptake of Contaminants. While information on diet and feeding distribution is not essential for determining current exposure for coastal species, it may provide the only basis for estimating exposure for pelagic species. In addition, such information is essential for a full risk assessment and for understanding the implications of episodic events. In principle, the uptake of contaminants by a population can be estimated from data on (1) diet and feeding distribution; (2) the contaminant burden of each prey species (and the way this varies in time and space); and (3) the efficiency with which the contaminants are absorbed by the species. However, sufficiently detailed information on diet and prey is rarely, if ever, available for marine mammals. Historically, coastal species have been well-studied compared to pelagic species, which are far less accessible. Determining exposure levels for pelagic populations will require extrapolation from similar coastal species, and modeling uptake from food sources. A better understanding of the movements and feeding patterns of pelagic species is also required.

Suggestions Regarding Existing Monitoring and Research Programs

Future sampling and monitoring efforts in major national and international programs should give higher priority to obtaining samples from free-ranging animals, particularly those whose age and reproductive history are known; rehabilitated animals; animals killed incidentally in fisheries (bycatch); and animals taken in directed hunting where this occurs. In addition, there is a need to collect information on body condition and stomach contents (both prey composition and levels of contaminants present) whenever possible. Increased coordination among existing projects should be encouraged, and historical data on contaminant levels in individual animals need to be made more widely available.

The Marine Mammal Health and Stranding Response Program of the National Marine Fisheries Service (NMFS) provides the infrastructure in the United States to determine exposure parameters in marine mammals. This program should be encouraged to incorporate studies or projects to measure contaminant burdens (total body burdens) in prey of marine mammals. The coordination of such studies or projects with potentially complementary ones conducted through the NMFS Office of Habitat Conservation, NMFS Science Centers, or relevant Department of the Interior agencies would enhance the efficiency of collecting data on prey species. Programs intended to monitor contaminant levels in marine mammals and their prey should incorporate assessments of trophic structure. Among the methods that could be used are those involving stable isotopes and fatty-acid profiles.

The Marine Mammal Health and Stranding Response Program has made substantial progress in developing QA protocols in the United States.

Efforts at international coordination in QA for sample collection, analytical methods, and data archiving are strongly encouraged. Such coordination would facilitate comparisons of data from a wider range of geographic areas and thus from animals with differing types and amounts of contaminants. These comparisons would, in turn, improve our ability to assess relationships between doses of exposure and observed biological effects and anomalies.

Measurement of Adverse Effects Subgroup Report

Subgroup Members: Castellini, Hansen, Wells (Rapporteur)

Issue Title: Measuring Adverse Effects in Marine Mammals

Issue Description and Rationale:

Risk assessment for marine mammals relative to contaminants will require integrated programs involving field and captive studies with marine mammals as well as laboratory studies using surrogate or model species. Controlled studies and experiments are needed to strengthen the correlative data that are already available. The following topics are of critical concern and therefore need to be addressed as quickly as possible.

Identification of Biological Responses. An organism responds to any external stressor or event in one of three ways: by adapting to exposure and compensating in some way; by removing itself from the area of exposure and thus eliminating the stress; or, ultimately, by experiencing the negative effects of the exposure. Multiple levels of response are possible, involving behavioral changes, physiological reactions, alterations in reproductive processes, and impacts on individual and population health. When applying contaminant models to individual animals, it is critical to establish levels of threshold response, response curves, reactions that are appropriate to the insult but not damaging, and responses that indicate damage to the animal. For example, the production of metallothionein (MT) is the appropriate response to increased metal exposure. The MT is used by the animal's system to chelate out the circulating metal and sequester the metal-protein complex in organs such as the liver or kidney. Thus, the presence of MT is an excellent biomarker for exposure to metals, but it also indicates that the animal's body has reacted appropriately to protect itself from high circulating metal levels.

Definition of Quantitative Relationships Between Contaminants and Biological Responses. To characterize exposure and biological responses to contaminants for any species, well-controlled experimental studies are a powerful tool. In the laboratory, the test species is exposed to varying doses of the chemical(s) in question. The animal's responses can be quantified, but they will depend on controlled variables, such as sex, age, stage of development, physical condition, the type and mixture of the chemical, environmental conditions, etc. In the field, test animals can be selected based on estimated or known exposure to the chemical(s) in question. While field studies are not as easily or thoroughly controlled as laboratory studies, they provide the advantage of involving animal subjects that are faced with a natural suite of stressors. A combination of laboratory and field techniques is used routinely in contaminant-wildlife studies, but has not been widely applied to marine mammals. It can be useful for establishing mechanistic links between contaminant chemistry and biological response.

As noted above with metallothioneins, biomarkers are useful for demonstrating that exposure to some sort of insult has caused a physiological response in the animal. Thus, biomarkers can be useful tools for addressing contaminant problems, provided, however, that the pathways between the exposure and the biochemical or physiological response being measured are well known. Understanding the mechanisms of biomarker production is critical, but it must be possible to make at least three key distinctions, as follows: (1) the difference between markers that demonstrate exposure and those that show or predict effect. That is, some markers may simply indicate that a species has been exposed to a chemical; others may predict responses not yet expressed by the organism or its offspring; and, finally, some may be the by-product of damage induced by the chemical; (2) the difference between persistent and ephemeral contaminants. Some chemicals may be extremely dangerous but very short-lived. If biomarkers could be developed for these chemicals, then their past presence could be verified even if the chemicals themselves have disappeared; and (3) the difference between single-

and multiple-chemical stressors. That is, any particular chemical may induce a particular biomarker event, but a mixture of chemicals may induce a distinctly different marker. Again, these would need to be investigated by means of carefully developed experimental studies or by extrapolation from other species and model systems.

Work with Marine Mammal Species That Are Difficult to Study. It is clear that certain marine mammal species that are at risk from contaminant exposure will never be adaptable to dose-response or controlled studies. For example, few offshore or large cetaceans can be studied with this type of approach. We urge a conservative risk-averse-management approach with these species. Under ideal circumstances, the goal is to study the species of interest directly. Since this will not be possible for many groups, model or surrogate species will need to become the focal points for contaminant studies.

Need for Adequate Statistical Power to Detect Effects. Regardless of whether a contaminant project is to be conducted in the laboratory or the field, it is essential that a statistical model be developed to determine the appropriate number of animals *before* the experimental protocol begins. It is of no benefit to study a sample that is too small for valid statistical results. Nor is there any benefit in sampling more animals than is necessary. In the laboratory, standard statistical methods of determining significance and power can be applied. In the field, it might prove necessary to adapt the mathematical models of epidemiology to understand the impacts of contaminants on whole groups of animals. This would involve stratification by age, sex, reproductive condition, season, location, etc.

An additional consideration is that the impacts of contaminants may be trans-generational. If this is known or suspected to be the case, the sampling scheme may require extended periods of time to allow for the long reproductive lives of individuals. Consideration should be given to conducting longitudinal studies of identified family lines.

Means to Resolve the Critical Uncertainties Surrounding the Issue

The "weight of evidence" concept has been useful in establishing the seriousness of concerns about contaminant risks in marine mammals. Controlled studies and experiments can be used to strengthen correlation data. Four broad types of study are identified and discussed below.

(1) Use of Captive and Free-Ranging Animals in Controlled Studies. The study of both captive and free-ranging animals is essential for quantifying contaminant effects in marine mammals. Non-lethal experiments on captive marine mammals allow for tightly controlled conditions; detailed longitudinal analyses; the use of animals that have been or are being rehabilitated; and, usually, ready access to sophisticated medical or research facilities. Diet, contaminant level, activity pattern, and a host of other variables can be controlled (including stress), making it possible to quantify effects accurately. In some cases, field studies can be longitudinal projects and allow for a compare-and-contrast scheme in which animals from highly contaminated areas are compared to animals from areas with low contaminant levels; individuals of different age, reproductive, or sex classes are compared; or the contaminant burdens of individuals can be tracked through time.

(2) Selection of Marine Mammal Model Species. As noted in the "Issue Description and Rationale" section above, it will probably never be possible to study many species of marine mammals in sufficient detail to make informed risk assessments on their behalf. Therefore, we need to identify species that can be studied, both for their own benefit and as models for less easily studied species. For example, historically, the most closely studied species have been those that are relatively easy to approach and that live near shore or haul out in accessible areas. Because of their proximity to human activities, these species also happen to be among those most at risk from contaminants. Thus, they need to be studied for their own sake and as models of animals exposed to high contaminant loads. The 1995 Bergen workshop of the International Whaling Commission recommended as model cetacean

species the bottlenose dolphin, beluga whale, harbor porpoise, and Amazon river dolphin. In addition to these, the identification of a model baleen whale species would be helpful in focusing field studies for this cetacean group. For pinnipeds, we recommend the harbor seal, California sea lion, elephant seals, fur seals, Weddell seal, and gray seal. These species, and others with large databases associated with them, can be studied in both captive and field conditions, sometimes close to human population centers. These species will allow detailed laboratory studies, field studies, and studies of individuals. Thus, they can be used to test hypotheses at both the individual and the population level.

(3) Development of Models of Lipid and Blubber Chemistry to Support Physiology-Based Pharmacokinetic Studies. Because many of the contaminants that affect marine mammals are lipophilic, they tend to be stored mainly in the blubber of pinnipeds and cetaceans. However, the ways in which lipid profiles in the blubber change during the year, by age or reproductive status or within the blubber layer itself, are poorly understood. Understanding of lipid and blubber chemistry must be improved if we wish to model contaminant pathways in marine mammals.

It is fortuitous that blubber biopsies provide data on many of the known xenobiotic compounds that raise health concerns. New contaminants that enter the oceanic system, however, may not be lipophilic. This would mean that blubber samples obtained from living cetaceans with a high-velocity dart and from pinnipeds with a simple needle are of no use for assays of these new contaminants. If a critical class of new compounds were muscle-specific, for example, how would samples be obtained? As new compounds are introduced to the marine environment, it will be necessary to determine their preferred sites of deposit so that effective and accurate sampling methods can be developed. As discussed previously, blood may be an appropriate tissue for sampling "short-lived" contaminants.

(4) Development of New Biomarkers for Rapid, Inexpensive, and Practical Screening of Response Biochemistry in Marine Mammals. The suite of biomarkers currently available for assessing contaminant exposure should be examined systematically and applied whenever useful to marine mammals. However, additional research is needed to develop sensitive, rapid, inexpensive, and field--adaptable biomarkers. Many of the procedures required for biomarker or contaminant samples are difficult to follow in field conditions. Furthermore, the assay costs can be restrictive. The net result is that the needed samples may not be taken, and when they are, they may never be analyzed. If biomarkers could be developed for tissue or blood samples that are easily obtained, stored, transported, and assayed, this would improve our ability to measure contaminant responses in non-laboratory situations.

Suggestions Regarding Existing Monitoring and Research Programs

Several existing research or monitoring programs can be used to address the issues and meet the needs identified above. Here we discuss six approaches that should be considered for enhancing the effectiveness of these programs with regard to measuring adverse effects of contaminants on marine mammals.

(1) Forge Collaborations between Marine Mammal Scientists and Ongoing Medical and Environmental Programs Designed to Quantify Contaminant Effects, Regardless of Whether These Involve Marine Species. The quantification of contaminant effects is well-established in the fields of medicine and environmental chemistry. Marine mammal scientists should be encouraged to establish collaborative links to programs in these fields. In the United States, such links could come from programs formally cosponsored by the National Institutes of Health or the Environmental Protection Agency and the Marine Mammal Commission or the National Marine Fisheries Service. Special training grants could be established for marine mammal scientists to work in appropriate medical or environmental laboratories. Numerous other avenues exist for developing collaborations, assuming that adequate support is made available by the relevant agencies involved in marine mammal

conservation.

(2) Develop Field Programs with Pinnipeds and Cetaceans That Involve Longitudinal Aspects and/or That Are Adaptable to Controls. Well-established field programs involving pinnipeds or cetaceans should be encouraged and supported to develop collaborative projects on contaminant issues. These programs offer the advantages of multi-year databases on recognizable individuals and/or access to large numbers of animals, whether through capture-release, salvage of bycatch, or ongoing directed killing operations.

For pinnipeds, some of the existing programs involve harbor seals, elephant seals, and fur seals worldwide, gray seals in the United Kingdom, California sea lions in the United States, and Weddell seals in the Antarctic. All of these projects include established infrastructure, long time-series of data, and reliable access. With regard to cetaceans, the bottlenose dolphin projects in the southeastern United States and at Moray Firth, Scotland, offer opportunities to work with free-ranging yet well-known individual animals. Both projects have good infrastructure, long time-series of data, and access. The beluga whale population in the St. Lawrence River of eastern Canada should be the subject of increased study. The bowhead and beluga whales hunted off the north coast of Alaska, minke whales taken in Japanese and Norwegian whaling, gray whales hunted in Russia, and the long-finned pilot whales killed in the Faroe Islands might be sampled through collaborative agreements with the appropriate hunting groups. These situations offer unique opportunities to obtain samples from animals that could be well used in contaminant studies.

(3) Use Captive Programs to Study Individual Marine Mammals in Detail. Collaborative research agreements should be established with facilities that maintain captive populations of marine mammals. Such agreements can facilitate in-depth studies of molecular biology, biochemistry, physiology, reproduction, behavior, and health of individual animals relative to contaminant exposure. The controlled environments in these facilities could provide opportunities for non-lethal experimentation and serial sampling over long time periods and, in some cases, across generations. Protocols should be established to take full advantage of specific events, e.g., sampling when an animal dies.

(4) Continue or Establish Marine Mammal Health and Stranding Programs in Which Large Numbers of Stranded or Rehabilitated Animals Can Be Examined. Programs involving the rehabilitation of stranded marine mammals should include research relevant to risk assessment. The animals in rehabilitation facilities represent a potentially valuable resource for studies of contaminant effects. Thus, allowance should be made for well-conceived studies using these animals. Animals in rehabilitation facilities might be used to identify health responses in situations where all potential risk factors other than contaminant exposure can be ruled out. Studies could be conducted in which contaminant loads and health are monitored as the animals are provided with "cleaner" food than is available in local waters. Consideration should be given to using animals in rehabilitation facilities particularly in circumstances where by-caught or hunted specimens are unavailable. Individuals that are fully rehabilitated would be preferable subjects for some studies; caution and careful design would be required to interpret results from animals that are seriously compromised by illness, injury, or acclimation to captivity.

(5) Focus the Sampling of Stranded Marine Mammals to Address Specific Research Questions. Stranded animals provide opportunities to sample a variety of tissues and collect data that can be used to evaluate contaminant effects. Currently, one of the major problems associated with sampling efforts is the lack of consistent protocols within and among the organizations that investigate strandings. All too frequently, the information collected is insufficient for interpreting contaminant data. Most of the organizations operate with inadequate funding, which limits sampling efforts and follow-up analyses. Responsible agencies should identify specific tissue needs, collection and handling protocols, necropsy procedures, and histopathological and toxicological analyses. Training in these

protocols should also be offered. In the United States, this effort could be coordinated through the NMFS Marine Mammal Health and Stranding Response Program. The effort should recognize and reflect the responsibilities of state agencies as well as U.S. federal agencies such as the U.S. Fish and Wildlife Service, the Environmental Protection Agency, and the National Oceanic and Atmospheric Administration.

(6) Standardize Protocols for Sampling Marine Mammals During Live-Capture Operations in Order to Facilitate Comparisons over Time and Across Study Sites. Live-capture operations provide unique opportunities to obtain samples from cross-sections of free-ranging populations. While specific research goals may differ from program to program, standardized protocols for collection and handling of a basic set of tissues for contaminant studies are needed to facilitate valid comparisons. In the United States, such standardization could be established and coordinated through the Marine Mammal Protection Act permit process (NMFS and U.S. Fish and Wildlife Service) and by coordination and planning meetings held by regional organizations.

Surrogate Approaches Subgroup Report

Subgroup Members: Colborn, Ross, Schwacke (Rapporteur)

Issue Title: Surrogate Approaches to Assessing Contaminant Effects on Marine Mammals

Issue Description and Rationale:

Why Is This Issue Important? Establishing cause-and-effect relationships between contaminants and marine mammal health or reproductive success is very difficult in the current legal and ethical paradigms. In the absence of conclusive studies on marine mammals themselves, the use of surrogate models can help overcome many of the uncertainties about such cause-and-effect relationships. Studies of surrogate models can provide insights about mechanism of action, the comparative risks presented by different chemicals, and dose-respon se relationships. In addition, control of experimental conditions makes it possible to eliminate confounding factors and reduce variability within treatment groups, thus providing the statistical power necessary to detect effects. The greater availability of species-specific reagents and the ability to carry out invasive studies represent additional advantages of using surrogate models. The selection of an appropriate surrogate model depends on the question being posed and the endpoint being assessed. For example, laboratory rodents may be useful for many endpoints, including immunotoxicolo gy, but a mustelid might be preferred for studies of delayed embryonic implantation in pinnipeds.

Surrogate studies and studies of marine mammals both need to draw upon the extensive mammalian toxicology literature. The usefulness of information generated from studies of surrogate models will depend upon certain assumptions and extrapolations. It is important to be aware of the assumptions and to critically evaluate the extrapolations at all stages of the research. While it is not necessary to address all unknown factors in surrogate study designs, it is essential to consider all of those that may affect extrapolation. For example, it is important to consider differences in exposure route, dose, metabolism, genetics, physiology, reproductive strategy, dietary composition, and sensitivity between a marine mammal in question and a surrogate species. Also, one needs to bear in mind the difference between chronic and acute toxicity. Ultimately, a combination of surrogate and marine mammal studies is needed to evaluate the risks from effects or potential effects of contaminants on free-ranging marine mammals. Sufficient information is not, and will not be, available solely from studies of marine mammals.

Evidence from both wildlife and laboratory studies identified a link between prenatal exposure to certain contaminants and urogenital development in males, thus flagging this issue for human health authorities. As a result, the Centers for Disease Control and Prevention (CDC) searched public health registries, hospital records, and other databases to estimate the incidence in the United States population of hypospadias, a visible impairment of the urogenital tract at birth in males. This condition is determined between gestation days 56 and 80 in humans and days 15 and 20 in the rat. The CDC reported a doubling of hypospadias in the U.S. male population between 1970 and 1990, to a 1990 rate of 1 in 125 male births (Paulozzi et al. 1997). This example supports the principle of using surrogate species to identify problems that might be present in marine mammal populations, but easily overlooked at the individual level.

What Is Generally Known about This Issue? Mammals are remarkably similar in many fundamental aspects of physiology. Their immune systems, endocrine systems, and reproductive physiology share many features. Evolutionary adaptation to different habitats has resulted in a great diversity of sizes and shapes within the mammals, but some of the physiological differences remain relatively minor. These include, for example, differences in cell-surface markers for leukocytes, or implantation strategies (e.g., direct vs. delayed implantation). Other differences may be more profound (e.g., in the organism's metabolic capacity to eliminate xenobiotics). Even these,

however, can be a question of degree rather than the presence or absence of a particular system. The similarities in physiological systems within mammals are greater than the dissimilarities, thereby allowing interspecies comparisons and providing a basis for the use of surrogate models. Assumptions about similarity have formed the basis for the wide use of rodent models in studying the potential toxicity of new chemicals and drugs to humans. Initial studies have shown that surrogate approaches can also provide toxicological information relevant to certain marine mammal issues

What, in General, Needs to Be Determined to Resolve the Critical Uncertainties? No single surrogate approach will be sufficient to explain the effects of the many types of contaminants present in environmental mixtures, or to elucidate the many types of responses to those effects on the part of organisms. Nor can surrogate studies be expected to entirely replace well-designed studies of captive or free-ranging marine mammals. Risk assessment for marine mammals will require a critical consideration of the accumulated evidence from different types of surrogate models (including humans), as well as studies of both captive and free-ranging marine mammals. Increased certainty about mechanistic insights from surrogate studies must be matched with increased ecological or "real world" relevance associated with studies of marine mammals. Using the "weight of evidence" from both types of studies, we will be better able to judge whether ambient levels of contaminants are affecting (or present a risk to) free-ranging marine mammals. We should also be better able to identify the chemical or class of chemicals which is having an observed effect on (or presents the greatest risk to) marine mammals.

Means to Resolve the Critical Uncertainties Surrounding the Issue

(1) Interspecies Extrapolation. The use of a surrogate species always assumes comparability of effects between a marine mammal species and the surrogate model in question. Extrapolation of results from laboratory studies to conclusions about effects on wild marine mammals requires critical assessment, although the uncertainty introduced by the extrapolation can be minimized through careful experimental design and selection of surrogate species. We have tabulated the more important sources of uncertainty and possible ways of resolving them (Table 7).

(2) Complex Mixtures. Ambient levels and patterns of contaminants in free-ranging marine mammals challenge efforts to understand the risk to these animals presented by specific chemicals. The use of surrogate models allows for the resolution of many critical uncertainties, with greater control over exposure regimes (chronic vs. acute; complex mixtures vs. simplified mixtures vs.single chemicals; comparability of TEFs among species). We have outlined in Table 8 the main sources of uncertainty and proposed means to resolve them.

(3) Mode of Action. Establishing the mechanisms, or modes, of action for contaminants in marine mammals is limited by ethical, logistical, and methodological considerations. Surrogate models allow for greater experimental control and levels of invasiveness which are required to understand the mechanism of action of a particular chemical or mixture of chemicals (Table 9).

What Are the Likely Consequences If This Issue Is Not Addressed?

The uncertainties surrounding this issue must be addressed in order to provide a credible evaluation of the effects of contaminants on marine mammals.

Table 7. Important sources of uncertainty in extrapolating results of studies from various other species to marine mammals, with potential means for their resolution

Source of Uncertainty	Proposed Means to Resolve
Differences in metabolic capacities to deal with contaminants	Explore the utility of physiology-based pharmacokinetic (PBPK) models to assess compartmentalization, elimination, etc., of contaminants in different species. Compare P-450 profiles in both surrogate and marine mammal species.
Comparability of mechanisms of action	Continue research to identify common mechanisms of action between laboratory species and marine mammal species. Examples: • Identification and characterization of *Ah* receptor in tissues of different species. • PCB metabolites and disruption of vitamin A, thyroid hormone and common carrier protein levels (e.g., in harbor seals and rats). • Common markers in reproductive endocrinology.
Comparability of physiological systems	Consider phylogeny, ecology, physiology, and available information for the proposed surrogate species prior to selection (e.g., delayed implantation as a reason for using mustelid instead of rat as surrogate for pinniped reproduction).
Validation of model species	Identify endpoints common to both surrogate and marine mammal species that may be predictive of additional effects which are impossible to measure in the marine mammal. Additional endpoints seen in the surrogate model will then provide grounds for speculation and guidance in the design of studies of free-ranging marine mammals.
Exposure regime	Consider duration of exposure, mode of exposure and lifestage(s) of subjects when designing experimental studies.

Risk assessment involves the determination of magnitude and probability of adverse effects related to contaminant exposure. Assignment of probability requires the quantification of uncertainty related to the estimation of effects, with decreased uncertainties resulting in more precise estimation of probability bounds. Because the ultimate goal of risk assessment is to provide the information necessary to make effective and informed risk management decisions, uncertainties must be minimized.

Table 8. Important sources of uncertainty in interpreting significance of exposure to complex mixtures of contaminants in marine mammals, with potential means for their resolution

Source of Uncertainty	Proposed Means to Resolve
Effect of complex mixture	Surrogate feeding studies (e.g., with laboratory rodents) using relevant mixtures with appropriate dietary and exposure controls.
Identification of chemical within mixture responsible for primary toxicity of that mixture	Identify evidence from existing studies, including laboratory and observational studies on terrestrial animals, fish-eating birds, or other relevant species. Perform single-chemical-class-exposure experiments with appropriate model species. Use TEFs to delineate relative risks of PCBs, PCDDs and PCDFs.
Possible synergistic or antagonistic interactions	Use simplified mixtures to explore and determine interactions (e.g., different combinations of PCB congeners, TBT with DDE, TBT with PCB126) in *in vitro* or laboratory-rodent studies.
Applicability of TEFs (are the largely rodent-based international TEF values applicable to marine mammals?)	Conduct comparative studies among wildlife and laboratory-animal species (e.g., using *in vitro* liver enzyme induction).

Ignoring the power of surrogate models, whether they be *in vitro* assays or experimental exposures of rodents to chemicals, will result in an endless debate about the hypothetical effects of food-chain contamination on marine mammals. If surrogate models are not used and existing information is ignored, we can expect a continued low level of confidence regarding effects; a low capacity to assess the chemicals with the greatest toxicities (greatest risk); a minimal understanding of mechanisms of action; an inability to assess risk to most cetaceans and to pelagic marine mammals generally (logistics limit sampling opportunities); and a diminished value of existing rodent literature. Failure to resolve the critical uncertainties outlined in the preceding section will result in a continued lack of confidence in our ability to assess effects, which will ultimately limit the usefulness of risk assessment altogether.

Suggestions Regarding Existing Monitoring and Research Programs

(1) Study Design in Assessing Effects of Complex Mixtures. There are lessons to be learned from existing studies on the effects of complex mixtures on animal groups other than marine mammals. Such studies may call attention to important contaminant issues, as was the case with DDT and the extirpation or near-extirpation of piscivorous birds from much of North America and Europe. Some of them may also provide examples of surrogate models for marine mammals. In a broad sense, these studies, or their methodological approaches, include

- Single-chemical laboratory-animal studies which identify problem chemicals and the effects of these chemicals, and which give a

Table 9. Important sources of uncertainty in determining mechanisms of action of contaminants in marine mammals, with potential means for their resolution

Source of Uncertainty	Proposed Means to Resolve
Most mechanisms of action are not well understood.	Conduct studies using *in vitro* exposures of cell cultures from marine mammals (e.g., tumor cell lines, kidney cell lines, peripheral blood cells). Conduct exposure studies using marine mammal immune cell-reconstituted Severe Combined Immune Deficient (SCID) mice. Share information from past or ongoing studies across agencies.

mechanistic foundation to other studies (e.g., on marine mammals).

- New techniques which help to identify the most potent contaminants in environmental mixtures, using a toxic equivalency approach (e.g., Ah- and estrogen-receptor mediated toxicities).
- Captive feeding studies or egg injection/egg painting studies in a laboratory setting (e.g., with mink, rats, fish-eating birds) based on environmentally relevant exposures.
- Field-based studies of free-ranging populations of animals which incorporate different levels of exposure (e.g., on river otters, fish-eating birds).
- Reviews of information already available on economically important animal groups (e.g., fish, ducks, other migratory species).
- Nationwide studies (e.g., in the United Kingdom or the United States) which address estrogenic activity in freshwater and coastal fishes.
- International research related to the issue of amphibian deformities and declines.
- The few important long-term monitoring studies of contaminants in sentinel species, such as the Herring Gull Monitoring Program in the Great Lakes.

(2) Institutional Issues Related to Assessing the Effects of Contaminants. In response to worldwide concern about the growing number of chemicals that are biologically active, the Environmental Protection Agency has a Congressional mandate to produce, by the year 2000, a set of *in vitro* and *in vivo* screens and assays to test synthetic chemicals for their possible endocrine-disrupting effects. This battery of tests will certainly not detect all of the possible mechanisms by which chemicals can interfere with development and reproduction. However, it is a start, and anyone designing models to predict negative effects of chemical exposure on marine mammals should follow closely the EPA's progress with this program.

Concurrent with the EPA initiative, an ad hoc interagency cooperative effort among the National Toxicology Program, the National Institutes of Environmental Health Sciences, the Food and Drug Administration, and EPA has developed a set of protocols for low-dose testing at physiologically and environmentally relevant ambient exposure levels. These multi-generational assays were designed to detect cryptic, long-term, or delayed effects on function. They include more sensitive endpoints than those used in traditional toxicology. In essence, this development is establishing a new discipline within the field of functional teratology. The new assays have revealed a number of developmental and reproductive effects of widely used

chemicals not previously reported.

There is a role for the National Science Foundation and other funding entities to support the basic research needed to determine where, when, and at what levels endogenous hormones control development and reproduction in marine mammals. This kind of information on laboratory animals provided insight into the fragility and sensitivity of the embryo and fetus, thus demonstrating the considerable utility of surrogate approaches to toxicology.

Case Study Subgroup Report

Subgroup Members: Aguirre, DeMaster, Matta (Rapporteur), Reif

Issue Title: Case Study — Contaminants in California Sea Lions off Southern California

Issue Description and Rationale:

This case study had two purposes. One was to illustrate how components of an ecological risk assessment approach can be used to evaluate the threat of persistent contaminants to marine mammals. The other was to identify critical uncertainties in available information. It was assumed that these uncertainties would become evident as we attempted to actually complete a risk assessment on a selected marine mammal species. The risk posed by organo chlorine contaminants to the California sea lion was chosen because this species has been relatively well studied and because risk-management decisions are actually pending with regard to contaminated sediments in the area. The case study follows the EPA ecological risk-assessment framework. It was developed using information available during the workshop and is therefore not complete.

Problem Formulation

Background. This risk assessment evaluates the potential ecological effects of DDT and PCBs on California sea lions. Beginning in the late 1960s, reproductive problems were observed at breeding colonies in southern California (De Long et al. 1973). At about the same time, information became available regarding the potential effects of DDT on reproduction, and the extent to which DDT was being discharged into the marine environment from local manufacturing sources. (In this case study, the term DDT refers to the sum of DDT and its metabolites, often referred to elsewhere as DDTs or EDDT). PCB concentrations mentioned here were generally quantified on the basis of common Aroclor mixtures. Unless otherwise specified, concentrations in sediments are stated in terms of dry weight, while tissue concentrations are expressed in terms of wet weight.

PCBs, DDT, and other contaminants were released into the marine environment of southern California primarily through sewage discharges along the Palos Verdes Shelf. These discharges began in 1937 and continue to the present. Large quantities of DDT were discharged through these outfalls as a result of pesticide manufacturing operations in the area between the late 1940s and the early 1970s (Mearns et al. 1991). PCBs from more than 7 sources in the Los Angeles area were also discharged through these outfalls and have resulted in widespread contamination. These contaminants associate with organic particles and persist for decades in the environment. According to Science Applications International Corporation (1998), discharges of these contaminants have declined dramatically over the last 30 years (from more than 100 tons per year of DDT in the late 1960s to 0.03 tons per year in 1985), but large areas of contaminated sediments remain. DDT concentrations in surface sediments currently exceed 1 ppm over 42 square kilometers of the Palos Verdes Shelf. Concentrations in sediment have declined since the 1970s by a factor of between 3 and 10, but concentrations of up to 91 ppm remain in surface sediments in some locations. PCB concentrations in sediments exceed 3 ppm around outfall discharges (Science Applications International Corporation 1998).

California sea lions in U.S. waters are managed by the National Marine Fisheries Service as a single population (i.e., stock) under the terms of the Marine Mammal Protection Act . Their population history and population dynamics are well known (Boveng 1988, Lowry et al. 1992, Barlow et al. 1997). Their numbers were severely reduced due to directed human management actions (i.e., intentional killing and bounty programs to reduce California sea lion numbers and promote the recovery of important commercial fisheries) late in the 19th century and early in the 20th century. The number of sea lions in California may have been as low as 5,000 animals at some point earlier in the present century. By mid-century, the recovery of the

population off California was well underway, with numbers reaching tens of thousands. Between 1984 and 1992, this population continued to increase at an annual rate of 5-8% per year. The current population likely includes 150,000 to 200,000 animals. Estimates of carrying capacity are not available for this species; therefore, it is not possible to specify the population level at which the net rate of increase will be zero (i.e., a stable population). It is very likely that the population rate of increase will decline during the next 25 years.

California sea lions have well-established breeding colonies in the California Channel Islands. They feed primarily on pelagic schooling fish (for example, anchovy, sardine, rockfish, and hake) and squid but are also known to eat a wide variety of other fish. Females and pups remain near the breeding grounds, where they presumably continue to be exposed to contaminants in food, while males can migrate long distances during the non-breeding season.

Contaminants of Concern. Although a number of contaminants are present in the marine environment of southern California (including trace elements and PAHs), this risk assessment focuses on DDT and PCBs. There is a large body of evidence that exposure to these contaminants is associated with adverse effects in a wide variety of species. Relevant information on the environmental chemistry, fate, and adverse effects is summarized in this section.

DDT and its degradation products, DDE and DDD, are not easily metabolized by microorganisms in the environment. DDT compounds are highly lipid-soluble, bind to organic matter in sediments, biomagnify, and persist for years in the environment. DDT compounds can be detected in filtered water samples, but are also associated with suspended particles. Depuration rates for DDT compounds are reported to be low, but they vary among species.

Although exposure to DDT can have acute lethal effects, sublethal chronic effects are much more common. Chronic effects of DDT and its metabolites include alterations in behavior and reproduction, which are thought to be the result of hormonal alterations, enzyme-system alterations, and alterations in calcium metabolism (Science Applications International Corporation 1998). DDT is known to disrupt reproduction in freshwater and marine fish, reducing fecundity, decreasing fertilization success, and reducing hatch success and larval survival (Hose and Cross 1994) at concentrations exceeding about 1 ppm (wet weight) in fish tissues. Reproductive problems in birds have also been associated with DDT exposure. DDT also has been associated with reduced immune-system function in fish (Science Applications International Corporation 1998). Within the past five years, it has been shown that DDE is a powerful anti-androgen (Kelce et al. 1995) and an anti-prostaglandin (Lundholm 1995, 1997). In humans, DDE has been associated with reduced milk production and a shortened lactation period at concentrations exceeding 3 ppm in maternal lipids (Rogan and Gladen 1985, Rogan et al. 1987, Gladen and Rogan 1995). PCBs can also adversely affect mammals (Safe 1994).

Each of the 209 PCB congeners differs in the number and position of chlorine atoms on the biphenyl ring, resulting in variable toxicity and persistence in biota. The relative persistence of PCBs generally increases with increasing chlorination. Compounds with few chlorines are thought to degrade rapidly. PCBs adsorb tightly to sediment particles and are lipid soluble. Some PCBs are metabolized by some species, and many are stored in lipid-rich tissues. Some PCB congeners can be excreted or eliminated during reproduction. They biomagnify and can be found at high concentrations in species at higher trophic levels.

Some PCB congeners can be acutely and directly toxic, but concerns about PCB toxicity are primarily related to chronic sublethal effects. PCBs are considered reproductive toxicants in fish, with reduced spawning success, reduced hatch success, and reduced larval survival. PCBs have also been associated with impaired immune-system function in fish and marine mammals. There is also recent evidence that some PCB metabolites could disrupt endocrine systems. Some PCB metabolites bind to the rat estrogen receptor (Korach et al. 1988).

Exposure to PCBs in combination with other contaminants has been associated with neoplasms in fish (Myers et al. 1998, Stehr et al. 1997, Varanasi et al. 1992). Johnson et al. (1998) found that *in utero* exposure to background levels of PCBs and their co-contaminants contribute to neurobehavioral and developmental deficits in newborns. These differences persist through school age.

Conceptual Model. (Pathways for contamination to reach target organisms: sediment Ö invertebrates Ö fish Ö sea lions.) Because California sea lions inhabit an area where sediments and fish are known to be contaminated with persistent, bioaccumulative organochlorine contaminants, the most significant pathway of exposure is likely through consumption of contaminated fish. Because the contaminants involved are known to disrupt reproductive- and immune-system function, and because there is recent evidence that these contaminants can cause effects such as cancer, the following assessment endpoints have been selected for this risk assessment:

- estimate risk of adverse reproductive outcomes in California sea lions due to contamination;
- estimate risk of cancer associated with exposure to contaminants in California sea lions;
- estimate risk of adverse immune-system effects in California sea lions due to contamination.

These assessment endpoints contain explicit expressions of attributes of the population of California sea lions that should be protected: reproductive output, survival, and immune-system function. They were selected based upon available information regarding the modes of action of the contaminants of concern. The three assessment endpoints can be evaluated using the following measurements: (1) direct measurements of adverse effects in California sea lions; (2) concentrations of contaminants in tissues of California sea lions; (3) concentrations of contaminants in fish and sediment from the area of concern; (4) threshold concentrations of contaminants found in laboratory or field studies to be associated with adverse effects (preferably from other pinniped species, but from other species such as rodents, if necessary and appropriate); and (5) if alternate species are used, creation of a food-web model to estimate the "dose" to sea lions to compare with data from experiments with other species.

A "weight of evidence" approach will be used to evaluate potential risk to California sea lions from organochlorine contaminants. Greatest weight will be given to actual measurements of adverse effects in these animals, if effects can be correlated with exposure concentrations. If actual measurements of adverse effects in California sea lions are not available, information from studies of other species can be used to predict the likelihood of adverse effects. These might include captive studies on other pinnipeds, or laboratory studies on rodents if diet, exposure route, metabolism, physiology, and reproductive strategy are considered sufficiently similar.

Exposure of California Sea Lions to PCBs and DDT

Two basic approaches are available to estimate exposure of California sea lions to PCBs and DDT. The first involves analyzing sea lion tissues from the area to determine body burdens and tissue residues. The second includes analysis of contaminants in prey and calculations of "doses" from expected food consumption.

Exposure data for anthropogenic contaminants in California sea lions are available from several sources. There are at least eight reports of contaminants sampled between 1969 and 1992, with the vast majority from California sites. Analyses have included primarily DDT, DDE, and PCBs from approximately 150 animals (O'Shea and Brownell 1998). Recently, additional data were collected from California sea lions that died after live stranding, including a subset of 25 animals that died from acute trauma (Gulland et al. 1996). The tissues were analyzed for chlordanes, PCBs, DDTs, and aromatic compounds (e.g., PAHs) (Stein, personal communication).

Because all California sea lions return to the Channel Islands to breed, and females and pups remain near the breeding grounds, it is difficult to establish reference conditions. All sea lions would

be expected to have been exposed to these contaminants to some degree. The lack of a control (relatively clean) population makes risk assessment a difficult task for this California sea lion population. However, data can be compared within subpopulations and age/sex cohorts. In establishing a link between exposure and effects, a gradient of contamination should be examined, if possible. This might involve collecting males close to and far from the areas of highest sediment contamination, or examining old vs. young females, who might be expected to vary in their levels of accumulated contaminants.

California sea lion prey are also contaminated with PCBs and EDDT. Concentrations of DDT in fish currently range up to 20 ppm or more (dry weight) (Brown et al. 1998), although they have declined dramatically since the 1970s. Data on concentrations of these contaminants are not available for all prey species. The most highly contaminated fish species include white croaker and dover sole, but these species do not constitute a large proportion of the diet of California sea lions (Antonelis and Fiscus 1980, Lowry et al. 1990, 1991). Nevertheless, concentrations of PCBs in white croaker have exceeded 4 ppm in some areas (Science Applications International Corporation 1998), whereas concentrations of DDT and PCBs in rockfish in comparison (a favored prey item of California sea lions) are approximately 10 times lower than those found in white croaker. Although anchovy (which are another favored prey item for California sea lions) have not been sampled recently, in the 1960s they contained up to 13 ppm total DDT; concentrations fell, however, during the 1970s (Anderson et al. 1975).

Effects of PCBs and DDT

Two lines of evidence can be examined for adverse effects on California sea lions. The first includes direct measurements of effects such as reproductive impairment or prevalence of carcinoma in California sea lions exposed to DDT and PCBs. Such direct measurements cannot separate the contributions of effects related to PCBs from those related to EDDT, because these contaminants co-occur in marine mammal tissue, prey items, and sediment throughout the area. Effects in sea lions would be evaluated on the basis of data collected from sea lions in several ongoing or recent studies, with the expectation that the total sample would include animals representing a gradient of exposure. This gradient would be used to assess the correlations between exposure and effects. The second approach relies on modeling to extrapolate from studies on species other than marine mammals. A theoretical approach to modeling is described here in lieu of a completed model, which was precluded due to time limitations at the workshop.

Studies of adverse effects of feeding DDT or PCB compounds to rodents are quite common. In order to apply the results of these studies to California sea lions, the following approach should be taken: (1) determine the no-effect dose and low-effect dose for rodents for the endpoint in question; (2) calculate the doses of PCBs and DDT obtained by California sea lions feeding in contaminated areas (by determining average and maximum concentrations of PCBs and DDT in prey species and estimating the quantity of prey consumed); (3) compare the doses in rodent studies (adjusted for body weight) to exposures received by sea lions (consider whether an extrapolation factor should be applied to account for the uncertainty due to species differences); and (4) conclude by reasoning that if marine mammal doses exceed rodent effect doses (toxicity reference values), then risk is indicated.

An alternate approach would be to convert toxic doses in rodents to toxic body-burden threshold concentrations in rodents. Tissue concentrations in relation to average lipid content in rodents would be compared to blubber concentrations in sea lions (using uncertainty factors, where appropriate, to account for species differences).

Effects on Reproduction. Elevated organochlorine concentrations have been associated with premature births in California sea lions, although cause-and-effe ct relationships have not been established (O'Shea and Brownell 1998). The initial study associated stillbirths and premature pupping in California sea

lions with high concentrations of organochlorines (DeLong et al. 1973). In sea lions sampled in 1970, concentrations that averaged 824 ppm DDT and 112 ppm PCBs in maternal blubber were associated with premature births. Mothers with normal term pups had mean concentrations of 103 ppm DDT and 17 ppm PCBs in blubber (DeLong et al. 1973). However, further research provided evidence that diseases, including leptospirosis and calicivirus infections, were also associated with the phenomenon of premature pupping (Smith et al. 1974, Gilmartin et al. 1976). Although it has not been conclusively demonstrated that the California sea lion population has been adversely affected by high exposure to DDT and PCBs, experimental evidence has shown that mixtures of organochlorines in the diet, including PCBs and EDDT, can impair reproduction in harbor seals during experimental situations (Reijnders 1986). Reported differences in DDT concentrations in 12 male California sea lions sampled in 1970 and seven males sampled in 1988–1992 were enormous, suggesting that a real reduction in organochlorine levels has occurred. Differences in the average ages of animals from the two samples could also explain in part the apparent changes in DDT concentrations, but information on the history of individuals (e.g., geographic foraging patterns, ages) from the more recent samples were not reported.

Cancer in California Sea Lions. Evidence linking anthropogenic contaminants to increased occurrence of neoplasms in marine mammals is sparse. A high incidence of neoplasms has been reported for the population of beluga whales in the St. Lawrence River, and these whales have been found to have higher concentrations of contaminants (including PCBs, other organohalogens, and metals) than beluga whales from less polluted high-latitude environments. St. Lawrence belugas also exhibit impaired reproductive and immune function (Béland et al. 1993, De Guise et al. 1994, 1995). Increased prevalence of hepatic neoplasms and related lesions are also found in fish inhabiting areas with high sediment concentrations of contaminants, including PCBs (Harshbarger and Clark 1990, Bowser and Martineau 1990, Hayes et al. 1990, Myers et al. 1991).

Recently, an 18% prevalence of neoplasms, the highest recorded to date in a pinniped population, was reported in California sea lions that stranded alive along the central California coast (Gulland et al. 1996). The specific neoplasm is thought to be of transitional-cell origin, and it occurs in the urogenital tract of young adults of both sexes (Gulland et al. 1996). Although the etiology of this carcinoma is unknown, chemical contaminants may play a role. One of the objectives of the present risk assessment is to assess the likelihood that the concentrations of contaminants, especially organochlorines (i.e., chlordanes, PCBs, and DDTs) and polycyclic aromatic compounds (i.e., benzopyrenes), found in sea lions are associated with an increased prevalence of transitional-cell carcinoma. It is known from rodent studies that these compounds have the potential to cause tumors.

Immunotoxicity. California sea lions inhabiting polluted coastal areas are known to accumulate high levels of organochlorines, which have been related to the occurrence of several immune-system abnormalities. These persistent lipophilic contaminants have been shown to adversely affect the immune system, leading to immunosuppression and in some cases increased susceptibility to infectious diseases in laboratory animals. De Swart et al. (1994) and Ross et al. (1996a) found that natural killer (NK)-cell activity and mitogen-induced proliferative T-cell responses were impaired in harbor seals fed fish from the Baltic Sea, which were more highly contaminated with PCBs and other organochlorines than "control" fish from the Atlantic Ocean. In addition, an increase was observed in polymorphonucle ar granulocytes, possibly suggesting an increased incidence of bacterial infections in the more contaminated Baltic Sea group (De Swart et al. 1994). Further studies demonstrated that seals fed Baltic Sea fish for two years had TEQ values 3.4 times higher than seals fed relatively uncontaminated fish from the Atlantic Ocean (Ross et al. 1995). These studies also demonstrated a contaminant-related suppression of delayed-type hypersensitivity and antibody responses in the seals fed fish from the Baltic. Contaminant levels (total PCB and TEQ)

were higher in many harbor seal populations in northern Europe at the time of the phocine distemper virus epizootic than the levels observed in the immuno-suppressed seals from Ross et al.'s (1995) study. It was speculated that contami nant-associated immunotoxicity in free-ranging harbor seals may have contributed to the severity of the epizootic (Ross et al. 1996b). T cells are important in the memory-based control of virus infections (i.e., the "learned" ability of these cells to recognize and attack cells infected by a pathogen which they have previously encountered, or "specific immunity") and in the overall immune response, while NK cells are important in the early clearance of virus infections in the absence of memory.

Risk Characterization

California sea lions are exposed to DDT and PCBs, as evidenced by accumulations of these compounds in blubber and other tissues. Data required for risk assessment with respect to reproductive outcomes and cancer may be obtained from several sources. The evidence available from marine mammals is limited, and in some instances controversial. Reijnde rs (1986) fed female harbor seals fish that were high or low in organochlorines and observed lower pregnancy rates in those with the more contaminated diet (daily intake of approximately 1.5 mg PCB). Inferences about impaired reproduction based on observational studies, such as those involving ringed and gray seals in the Baltic Sea (Helle et al. 1976a,b) and California sea lions (DeLong et al. 1973), need to be qualified because it is not possible to control for extraneous variables such as concurrent infectious diseases. Also, conclusions from such observational studies may be biased by the reciprocal effects of impaired reproduction on contaminant burdens. Given the limited database available for risk assessment from other pinniped species, data from experimental laboratory animals such as rodents will be required for dose-response estimation of effects. The uncertainty surrounding extrapolation of rodent data to pinnipeds will need to be factored into risk characterization.

Similarly, the evidence for effects of contaminants on cancer risk or on reliable markers for cancer risk in pinnipeds is extremely limited. Some use has been made of assays for induction of cytochrome P450 enzymes, induction of mutant oncogenes, and DNA adducts to substances such as benzo-"-pyrene. However, the predictive value of these markers is uncertain, and they are not considered reliable indicators of cancer risk. Therefore, dose-response data from rodent models will also need to be included in the risk characterization for cancer endpoints.

In a "weight of evidence" approach, epidemiologic evidence from the target population can contribute to the overall assessment of risk. For example, tissue concentrations of DDT, PCBs, and polycyclic aromatic compounds (PACs) are being compared between stranded animals with transitional-cell tumors and those with other, non-neoplastic disorders (Stein, personal communication). This approach can be used directly for risk assessment, provided that potentially confounding variables such as age, sex, pregnancy, stage of lactation, etc. are appropriately controlled and that technical issues regarding accumulation of xenobiotics in affected-animal tissues are resolved. However, the usual approach in assessing causality for environmental contaminants and disease requires replication in other populations, determination of temporal sequence, and adequate biological plausibility, all of which may be difficult to achieve. Thus, complementary information, based on other forms of evidence, should be incorporated into risk characterization in order to expand the framework for risk-management recommendations.

Uncertainty Analysis. There is considerable uncertainty involved in characterizing the risk posed by persistent contaminants to the U.S. population of California sea lions. Therefore, it is critical that the risk-characterization process incorporate uncertainty in a precautionary manner. That is, as uncertainty in components of the process increases, or as overall uncertainty increases, proposed management actions should become more risk-averse. Several approaches have been reported in the literature.

These include the use of (1) a multiplier (e.g., a factor of 10 or one-tenth) in establishing thresholds for one taxon based on laboratory studies from disparate taxa; (2) the 10th or 90th percentile for one or more of the input parameters in modeling the potential adverse effects; (3) Bayesian or Likelihood techniques that produce prior distributions for output parameters of interest, where management decisions are based on either conservative interpretations of posterior distributions or objective loss functions; and (4) simulation studies that incorporate uncertainty into the process of "tuning" the output to meet specified management objectives. Given the arbitrary nature of the first two approaches, efforts should be made to incorporate uncertainty objectively, as is done when using the latter two approaches. As noted earlier, uncertainty exists in virtually all of the input parameters used to characterize risk, such as uncertainty in quantifying the dose-response relationship for specific age and sex classes of sea lions, the number and magnitude of chemicals to which sea lions are exposed, and the degree to which density-dependent life history responses confound our ability to detect contaminant-related changes in survival and reproduction. In addition, there is uncertainty in other, less obvious input parameters, such as stock structure (i.e., rates of exchange between sea lions in Mexico and U.S. waters), seasonal aspects of the dose-response relationship, and age- and sex-specific responses to specific contaminant levels.

Interpretation of Ecological Significance. One problem in trying to evaluate which measures of response to contaminants are most important has to do with the current dynamics of the California sea lion population and the risks from other factors. This risk assessment attempts to identify additional risks to the population from exposure to contaminants. The population abundance has been increasing for more than 40 years. How the population is responding to the short-term effects of the recent El Niño event is unknown. However, it is anticipated that the population will respond as it did to the major El Niño in 1983. That is, after a few years, pup production will start to increase annually, and after 3-7 years, the population will have recovered to its pre-El Niño level and continue increasing. Therefore, unless the effects of contamination on survival and reproduction of this population are unexpectedly severe, any adverse effects due to contamination will likely be reflected in a slowing of the population rate of growth. Unfortunately, as the population continues to increase toward its carrying-capacity level, the population rate of growth will decline until it reaches zero. Because of this situation, a slowing in the observed growth rate of California sea lions cannot be taken as conclusive evidence that persistent contaminants are adversely affecting this population. Rather, it will be necessary to estimate the expected reductions in reproduction or survival to determine the likely impacts of contaminants on California sea lions.

Despite the lack of definitive scientific evidence of a causal relationship between contaminant levels and effects on reproduction or survival in California sea lions, there are studies suggesting that organochlorines have adverse effects on other marine mammals. These studies support the hypothesis that organochlorine exposure has increased the susceptibility of sea lions to diseases, including those that can cause abortions and other endocrine disorders. It is evident that much more research is needed to understand the ecological significance of persistent contaminants and their role in relation to other factors, including changes in prey availability, infectious diseases, algal blooms, global warming, El Niño and related events, and population dynamics of the species of concern. Qualitative and quantitative appraisals are needed of the actual and long-term effects of organochlorines and other contaminants. Both field studies and toxicity tests with surrogate species can be used to evaluate linkages between contaminant concentrations and effects on ecological and physiological receptors.

Field studies of California sea lions may confirm the link between pollution and ecological effects. Evidence can be obtained by investigating the reduction of population size or the association of sea lions with stressed habitats along the California

coast. More studies regarding community biodiversity and trophic structure may suggest answers to some questions and help explain recent die-offs possibly caused by domoic acid and other biotoxins. Dose-response studies may link contaminants and other etiologic agents to the incidence of tumors or other lesions.

Summary

Risk assessment is used to evaluate the risk posed by a particular agent or agents to the health or maintenance of a population, species, or ecosystem. In this case study, we have attempted to highlight the elements that should be included in a risk assessment. We have used the population of California sea lions in U.S. waters as the population of interest. As noted in this example, risk assessment requires the integration of information on (1) current sources of exposure; (2) current distributions and concentration patterns of the contaminants of concern; (3) the dose-response relationship for DDT and PCBs, derived either from field studies of California sea lions or from whole-animal field and laboratory studies of other pinnipeds (or other mammals); and (4) mechanisms by which contaminants can compromise the survival or reproduction of model species, obtained from *in vitro* cell-culture studies. Our relatively cursory risk assessment indicates that DDT and PCBs may pose considerable risks to the population of California sea lions in U.S. waters. This conclusion is based on a preponderance-of-evidence approach (sometimes referred to as the "weight of evidence" approach) in the absence of detailed, controlled dose-response studies using California sea lions as the study subjects. Such an approach is precautionary and scientifically defensible. Finally, we have not included in this report a detailed section on the relative merits of a suite of actions directed at mitigating the effects of contaminants on California sea lions (often referred to as a risk-management evaluation). Such an evaluation would require better information on current sources, exposure levels, and dose-response relationships, including those for dose levels considerably lower than those observed in the early 1970s.

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Working Group on Future Trends

Working Group Members: Fair, Geraci (Leader), Goldberg, Jarman, Kucklick (Rapporteur), Krahn, Muir, Norstrom, Phinney, Tanabe, van Leeuwen, York.

This working group did not divide into subgroups, and its report consists of a unified document in a format somewhat different than those of the other working groups. The group considered four major topics: (1) projected "new" contaminants of future concern; (2) future trends with contaminants currently known to be issues for marine mammals; (3) future needs to improve and insure consistency of sample collection and analysis; and (4) future management needs.

Background and Rationale

The problems of persistent organic pollutants will remain well into the foreseeable future. A general decline in levels of persistent organic pollutants in the marine environment is not anticipated. There is every likelihood that the environmental trends of halogenated organic compounds, such as polybrominated diphenyl ethers and chlorinated paraffins, will parallel production trends, as demonstrated with well known chemical contaminants such as PCBs (polychlorinated biphenyls) and DDT (dichlorodiphenyltrichloroethane). While the environmental levels of some compounds may be slowly declining, many are still within the ranges where subtle toxic effects are to be anticipated. Trends in contaminants must be placed in a regional context, and rates and directions of change are often region-specific. For example, in the Southern Hemisphere the concentrations of PCBs appear to be increasing. The rates of change of many compounds in the Southern Hemisphere are poorly known, and this region may be at future risk.

Much of the research on contaminants and marine mammals has focused on the problem of persistent organochlorine chemicals such as PCBs and DDT, which are a continuing and global problem. Potential problems caused by other persistent, toxic, and bioaccumulative substances (PTBSs) cannot currently be addressed due to the lack of basic information on their production, use, exposure, and effects (Environmental Protection Agency 1998). It is currently estimated that there are roughly 2400 lipophilic and persistent chemicals, of which 390 are PTBSs. In order to prevent long-term pollution from these largely unknown chemicals, chemical industries should disclose basic information on such compounds, and this information should be made widely available. This will require enhanced international cooperation, preferably within the existing framework of chemical contaminant programs, such as the Existing Chemicals Program of the Organization for Economic Cooperation and Development (1991) and the Program on Persistent Organic Pollutants of the United Nations Environment Programme (1998).

Monitoring studies are essential to the description and understanding of pollutants. It is necessary to exploit existing analytical techniques to identify as many anthropogenic compounds as possible in marine mammal tissues in order to expand the identification of existing and new chemicals that accumulate in, and pose potential threats to, these species.

"New" Contaminants of Future Concern

Persistent, Toxic, and Bioaccumulative Compounds Not on the Standard Persistent Organic Pollutants (POPs) List That Are Used or Produced in Large Quantities. A number of compounds not on the standard POPs list are still used or produced in large quantities. Examples include polychlorinated naphthalenes and polychlorinated diphenyl ethers; other examples are listed in the International Whaling Commission's Bergen workshop report (Reijnders et al. 1999). Organo-metallic compounds such as tributyltin, although phased out in many marine-oriented applications, are still used in some areas.

Non-Persistent Chemicals That Could Present a Risk to Marine Mammals Through Their High Bioaccumulation Potential and Heavy or Wide-

spread Use. This is a potentially very large group of chemicals consisting of, for example, pesticides, biocides, and pharmaceuticals.

Eutrophication and Biotoxins. Biotoxins from algal species have been implicated in die-offs of manatees (O'Shea et al. 1991, Bossart et al. 1998), humpback whales (Geraci et al. 1989), dolphins (Geraci 1989), seals (Banish and Gilmartin 1992), sea otters (DeGange and Vacca 1989), and possibly California sea lions (Anonymous 1998). These events may be exacerbated by eutrophication in coastal waters. One of the most pervasive marine pollution problems on a global basis involves the eutrophication (particularly the accelerating inputs of nitrogen and phosphorus compounds) of coastal waters via the atmosphere, rivers, and outfalls. Eutrophication of coastal waters is continuous and expanding. Consequent increases in plant productivity are evident from the increasing frequency and geographic range of algal blooms (including toxic algal blooms), anoxic and hypoxic zones in the water column and sediments, and alterations to the makeup of communities of organisms. These consequences can affect marine mammals in the following ways: (1) mortality through the ingestion of toxic algae in foods; (2) alteration of their normal food supplies through changes in the make-up of their food webs; and (3) diminished oxygen in their environmental waters (Hallegraeff 1993, Anderson 1995).

Polymers and Low Molecular Weight Monomers. These compounds are released in huge quantities into the ocean, but their fate is uncertain. Vinyl chloride is one example of a compound that may be an emerging chemical threat.

Chemicals That Cause "Endocrine Disruption." Endocrine disruption remains a potentially important environmental problem and needs to be included in future marine mammal research. New technologies should be developed to identify such compounds and their effects on marine mammals. Support of programs for compilation of information on these compounds, including physical properties, applications, and usage, is important for predicting future trends. However, these chemicals are not included as a separate category in this working group report for the following reasons: (1) the structural diversity of chemicals implicated as "endocrine disrupters" is very large, and marine mammal research is better served by focusing research questions on specific chemicals and their effects, including endocrine system effects; (2) many suspected "endocrine disrupters" are included under existing chemical headings (e.g., PCBs, tributyltin, etc.); (3) for carnivorous marine mammals, metabolism of contaminants by prey species such as fish confounds the ability to make a direct connection between exposure to potential endocrine disruptive chemicals and reproductive and developmental effects.

Future Trends With Currently Known Contaminants

The major classes of persistent organochlorine contaminants bioaccumulating in marine mammals, historically and at present, are PCBs, DDTs, toxaphene, chlordanes, HCHs (hexachlorocyclohexanes), and their metabolites. The relative distribution of these substances may vary from one geographical location to another and from inshore to offshore. However, PCBs and DDTs are the groups of contaminants with the highest concentrations in marine mammals almost everywhere. Low amounts of many other organochlorines, such as dieldrin, chlorobenzenes, mirex, chloroparaffins, PCNs (polychlorinated naphthalenes), PCDDs (polychlorinated dibenzodioxins), and PCDFs (polychlorinated dibenzofurans) are often also present. There may be locations where these contaminants accumulate to levels of concern in marine mammals, but judging by current research, they generally are much less important toxicologically than the first group of compounds and their metabolites.

PCBs and DDTs are the classes of persistent bioaccumulating toxic compounds of most concern to marine mammals (reviewed by O'Shea 1999). PCBs have a potentially broad range of toxic effects (Safe 1984, 1994). These include effects on immune, endocrine, and nervous system function,

resulting in interference with growth, development, sexual differentiation, behavior, and resistance to disease. In addition to the PCBs themselves, two classes of metabolites, (methylsulfone-PCBs and hydroxy-PCBs) have been shown to be persistent and potentially toxic. Methylsulfone-PCBs accumulate selectively in tissues such as lung and liver in some mammals, but this is very species-specific. Their concentration is typically on the order of 3 to 5% of total PCBs in marine mammals. Some methylsulfone-PCBs are potent cytochrome P450 enzyme inducers. They have been found to decrease pup survival and reduce thyroxine levels in blood of mink dosed with a mixture similar to that found in Baltic seals. However, their toxicity is not well understood. Hydroxy-PCBs have a structure very similar to that of thyroxine, and they have been shown to bind competitively to the thyroxine transport protein, TTR. This results in lowered circulating levels of thyroxine and vitamin A. The consequences of this are unknown. Hydroxy-PCBs have been found at concentrations similar to those of the PCBs themselves in the plasma of humans, polar bears, and experimental laboratory mammals, but as yet there are no data for their occurrence in marine mammals other than the polar bear. Members of the DDT family and metabolites have well-documented effects on endocrine systems, especially corticostero id synthesis in the adrenal gland. The compound o,p'-DDD was even used to treat Cushing's disease, an adrenal dysfunction in humans (Gutierrez and Crooke 1980). A methylsulfone metabolite of DDE is a potent adrenocortolytic agent in mice and mink, but there appear to be large species differences.

Long-term trends of PCBs and DDTs in the environment must be understood in a global context. Although both groups of compounds have been controlled in the major industrialized countries of the world for more than 20 years, this should not lead us to believe that the problems have gone away. DDT continues to be manufactured and used in large amounts in some tropical countries (Anonymous 1996). It is likely that PCBs continue to be manufactured in the former Soviet Union. An enormous inventory of PCBs is still in use in electrical equipment around the world or is improperly stored in waste dumps. PCB emissions to the atmosphere from large urban areas with no particularly identifiable source are probably large. Land and water surfaces all over the world function as temporary sinks for quantities of these compounds, effectively retaining and recycling them back into the oceans, via the atmosphere, for decades. The upper levels of ocean sediments also serve as an important reservoir for PCBs, which then can return to the food chain when ingested by benthic organisms. Rates of PCB destruction in the environment appear to be extremely low. Therefore, environmental decreases are mainly due to effective removal from recycling by physical processes such as burial in sediments or redistribution.

The voluntary ban on sale of PCBs for open uses in the United States in the early 1970s had an almost immediate effect on PCB levels in the environment close to highly industrial urban areas. Exponential rates of decline at half-lives on the order of three to five years were recorded throughout the 1970s in biota from the Great Lakes, nearshore marine environments of North America, and the Baltic Sea (Addison et al. 1986, Blomkvist et al. 1992, Falandysz 1994, De Vault et al. 1996). However, these rates began to slow in the 1980s, and in the 1990s levels are no longer declining at statistically meaningful rates (Beck et al. 1994, Tanabe et al. 1994a, De Vault et al. 1996, Muir et al. 1996). Marine environments remote from sources were never as highly contaminated as those close to sources. However, the effects of production controls have been limited by the fact that loading to the global marine system is governed largely by long-range physical transport by air and water and global-equilibration processes, and not by proximity to sources (Tanabe et al. 1983, 1994b, Simonich and Hites 1995). This dampening effect explains why there have been timelags of five or more years for peak organochlorine concentrations to occur in remote environments (e.g., the 1976 peak in the northern fur seal [Tanabe et al. 1994a]). The peak may have occurred even later in the Arctic, which is isolated from mid-latitude atmospheric circulation in summer. For example, concentrations of PCBs in polar bears from Hudson Bay were higher in 1984

than they were in 1969 or 1992 (Norstrom et al. 1998). Rates of decline subsequent to the peak were also much slower than at mid-latitudes.

The situation for DDT is less clear, possibly because of the continuing influx to the environment from Asia. Dramatic declines occurred in the 1970s in the marine environment close to areas of use in the United States and Canada, and also in the Baltic Sea. Similar to PCBs, declines in DDT in remote environments were less dramatic in the 1970s. However, declines may be continuing in some remote areas. This regional behavior may be driven by the large source to the atmosphere from Asia, which would not be expected to influence all areas in the Northern Hemisphere equally.

The "cold condensation effect" has gained prominence in describing transport processes to polar environments (Wania and Mackay 1996). This effect is driven by the fact that some persistent compounds are more soluble in cold than warm water. It has been elegantly demonstrated for HCHs. Recent evidence shows that "old" water under the north polar ice cap is out of equilibrium with air over the ice, and it outgasses when it reach es open water, becoming a net source to the atmosphere (Jantunen and Bidleman 1995, Hargrave et al. 1997). This reverse of the cold condensation effect demonstrates the point that thermodynamic equilibration processes are the driving force in planetary distribution of some contaminants. It would be a mistake to believe that the cold condensation effect simply moves organochlorines from warm to cold environments. There is no evidence that the net movement from warm to cold environments is occurring at a perceptible rate for the less volatile and less water-soluble contaminants such as PCBs and DDT. Concentrations of these compounds have remained consistently lower in the water and the biota of the Arctic than in those of mid-latitudes over the past ten years (Muir et al. 1992, Iwata et al. 1993, Norstrom and Muir 1994).

Atmospheric circulation within the temperate zones of the Northern and Southern Hemispheres is much more rapid than exchange between the hemispheres because the tropics constitute a barrier to air exchange. The long-term prospect of net movement of persistent organochlorine chemicals from the Northern to the Southern Hemisphere therefore must be considered. Such movement may explain PCB trends in minke whales. Northern minke whales have much higher levels of PCBs than southern minke whales (Tanabe, pers. comm.). While there are no significant temporal trends in recent PCB concentrations in the northern population, there are indications of an increase in PCBs in the southern minke whale population.

The shift to a more nearly global steady-state concentration is likely the dominant process in the long-term trends of PCBs and DDTs in the marine environment. We can expect future rates of change to be slow, based on the history of the last decade. The rate of decline depends to some extent on whether the present concentrations are being maintained by sources that can be controlled by destroying inventory and preventing further manufacture and use. However, it is possible that recycling of historically emitted PCBs and DDT is the major factor in maintenance of present environmental concentrations.

Future Needs to Improve and Ensure Consistency of Sample Collection and Analysis

Sample Collection (and Storage). The development of a universal protocol for sample collection that includes all basic (Level A) data and supporting documentation (Geraci and Lounsbury 1993) should be encouraged. We emphasize the value of using animals from fishery bycatch, hunting, live-capture operations, and fresh strandings, and samples collected by biopsy darting. It is important to note that samples from stranded animals that may be suffering from disease and have poor nutritional status may not be representative of healthy animals. We encourage further development of biopsy techniques to overcome the limitations of small sample sizes, the lack of associated life history information, and the questionable comparability between biopsy samples and underlying blubber samples. We recognize the need to investigate storage stability of contaminants and tissues and to develop appropriate recommendations.

Sample Analysis, Data Reporting, and Archiving. As analytical techniques are developed, data comparability among laboratories should be assured on a global scale. We recommend that laboratories adopt a performance-based quality-assurance program that incorporates standard reference materials (SRMs) and participation in intercomparison exercises in their protocols. SRMs should be developed for emerging chemicals, and existing SRMs should be analyzed and certified for these emerging classes of chemicals.

We recommend that chemical analyses of marine mammal tissues generate quantitative data on as large a number of compounds as possible. This will provide robust data sets for future use. We encourage the development of protocols stipulating quantitation of a minimum number of compounds of persistent contaminants, such as PCBs, in order to enhance the comparability of data sets for purposes of risk determination and trend analysis. We recognize that in some instances (e.g., damage assessment, risk assessment, or environmental emergencies such as oil spills) a smaller number of analyses may be appropriate (e.g., screening analyses) as long as the quality assurance (e.g., SRMs) supports the data.

Concerns Regarding Developing Nations

Many of the contaminants that are of concern in relation to marine mammal populations have been banned in industrialized countries. Their continued use in and export from developing nations represents a risk to marine mammal populations. For example, India still produces 4,000 metric tons of DDT per year (Anonymous 1996). The use of PCBs continues in the former Soviet Union, although decisions to terminate their manufacture may have been reached very recently in the Russian Federation or in one or more of the newly independent republics. In general, there is a lack of information on production and use of POPs in developing nations.

As a step toward understanding the distribution of POPs in the Southern Ocean, we strongly recommend that contaminant studies be incorporated into ongoing life history investigations of marine mammals. Emphasis should be on species and populations that can be considered sentinels or indicators of regional contaminant burdens. We recommend that multidisciplinary studies be conducted on "sentinel" marine mammal populations and that these studies include investigations of contaminants, life history, biomarkers, and animal health. We encourage institutions and agencies to work together in this regard.

Future Monitoring and Research Needs

Emissions. Most monitoring studies have been preoccupied with water and tissue analyses rather than with collecting data on global use and release of persistent chemicals. More effort should be devoted to identifying emission sources and calculating environmental loads. This information is crucial to the interpretation of monitoring data and to understanding the spatial and temporal distribution of chemicals in biota. Such information should be available through various programs including the OECD, UNEP, and International Program on Chemical Safety (IPCS).

Contaminant Levels in Marine Mammals. The study of individuals has been and will remain essential for understanding animal health in relation to contaminants. However, to understand effects on marine mammal populations, information on individual animal health must be supplemented with information on habitat quality, life history, and trophic relationships. Population studies will require multidisciplinary collaborative efforts among government, academic, and non-government organizations. Specific research and monitoring goals and needs should include the following:

- Species should be sampled in both nearshore and pelagic environments, following suitable protocols. Such species might include ringed seals, fur seals, striped dolphins, bottlenose dolphins, bowhead whales, minke whales, and beluga whales.
- Monitoring of marine mammal populations that are recovering, stable, and declining is ex tremely valuable and should continue. Recovering populations provide reference values for effect thresholds, and these may constitute the

only measures of the consequences of contamination.

- Archiving of tissue specimens in dependable, long-term programs is essential. Retrospective analysis depends on specimen banking; thus support for tissue archiving must also be dependable and long-term. Samples used for estimating temporal trends should be collected and maintained in a way that maximizes the number of contaminants that are measurable by the best available techniques. Sampling protocols ideally would include blubber, liver, and plasma/serum. As these samples are analyzed, researchers should explore for the widest possible array of compounds with properties that may fall outside the realm of those currently examined (e.g., hydroxy and methylsulfone PCBs).
- Collecting, analyzing, and archiving samples for biomarker determinations.
- Developing and refining food-chain models to (1) assess and validate the exposure of mammals to a limited number of well-known chemicals; and (2) select new priority chemicals on the basis of emission, physico-chemical, and toxicological data.
- Monitoring of marine mammal tissues for well-known contaminants such as PCBs and DDTs should continue. Such information is crucial to understanding processes related to the fate, behavior, and population-level effects of these chemicals. Furthermore, data on these "old" compounds will be crucial for validating models developed for the assessment of risks associated with new contaminants.
- Contaminant concentrations in tissue samples should be interpreted in the context of animal ecology and habitat quality. We encourage the use of existing data and the development of new data sets on the levels of contaminants in prey. Such data will facilitate interpretation of trends in current contaminants and estimation of exposure to compounds not yet identified. It is also important to encourage the integration of all monitoring studies with hypothesis-driven research.
- Efforts to examine the global fate and transport of persistent organic contaminants should be supported. In this regard, we recognize that there is a large data gap in the Southern Hemisphere. As discussed earlier in this report, levels of HCHs are high in the Arctic due to the physical properties of this group of compounds. Also, the production of PCBs in the former Soviet Union has global implications.
- Much more research is needed before we can interpret the relationships between measured contaminant concentrations and measured biological effects in marine mammals. Studies must include laboratory experiments on appropriate animal models and surrogate species, risk-assessment modeling, and controlled experimental work with marine mammals that is designed to address critical research questions, applies sound scientific criteria, and has statistical validity.

Future Management Needs

Provide Contaminant Information in More Easily Understood Formats. Information needs to be developed in the form of interpreted data sets that are useable by policy makers, environmental managers, and the general public, preferably in a GIS format. Programs are needed to heighten awareness and to inform the public and policy makers of the significance of contaminants in marine mammals, including "old" contaminants such as DDT and PCBs that are still found in the environment. Among the tools available for this purpose are the mainstream media, web pages, the Internet, and liaisons with government agencies and international non-government organizations, e.g., World Wide Fund for Nature (WWF), International Union for the Conservation of Nature and Natural Resources (IUCN–World Conservation Union), and Center for Marine Conservation.

Develop Stable Funding Support. Stable funding is essential for these long-term research and monitoring efforts. For example, there is recognized value in the continued monitoring of contaminants in marine mammal populations that are at equilibrium or recovering. An easier mechanism must be developed to facilitate the transfer of funds among

federal and state agencies and non-government organizations. New funding opportunities such as partnerships with industries, foundations, and other benefactors should be identified.

Convene Expert Panels Periodically. An international group of experts should be convened on a periodic basis to review and interpret contaminant data on marine mammals. The output of such meetings would be a status report, including databases and comparisons.

Increase Information Transfer. It is necessary that the Marine Mammal Commission and other responsible agencies facilitate a dialog among federal agencies and international agencies such as the OECD, IPCS, and UNEP. These organizations should be able to provide basic information on the production, use, physico-chemical properties, toxicology, and ecological effects of anthropogenic chemicals in the environment. Such information is crucial for anticipating future problems associated with chemical contaminants in the marine environment.

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Section IV. EXTENDED ABSTRACTS OF PLENARY PRESENTATIONS

Persistent Ocean Contaminants and Marine Mammals: A Retrospective Overview

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Introduction

In this paper we present (1) a chronological summary of knowledge about marine mammals and persistent ocean contaminants, beginning with the 1940s; (2) a summary of major findings and advances for each decade in the context of larger events in the related fields of environmental policy, toxicology, and technology; (3) indices of the cumulative amount of information on contaminants and marine mammals reached each decade based on compilations of information appearing in about 420 publications in the scientific literature (exclusive of studies of oil) as documented by O'Shea (1999); and (4) examples of progress made in the application of the scientific method to problems in the study of contaminants and marine mammals. The amount of available information on the topic is growing at a very rapid pace, but much of this information is not well integrated. Increasing applications of sophisticated technology reveal traces of greater numbers of contaminants, as well as an escalating multitude of stressors on the marine environment. In combination, these revelations make interpretation of observed effects and predictions of future effects of contaminants on marine mammals an extremely complex task.

The 1940s and 1950s

During this period, much of the world was involved in and recovering from massive military conflicts. Experience in mobilizing technology to serve these conflicts was followed by great faith in the applications of research in chemistry and physics to further global social and natural resource development. Production of PCBs escalated markedly after World War II, and the era of large-scale use of organochlorine pesticides was also ushered in during this period. A Nobel Prize was awarded in 1948 for discovery of the insecticidal properties of DDT, a compound which saved millions of people from death due to insect-borne pathogens, but which was to be found in the blubber of marine mammals in remote reaches of the world beginning nearly 20 years later; DDT subsequently has become one of the most intensively studied persistent contaminants. With the exception of radionuclide fallout from the use and testing of nuclear weapons, there was little recognition or concern about environmental contaminants during this period, particularly with regard to marine mammals. The scientific literature of the time contains no information on contaminants in marine mammals, although retrospective studies of commercial oils show that seals and whales contained metabolites of DDT in the 1950s (Addison et al. 1972) and that mercury was present at low levels in Southern Ocean fin whales

in the 1940s (Nagakura et al. 1974).

The 1960s

This decade witnessed an awakening of concern about the impacts of persistent environmental contaminants on human health as well as on wildlife and ecosystems. In Japan, local effects on human health from contamination of seafoods with mercury and rice with PCB oils provided prime examples that attracted worldwide attention. In 1966, the first two publications on contaminants in marine mammals reported DDT and metabolites in Antarctic seals (Sladen et al. 1966, George and Frear 1966), providing the first published documentation of what has become a major, long-lasting issue studied throughout the oceans of the world. By the end of the decade the presence of mercury, DDT, DDE, DDD, dieldrin, and PCBs in tissues of marine mammals had been documented, with published organochlorine data based on about 50 individual pinnipeds belonging to six species, four harbor porpoises, and one common dolphin. Mercury was the only toxic element reported in marine mammals in the 1960s, based on examination of 32 ringed seals from Finland.

The 1970s

Observational data on the presence of contaminants in tissues of marine mammals began to expand rapidly in the 1970s, a time of much formal recognition of the dangers of chemical contamination, as reflected by worldwide advances in environmental legislation and policy. These included a number of formal restrictions on production and use of PCBs and certain organochlorine pesticides in some parts of the world. The number of potentially toxic elements reported in pinniped tissues expanded to 18, and data on elements were reported for at least 1,410 individual marine mammals, including 10 species of pinnipeds and 17 species of cetaceans. Organochlorines were reported for 1,230 marine mammals, including 11 species of pinnipeds and 26 species of cetaceans. The list of organochlorines reported in marine mammals expanded to about 26 compounds or groups of compounds over the course of the 1970s. Organochlorine residue studies began to show patterns of accumulation with sex and age, geographic trends, and "hot spots." Characteristic statistical distributions placed most values in the low to moderate range, with a few more heavily contaminated individuals. A few attempts at experimentation were made in very limited studies of metals in seals. Some *in vitro* experiments were conducted with pinnipeds; these could be classified as early "biomarker" studies. The first observations suggesting associations between organochlorines and reproductive problems in female pinnipeds were published. These were made on seals in the Baltic (e.g., Helle 1976a,b) and on California sea lions (DeLong et al. 1973). However, cause-and-effect interpretations of these observations were not possible. Despite advances in other areas of marine toxicology, no dose-response relationships had been established for marine mammals, nor had any experimental tests of hypotheses been conducted.

The 1980s

This decade was marked by (1) ever-expanding information on chemical residues in the bodies (primarily blubber) of marine mammals; (2) landmark experimental approaches with captive pinnipeds; (3) more sophisticated analytical technologies allowing quantification of individual PCB congeners and corresponding hypotheses about metabolic capacities of marine mammals, and the possible vulnerability of their endocrine physiology; and (4) application of biomarkers to the study of contaminants in marine mammals. In the first full toxicological experiment with marine mammals, Reijnders (1986) showed that reproduction in captive female harbor seals was impaired when the diet consisted of fish with higher concentrations of mixed organochlorines. Tanabe (1988), Tanabe et al. (e.g., 1987, 1988), and Boon et al. (e.g., 1987, 1992) showed that certain PCB congeners were especially recalcitrant to metabolism in marine mammals (based on comparison of residue profiles in marine mammals with those in their food and those in terrestrial mammals). These studies suggested that this recalcitrance was linked with mixed-function oxidase systems and possible endocrinological effects. The

hypothesis that organochlorines interfere with adrenocortical metabolism was established based on pathology in seals (Bergman and Olsson 1985). Biomarker studies were initiated primarily in the latter half of this decade, with biomarker information of some kind available for at least 83 individuals in eight species of cetaceans and 90 individuals in five species of pinnipeds (Fossi and Marsili 1997). Much residue data accrued in the 1980s, and many more extensive studies verified strong patterns of accumulation for some organochlorine compounds by sex and age across species: concentrations in blubber increase with age in males but reach a plateau or decline after the age of first reproduction in females. This is because females transfer compounds to young, primarily via lactation. Observational data on contaminant residues continued to overshadow experimental investigations, with organochlorine analyses embracing another 2700 individuals (in 12 pinniped species, 33 cetacean species, and other groups); toxic element determinations were carried out on the tissues of more than 1,700 individual marine mammals.

The 1990s

Deepening concern and debate about the influence of contaminants on disease processes have come to the forefront in the present decade. An additional focus has been on the possible importance of endocrine disruptors to population dynamics. Studies of beluga whales in the St. Lawrence River system (e.g., Béland et al. 1993, Martineau et al. 1994) and pinnipeds in the Baltic (e.g., Bergman et al. 1992) showed various pathologies strongly suggestive of contaminant impacts. Die-offs of bottlenose dolphins on the Atlantic coast of the United States in the late 1980s (e.g., Geraci 1989, Lipscomb et al. 1994, Duignan et al. 1996) were followed by additional dramatic events involving pinnipeds and small cetaceans in Europe and the Gulf of Mexico during the 1990s (e.g., Domingo et al. 1990, Aguilar and Borrell 1994, Lipscomb et al. 1996, Taubenberger et al. 1996). Studies of these events included designs that statistically partitioned cause of death and organochlorine residue data among variables such as age and sex to test hypotheses about linkages between disease and contaminants, with sometimes conflicting results (e.g., Hall et al. 1992, Aguilar and Borrell 1994). Die-offs associated with morbillivirus infections were very dramatic and attracted much attention and speculation about links with contaminants, but such infections are typically associated with high mortality in naive mammalian populations even when contaminants are not present. Concern about disease-organochlorine relationships prompted some additional rare examples of experimental approaches using captive harbor seals. These studies showed organochlorine-linked effects on immunocompeten ce (e.g., Ross et al. 1995, 1996, Harder et al. 1992, De Swart et al. 1996). Biomarker studies continued to increase in the 1990s, with some form of sampling published for at least 181 cetaceans in seven species and 384 pinnipeds in nine species. Biomarker studies began to include organochlorine determinations from biopsies or tissue samples from the same individuals, and results generally showed a correspondence with mixed function oxidase activity. Other observation-level data continue to accumulate during the present decade, with analytical chemistry techniques greatly expanding the numbers of individual compounds (especially industrial chemical congeners) and elements reported in marine mammal tissues at ever finer levels of sensitivity. Butyltins in tissues of marine mammals were first reported in the 1990s and are becoming commonly reported (e.g., Kannan et al. 1998, Tanabe et al. 1998), but they are of uncertain toxicological significance to these species. Some 50 toxic elements have been found in marine mammals thus far in the 1990s, based on assays from at least 5,556 individuals. Publications document that more than 5,529 individuals have also been examined for organochlorines in the 1990s, including 17 species of pinnipeds, 40 species of cetaceans, polar bears, sea otters, and manatees. Comparisons of recent data with patterns from past decades have been made, but temporal trends are uneven among species, regions, and specific contaminants. Despite the existence of an enormous database on contaminant residues (contaminant data are now reported for more than 18,000 individuals), it has not been firmly established what

concentrations of any particular substance in blubber are diagnostic of detrimental health effects in marine mammals. The database itself is strongly biased, with a huge proportion of the samples taken from the Northern Hemisphere, particularly Canadian and European waters. The data are also biased by species: 77% of the individual pinnipeds examined for organochlorines and 73% of those examined for toxic elements are from just four species; 67% of all cetaceans examined for organochlorines and 64% of those sampled for toxic elements have been from only five species. Few data exist for other contaminants in marine mammals, such as radionuclides or PAH's, but surveys conducted thus far do not suggest significant problems. Attempts to predict future impacts of known and possibly "new" contaminants on marine mammal species and populations are exacerbated by the many other changes in the marine environment. Confounding factors can include harvesting and bycatch (including historical impacts), depletion of fisheries and other food chain shifts, eutrophication in nearshore environments, global temperature flux, introductions of exotic species, ship traffic, noise pollution, and toxic algal blooms.

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Reproductive and Developmental Effects of Endocrine-Disrupting Chemicals on Marine Mammals

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Introduction

A large number of xenobiotics with endocrine-disrupting properties have been detected in marine mammal tissue (Wagemann and Muir 1984, Aguilar and Borrell 1995, Colborn and Smolen 1996, Reijnders 1996). Although most of the species known to be contaminated in this way are coastal, considerable concentrations of such compounds have even been detected in at least one cetacean that forages in deep water, the sperm whale (de Boer et al. 1998). Only in a few studies have observed reproductive disorders been found to be associated with certain chlorinated hydrocarbons and their metabolites. Among these studies are those involving ringed and gray seals in the Baltic Sea (Helle 1980, Bergman and Olsson 1985), beluga whales in the St. Lawrence River (Béland et al. 1992), harbor seals in the Wadden Sea (Reijnders 1980), and California sea lions in the eastern Pacific Ocean (DeLong et al. 1973). The findings of these studies, although strongly suggestive, have not been conclusive. The etiology of the observed disorder has usually been uncertain, and proof of a causal relationship between exposure to a specific contaminant and an impact on the reproductive or endocrine system has remained elusive.

This paper discusses the issue from an epidemiological point of view. My focus is on marine mammal species in which disorders in hormone concentrations, reproductive problems, or pathological conditions associated with hormonal imbalance have been observed. An overview of associations between organochlorines and marine mammal reproduction and endocrinology is presented in Table 10. I conclude with some comments on the possibilities of monitoring and evaluating problems related to xenobiotic-induced reproductive impairment and endocrine disruption in marine mammals.

Basic Pharmacology and Physiology of Hormones in Reproduction and Early Development

Hormones are messenger compounds. Their release leads to functional changes in an organism's cells, tissues, and organs. They are produced by endocrine organs and delivered into the bloodstream. A small percentage of hormones circulate freely, but the majority are bound to transport proteins. The free hormones diffuse into the tissues and cells. Target cells possess specific receptor molecules which bind to particular hormones, leading to activation of the receptor. Steroid hormones as well as thyroid hormones play important roles in reproduction and early development. They are discussed separately here.

In vertebrates, sex hormones belong to a group of steroids which are synthesized from cholesterol. Steroids can be divided into four functional groups: the three sex hormone groups (androgens, estrogens, and progesterone) and the glucocorticosteroids. The latter plays a role in regulating metabolism and growth and in osmoregulation.

In mammals, steroid hormones are mainly synthesized in the adrenals, gonads, and placenta. The production of each of the sex hormones is localized in a specific gland such as testosterone in the testes, estrogens and progesterone in the ovaries, and progesterone, estrogens, and testosterone in the adrenals. These organs can, in many species,

Table 10. Associations between organochlorines and marine mammal reproduction and endocrinology

	Where in Process	Species	Location	Mode of Action	Certainty of Mode of Action*	Contaminants	Certainty of Contaminants*
Reproductive Disorders							
Implantation failure[1]	implantation	harbor seal	Wadden Sea	hormone metabolism enhancement	2	PCBs/-metabolites	2
Failed implantation or fetal development[2]	implantation	beluga whale	St. Lawrence River	unknown	4	organochlorines	4
Stenosis and occlusions[3]	post partum	gray and ringed seals	Baltic Sea	organochlorine induced uterine pathology	3	PCBs/DDE/MSFs	3
Sterility[4]	unknown	gray and ringed seals	Baltic Sea	pathologic sterility	1	PCBs/DDT/-metabolites	3
Premature pupping[5]	late gestation	California sea lion	Southern California Bight	microsomal enzyme induction; steroid mimicking	3	PCBs/DDT	3
Hormonal Disorders							
Low vitamin A /thyroid hormones[6]	all ages	harbor seal	Wadden Sea	binding competition	1	PCBs/-metabolites	2
Reduced testosterone[7]	adults	Dall's porpoise	North Pacific Ocean	unknown	4	DDE/possibly PCBs	4
Lowered estradiol level[8]	implantation	harbor seal	Wadden Sea	enhanced hydroxylation	3	PCBs/-metabolites	2
Morphological Disorders							
Skull lesions (osteoporosis, paradentitis)[9]	predominantly adults	harbor and gray seals	Baltic Sea, Wadden Sea	infection/ hyperadrenocortical	2	PCBs/DDT/-metabolites	3
Exostosis[10]	all ages	harbor seal	Baltic Sea, west coast of Sweden	unknown	4	PCBs/DDT/-metabolites	3
Testis abnormalities[11]	immatures and adults	minke whale	Southern Ocean	unknown	4	organochlorines	4
Adrenal hyperplasia[12]	unknown	beluga whale	St. Lawrence River	unknown	4	organochlorines	4
Hermaphroditism[13]	fetal	beluga whale	St. Lawrence River	genetic/environmental	4	PCBs/DDT	4

*1= definite, 2 = probable, 3 = possible, 4 = unknown

[1]Reijnders et al. 1986, Reijnders, this report; [2]Martineau et al. 1994; [3]Helle 1980, Helle et al. 1976; [4]Bergman and Olsson 1985; [5]DeLong et al. 1973; [6]Brouwer et al. 1989; [7]Subramanian et al.1987; [8]Reijnders, this report; [9]Bergman et al. 1992, Stede and Stede 1990; [10]Mortensen et al. 1992; [11]Fujise et al. 1998; [12]Béland et al. 1992; [13]De Guise et al. 1994.

also produce small quantities of the other sex hormones. The specific pathway of biosynthesis in mammals is in the following order: cholesterol → pregnenolone → progesterone → androstenedione → testosterone → estradiol (Fig. 5).

The hypothalamus and pituitary are responsible in most vertebrates for the regulation of hormone concentrations and the timing of reproduction and sexual development. The hypothalamus produces a peptide, gonadotropic releasing hormone (GnRH), which stimulates the pituitary to synthesize follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones regulate the synthesis of progesterone, estradiol, and testosterone. Through the production of FSH and LH, the synthesis (positive and negative feedback) of sex hormones and gonadal development are regulated.

Thyroid hormones are important in the structural and functional development of sex organs and the brain, both intrauterine and postnatal. They are

synthesized by the thyroid gland under stimulation through the thyroid stimulating hormone produced by the pituitary. The form that is most important as a biological parameter is thyroxine (T4, tetraiodothyroxine).

Disorders in Hormone Concentrations

A negative correlation has been observed between testosterone levels and tissue concentrations of DDE and possibly PCBs in Dall's porpoises (Subramanian et al. 1987). In a semi-field experiment with harbor seals, Reijnders (1986, 1990) found that lower levels of 17ß-estradiol occurred around the time of implantation. A possible explanation for the observed lower hormone levels in both Dall's porpoises and harbor seals would be an increased breakdown of steroids as a consequence of PCB- or PCB metabolite-induced enzyme activity. Enhanced steroidogenesis caused by some metals and a PCB mixture (Arochlor 1254) has been demonstrated *in vitro* for gray seals (Freeman and Sangalang 1977). Furthermore, increased metabolism of PCBs as a result of P450-enzyme induction has already been demonstrated in marine mammals (Tanabe et al. 1988, Boon et al. 1992). A second explanation might be that PCB and DDE, or metabolites thereof, bind to hormone carrier proteins and/or hormone receptors. If such binding were to occur, either tissue metabolism of steroids would be hindered or binding of steroids to receptor proteins in target tissue would be impeded. It is conceivable that both mechanisms — increased steroid breakdown and the binding of xenobiotic compounds to carrier proteins or hormone receptors — could operate in tandem.

Figure 5. Major steroidogenic pathways in mammalian endocrine tissues. Black arrows indicate P450-mediated conversions.

With respect to the first mechanism, Troisi and Mason (1998) found experimentally that rates of progesterone and testosterone metabolism in harbor seals were negatively correlated with both PCB concentrations and the level of P450-enzyme induction. Applying the findings by Troisi and

Mason (1998) to Dall's porpoise, the lowered levels of testosterone reported by Subramanian et al. (1987) could be explained by a hindrance in the transformation of precursors for testosterone by PCB or DDE metabolites. This postulated explanation obviously needs further testing.

Concerning the second mechanism, it is known that PCB metabolites, in particular PCB-methyl sulfones, bind to uteroglobin (Patnode and Curtis 1994). Since we did not find any receptor interference in *in vitro* pilot experiments with harbor seal blood, this mechanism is not considered a likely explanation for the observed reproductive failure in this species. The finding by Troisi and Mason (1998) of decreasing metabolism of progesterone and testosterone in liver microsomes does not allow a conclusion about a possible impediment of the transformation of progesterone and testosterone, that might in turn have led to the lower estradiol levels observed in harbor seals. This is because those transformations occur in the reproductive organs. However, it has been found by Funae and Imaoka (1993) that sex-dependent cytochrome P-450 isoenzyme patterns exist. It is known that induction of CYP1A(2) causes increased hydroxylation of estradiol, leading to enhanced excretion and hence lower levels of estradiol. It has been demonstrated that induction of CYP1A is significant in harbor seals (Boon et al. 1987). I postulate that enhanced breakdown of estradiol, through enzyme-induced metabolism by organochlorines, is a plausible mode of action to explain the lower levels of estradiol observed in harbor seals. Estradiol has a priming effect on the proliferation of the endometrium, in effect preceding proliferation of the luminal and glandular epithelium under the influence of progesterone. The lower levels of estradiol could have impaired endometrial receptivity and prevented successful implantation of the blastocyst. Further studies are planned to investigate this possibility.

Decreased levels of thyroid hormones have been found in harbor seals (Brouwer et al. 1989) as a consequence of competition between a hydroxylated metabolite of PCB-77 and thyroid hormones for binding to a transport protein, transthyretin (TTR). Such a hypothyroid condition can have significant effects on early development and later reproductive performance. Fetal accumulation of hydroxylated PCB-metabolites has been found to occur in experimental animals and may also occur in seals (Brouwer et al. 1998). Given that thyroids are involved in the development of Sertoli and Leydig cells (spermatogenesis), brain development, and early development of the sex organs, further research in this area is warranted.

Green et al. (1996) and Green (1997) provide additional information on lactational transfer of PCB-methyl sulfones (PCB-MSFs) from gray seals to their offspring. They report that the summed concentrations of PCB-MSFs (in lipid) are approximately 5% of the total PCB concentration. A similar ratio was found in seal milk. The uptake of PCB-MSFs is therefore quantitatively important. Moreover, the pups excrete only approximately 0.5% from the amount they ingest. In contrast to the mobilization of PCB-congeners from maternal blubber to milk, which is negatively correlated with congener-lipophilicity, the metabolization of PCB-MSFs is independent from the degree of chlorination as well as the chlorination pattern. The ratio PCB-MSFs/total PCBs is therefore higher in milk than in blubber. This metabolization process, offering a certain protection against the more lipophilic PCBs, obviously does not work in the case of the PCB-MSFs. The significance of this finding remains unclear.

Reproductive Disorders

Clear cases of hermaphroditism were observed in about two out of 120 examined beluga whales in the St. Lawrence River (De Guise et al. 1994, P. Béland, pers. comm.). This condition has been attributed to hormonal disturbance in early pregnancy, whereby normal differentiation of male and female organs was disrupted. Research is ongoing to test that hypothesis and to acquire information on the underlying mechanism.

In examined mature female Baltic seals, 30% of the gray and 70% of the ringed seals exhibited partial or complete sterility, caused by stenosis and

occlusions. Recent studies suggest that PCB- and DDE-methyl sulfones are the toxic compounds responsible for these abnormalities (Olsson et al. 1994). A plausible hypothesis is that early pregnancy is interrupted, perhaps via decreased uteroglobin binding due to the methyl sulfone-occupation or low hormone levels, followed by development of pathological disorders. Toxic effects in several steps in the brain-hypothalamic-hypophyseal-adrenal-placental axis could be involved in the latter stage (Reijnders and Brasseur 1992). Further investigations are needed to elucidate this phenomenon. DeLong et al. (1973) found premature pupping in California sea lions to be associated with high PCB and DDE levels. The concurrent finding in the animals of pathogens with known potential to interfere with pregnancy rendered it impossible to attribute causation specifically to either of the organochlorines.

Abnormal testes — transformation of epididymal and testicular tissue — have been observed in North Pacific minke whales (Fujise et al. 1998). A possible relationship with levels of organochlorines has been postulated. Further histological examinations and pathology assessments are being conducted to investigate this phenomenon.

Other Hormone-Related Disorders

Besides sterility, a suite of pathologies and disorders have been observed in Baltic seals. These include exostosis in harbor seal skulls (Mortensen et al. 1992) and osteoporosis in gray seal skulls (Olsson et al. 1994). Similar disorders have been found in harbor seals in the Wadden Sea (Stede and Stede 1990). This disease complex is characterized as hyperadrenocorticism. It is unclear whether hyperadrenocorticism is manifested in early development.

The research field between reproductive biology and immunology is in an early stage of development. It is known that both the humoral (antibodies) and cellular (lymphocytes) aspects of the immune system are regulated by estrogens and androgens (Grossman 1985). Disruption in steroid hormone balance might therefore lead to malfunctioning of the immune system. Of relevance in the present context is the role of progesterone and estradiol in preventing the maternal-fetal rejection response. This relationship could help explain the observed problems of harbor seals in the Wadden Sea as these problems occurred at around the time of implantation. Also, corticosteroids (Wilckens and de Rijk 1997) and thyroid hormones (Brouwer et al. 1989, 1998) are involved in immune functioning. The possible effects of xenobiotic-caused thyroid and corticosteroid hormone imbalances on early development and reproduction are insufficiently known.

Monitoring and Evaluation of Effects and Related Research Needs

There are serious impediments to monitoring and evaluating hormone-related xenobiotic effects. Firstly, the majority of the present tests do not measure transgenerational influence, yet several disorders occur only in the adult stage or offspring. Secondly, gene *expression* is affected, not gene *constitution*. Therefore, mutagenicity endpoint tests are not particularly relevant. Thirdly, many tests are *in vitro*, and this complicates investigations of disruption in neurobehavioral function and reproductive morphological development. Finally, some xeno-estrogens only become biologically active after *in vivo* metabolism (e.g., methoxychlor).

Development of hormone-responsive cell cultures as biomarkers could provide a partial solution (see Colborn et al. 1993). Biomarkers are available to measure exposure to xenobiotics, including xeno-estrogens (Fossi 1994). A series of recent studies describe techniques to investigate metabolism of PCBs, PCDDs, PCDFs, and toxaphenes in marine mammals (Troisi and Mason 1997, Boon et al. 1997, 1998, Letcher et al. 1998). These provide opportunities to measure exposure of marine mammals to, for example, xeno-estrogens and other endocrine-disrupting contaminants. However, preparatory research has to be carried out to adapt existing biomarker protocols for use in marine mammal studies. This includes sampling

tissue, particularly of neonatal and juvenile animals, to analyze for (1) thyroid hormones and vitamin A (important in cell differentiation) in blood and brain; (2) thyroid hormones and vitamin A in brain and liver; (3) estrogen-receptor binding capacity of ovarian, brain, and liver tissue; (4) glial fibrillary acidic protein and synaptophysins in brain; (5) P450-enzyme induction (i.e., CYP-1A) in liver, brain, and uterus; and (6) levels of xeno-estrogens in blubber, blood, liver, and brain.

To enhance evaluation of the results, in terms of biological significance, it is important to carry out investigations and compare trends in animals from relatively unpolluted as well as highly polluted areas. Initially, a choice has to be made for model compounds as well as for model species occurring over a gradient of pollution. Studies in relatively clean areas serve mainly to obtain reference values for exposure to contaminants and indicators for the status (functioning) of the studied population. Studies in highly polluted areas make it possible to carry out research on pathology in neonatal and juvenile animals. In combination with analyses for contaminants and associated physiological and pathological responses, these studies will facilitate development of a *multiple response concept* as described by Reijnders (1994) and Reijnders and de Ruiter-Dijkman (1995). The development of techniques to extrapolate observed individual responses, and thereby evaluate population-level and possibly ecosystem-level effects, is equally important.

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Contaminants and Marine Mammal Immunotoxicology and Pathology

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Recent mass mortality of marine mammals in different locations, involving a number of different species, has attracted public attention and prompted scientific investigations (Geraci et al. 1982, Osterhaus et al. 1988, Kennedy et al. 1991, Domingo et al. 1990). Consistent findings of relatively high tissue concentrations of environmental contaminants have raised questions regarding the role of these compounds in the development of disease processes. In particular, there has been concern about the host's ability to develop a protective immune response against pathogens. Contaminant-induced immunosuppression was proposed as a possible explanation for the high incidence, diversity, and severity of lesions caused by opportunistic and mildly pathogenic bacteria in beluga whales from the St. Lawrence estuary in Canada (Martineau et al. 1988, De Guise et al. 1995). Although many contaminants of the marine environment (organochlorines, heavy metals, polycyclic aromatic hydrocarbons) are well characterized as immunotoxicants in laboratory rodents, it is a significant challenge to demonstrate immunotoxic effects on marine mammals exposed to these agents. Among the reasons are that, for marine mammals, (1) the existing immunologic database is limited, (2) few assays have been developed and there is limited availability of reagents with which to evaluate immune function, (3) most natural populations are outbred and therefore have considerable genetic diversity, and (4) logistical and ethical considerations must be taken into account.

Marine mammal immunology is a relatively new, rapidly evolving discipline. Its importance has become more widely appreciated in recent years for at least two reasons. One is the realization that the immune system plays a central role in general health. The other is the realization that immune function can be influenced by factors such as pollution or stress. It is important to understand the complexity of the immune system and its functions, and to recognize that no single assay can evaluate the immune system as a whole. Different assays can evaluate different components, mechanisms, or functions. Some laboratories have committed themselves to developing and standardizing assays and reagents to evaluate particular aspects of the marine mammal immune system, e.g., humoral, cell-mediated, or non-specific immunity. Methods such as cryopreservation have been validated in order to standardize the collection and transportation of samples, regardless of when, where, or from what population they have been obtained. This facilitates simultaneous analysis and enhances the possibilities for making valid comparisons.

Direct determination of the effects of environmental contaminants on the immune system of wild marine mammals is made difficult by logistical and ethical considerations. Nevertheless, several approaches have been used. *In vitro* exposures of beluga immune cells to heavy metals and organochlorines demonstrated the susceptibility of those cells to toxic compounds at levels comparable to levels found in the tissues of wild belugas (De Guise et al. 1996, 1998). When immune cells from belugas were exposed *in vitro* to mixtures of organochlorines, at levels at which the individual compounds would have had no effect on immune function, synergistic effects were observed (De Guise et al. 1998). This observation called attention to the often overlooked or unsuspected dangers posed by environmentally relevant complex mixtures.

Another approach is to use animal models. For example, the potential immunotoxicity associated with exposure to complex mixtures of contaminants has been assessed by feeding one group of rats highly contaminated blubber from St. Lawrence belugas and another group much less contaminated blubber from Arctic or Subarctic belugas (De Guise et al. unpublished data). Similarly, one can compare groups of rats fed either oil extracted from highly contaminated Baltic Sea fish or oil from much less contaminated Atlantic Ocean fish (Ross et al. 1997). The *in vivo* effects of exposure to environmentally relevant concentrations of pollutants were demonstrated experimentally in semi-field conditions where one group of harbor seals was fed highly contaminated Baltic Sea fish and another group was fed much less contaminated Atlantic Ocean fish (De Swart et al. 1994). The animals fed Baltic Sea fish exhibited impaired natural killer (NK) cell activity and T-lymphocyte function (de Swart et al. 1994) as well as delayed-type hypersensitivity (Ross et al. 1995). These experimental study designs probably provide the best means of investigating the effects of exposure to environmental contaminants through food consumption. The perinatal transfer of contaminants (through the placenta and milk) that occurs in nature, and that affects the highly vulnerable developing immune system, is not accounted for, however, in such semi-field experiments.

Field studies of wild populations of marine mammals are logistically difficult. However, in a study of free-ranging bottlenose dolphins in Florida, decreased lymphocyte proliferation was associated with increased blood concentrations of organochlorines (Lahvis et al. 1995). In Alaska, northern fur seal pups were captured, studied, and released. Mitogen-induced proliferation of blood lymphocytes, along with other immunoassays and health parameters, were evaluated and compared with whole-blood levels of organochlorines. Lymphocyte proliferation was negatively correlated with increasing concentrations of nine PCB congeners (Beckmen, unpublished data). Moreover, pups of primiparous dams, having the highest measured blood levels of organochlorines, had significantly lower antibody production in response to vaccination with tetanus toxoid than pups of old dams, with low blood levels of organochlorines (Beckmen, unpublished data).

The impact of environmental contaminants on the health of wild marine mammal populations cannot be fully understood until the effects of those contaminants on the immune response have been properly assessed. Deleterious effects on immune functions have been observed in marine mammals *in vivo*, as well as in animal models and in marine mammal cells *in vitro*. Future studies should be directed toward contaminant exposure at critical life stages, such as at and near the time of birth, and effects on the developing immune system. Identification of a subpopulation or cohort at greatest risk (highest exposure and critical life stage) can help focus research and diagnostic efforts to determine whether adverse health effects are ongoing or imminent. In addition, studies of the "normal," protective marine mammal immune response to common pathogens should provide a better understanding of the relationship between a potentially suppressed immune system and the development of diseases (possibly including epizootics).

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Assessing the Risks to Marine Mammal Populations from Exposure to Contaminants

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Introduction

A well-defined methodology has been developed for assessing the risks to human populations associated with exposure to contaminants. Methods derived from environmental epidemiology and environmental health policy (Hertz-Picciotto 1996) are used to estimate the risks of exposure and the likely response to exposure, taking account of the characteristics of humans who have been exposed and the environmental variables which are associated with exposure (Stallones 1988). In many cases, exposure probabilities and responses cannot be estimated directly because the potentially exposed group is too small, or there is a long latency period between exposure and response. As a result, estimates of risk are often based on the shape of dose-response curves derived from experimental studies of laboratory animals which are believed to be suitable surrogates for humans.

The assumptions made in this risk assessment process are often not directly testable, yet the needs of policy makers are usually so great that this approach is considered to be an acceptable way of evaluating the health costs of different actions. The U.S. National Research Council developed a conceptual framework (National Research Council 1983) for the process of risk assessment (Figure 6) that has been widely accepted. This framework has been extended by the U.S. Environmenal Protection Agency (EPA 1992) so that it can be used to assess the risks to the environment from contaminant exposure, and Monte Carlo techniques can be used to take account of uncertainties in assumptions about parameter values and processes (e.g., Cohen et al. 1996).

However, there is a fundamental difference between the two approaches. Human risk assessment is primarily concerned with the risk to *individuals* from exposure. Environmental risk assessment usually requires an additional step, that is estimating the consequences of the individuals' responses to exposure for the *dynamics* of their population, and, ultimately, the consequences of these changes in population dynamics on ecosystem functions. The impacts of contaminants on individual animals or plants may, of course, be considered important when they involve species (like many marine mammals) which have enormous popular appeal, or endangered species (where the loss of even a few individuals could prejudice survival or recovery).

In this document we consider how the EPA risk assessment approach can be used to evaluate the potential impacts of exposure to specific contaminants (or combinations of contaminants) on marine mammal populations. We assume that potentially hazardous compounds have been identified, and focus our attention on exposure and

exposure-response assessment, and on risk characterization (Fig. 6). We show how mathematical models developed to understand the effects of parasites on the population dynamics of their hosts can provide useful insights, and help in the estimation of key parameters.

Exposure Assessment

We can obtain some information about exposure from surveys of the concentration and total burden of contaminants in the bodies of animals found dead or killed for other purposes. However, for contaminants which accumulate in particular tissues – and many of the contaminants of concern for marine mammals fall into this category – such measurements do not provide a direct indication of exposure. Thus it is necessary to model the ways in which the contaminants have accumulated. There is a large body of literature that documents models of the kinetics of contaminants in animal tissues (see, for example, Walker 1990), but many of the parameters of these models are difficult to estimate and likely to be species-specific.

We propose an alternative approach using stochastic models developed to describe the accumulation of macroparasites in their hosts (Tallis and Leyton 1966, 1969). Such models have not been used widely in parasitology because their assumptions are usually considered too restrictive (see, for example, Roberts et al. 1995). However, most of the assumptions are met in the case of exposure to contaminants. The distribution of contaminant burdens (and not the distribution of concentrations,

Figure 6. The U.S. National Research Council conceptual framework (National Research Council 1983) for the process of risk assessment, extended by the U.S. Environmental Protection Agency (EPA 1992) to assess the risks to the environment from contaminant exposure.

which is what is normally reported in the literature) among "hosts" is a function of exposure to contaminated prey, the distribution of contaminant burdens in prey, the rate at which contaminants are

metabolized by the "host," and contaminant-induced mortality. Tallis and Leyton were able to show that, even if the distribution of parasites in a vector is symmetrical, the distribution of parasites in their hosts is likely to be skewed, and will in many cases follow a negative binomial distribution. There is a great deal of data (summarized in Hudson and Dobson 1995) suggesting that parasite burdens often provide a good fit to the negative binomial. However, concentrations of contaminants are continuous variables and more often conform to a lognormal distribution, suggesting that accumulation is the result of a number of processes whose effect is multiplicative.

The use of models of this kind allows us to predict the distribution of contaminant burdens in marine mammal populations from information on their diet and the distribution of contaminants in their prey. Although such information is not easy or inexpensive to acquire, it can be obtained much more readily than information on the total body burden of a large and representative sample of marine mammals.

Exposure-Response

The next stage in the risk assessment procedure is to estimate the effect of a specific level of individual exposure on the probability of survival or fecundity for that individual (i.e., we need to know the form of the dose-response curve). As with humans, it is rarely practicable to estimate such curves directly. Instead, we must rely on analogy with related species to provide insight into the form of the dose-response relationship.

We may be able to obtain further insight from an analysis of the distribution of contaminant burdens or concentrations in "hosts." Contaminant-induced mortality does affect the observed distribution of contaminants in survivors, but the effects of different exposure-response relationships are relatively small and would be extremely difficult to detect, even with (unreasonably) large sample sizes (Fig. 7, upper). However, the distribution of contaminants in animals that die (for example during mass mortality events induced by viral infection, in which contaminants are believed to play a contributory role) depends on the form of the exposure-response curve (Fig. 7, lower). This suggests that a more detailed analysis of the distribution of contaminants in animals found dead could be useful.

Risk Characterization

In order to complete the risk characterization we need some insight into the potential effects of contaminant exposure on "host" populations. This can be obtained by analyzing simple mathematical models of the processes involved. In such models, changes in "host" abundance are assumed to depend on the "host's" maximum potential rate of increase, the nature of self-regulation within the "host" population, and the mortality (or reduction in fecundity) induced directly or indirectly by contaminant exposure. Changes in contaminant burden are assumed to depend on the rate at which contaminants enter the environment, the rate at which they are metabolized by "hosts," and the input of contaminants from "natural" and contaminant-induced "host" death.

For contaminants which do not accumulate, it can be assumed that concentrations in tissues reflect exposure. Hallam et al. (1983a, b) developed deterministic models which can be used to investigate the response of marine mammal populations to non-bioaccumulating contaminants, and Gard (1990) extended these models to take account of stochasticity. The basic equations are

$$dM/dt = M(r_0 - ß(p) - kM)$$
$$dp/dt = q - (b+z)p - ß(p)$$

where M is the abundance of the marine mammal, r_0 is the rate of increase at low population density, p is the average concentration of contaminants in

Figure 7. An analysis of the distribution of contaminant burdens or concentrations in "hosts"; the effects of different exposure-response relationships are relatively small and would be extremely difficult to detect, even with large sample sizes (upper figure); the distribution of contaminants in animals that die depends on the form of the exposure-response curve (lower figure).

the marine mammal population, ß(p) is the exposure-response relationship, r_0/k is the equilibrium size of the marine mammal population in the absence of contaminants, q is the rate at which contaminants enter the environment, and b and z represent natural mortality and metabolism of contaminants, respectively.

For contaminants which accumulate, "host" response is likely to be related to the burden of contaminants in individual marine mammals. In this case, equations first developed by Anderson and May to model the effects of macroparasites on their hosts (Anderson and May 1978, May and Anderson 1978) are more appropriate:

$$dM/dt = M(a - b - kM) - ß(P)$$
$$dP/dt = qM - (b+z)P - ßME\{i^2\}$$

In these equations, P is the total quantity of contaminant in the "host" population, and E{i} is the expectation that an individual marine mammal will have a burden i. This model therefore takes explicit account of the observed distribution of contaminant burdens. If this distribution is log-normal, $E\{i^2\} = (P/M)^2$; if it is Poisson, $E\{i^2\} = (P/M)^2 + P/M$; and if it follows the negative binomial, $E\{i^2\} = (P/M)^2 (k+1)/k + P/M$. Other parameters represent the same quantities as in the Hallam model.

Both models predict that the presence of contaminants can reduce the rate of recovery of a depleted population, and reduce its equilibrium size. The magnitude of these effects is directly related to the size of q and the form of ß.

Another potentially fruitful approach here is to adapt moment methods, developed for macroparasites to approximate the stochastic dynamics of pollutants without assuming an empirical distribution (Grenfell et al. 1995.)

Multiple Contaminants and Multi-Species Interactions

The Anderson and May model can be modified relatively easily to take account of the effects of combinations of contaminants (see, for example, Dobson 1985), and their additive, multiplicative, and interactive effects on "host" mortality.

It is also possible to expand these models to take account of the fact that contaminants are ingested by many species in the marine ecosystem. Such multiple-host, multiple-parasite models are in their infancy, but it is widely recognized that this will be a major growth area in epidemiological research over the next decade. An interesting implication from analyses of this kind is that an increase in the abundance of marine mammal species which are less susceptible to the effects of contaminants may benefit more susceptible species (provided that there is not competition between them for other resources). This is because the less susceptible species "lock up" or sequester a significant proportion of contaminant inputs, thus reducing the exposure of more susceptible species. The large quantities of organochlorine contaminants which accumulate in the bodies of sperm whales (Law et al. 1997), for example, may serve this purpose.

Implications For Future Research

We suggest that the considerable insight into the potential effects on marine mammals of exposure to contaminants can be obtained using mathematical models originally developed to analyze the effects of macroparasites on their hosts. In addition, analysis of the distribution of contaminants in animals found dead, particularly after mass mortality events in which contaminants are implicated, can provide much needed information on the form of the relationship of response to exposure.

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Overview of Federal and International Programs on Contaminants in Marine Mammals

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Many marine mammal species bioaccumulate a variety of environmental contaminants as a consequence of feeding at high trophic levels in the food web. Similar accumulation of toxic substances has been associated with deleterious health effects in a range of wildlife. Numerous state, federal, private, and international programs address contaminant issues in marine mammals. Such programs can focus on the effects on individual species, ecosystems, habitat quality, or human health, and they can involve research, monitoring, surveys, or the archiving of data or tissues. This paper provides an overview of several prominent federal and international programs which directly, or indirectly, involve investigative activities relating to environmental contaminants and marine mammals. We also discuss potential avenues for scientific coordination.

Most U.S. federally funded marine mammal programs that address contaminants and their effects reside in the Department of Commerce (DOC), the Department of the Interior (DOI), and the Environmental Protection Agency (EPA). Both the DOC and DOI have trust responsibilities for marine mammals and their ecosystems, whereas the EPA has regulatory authority for the protection of the natural environment and human health. The DOI's Fish and Wildlife Service (FWS) has stewardship responsibility for sirenians, sea otters, walruses, and polar bears. All other marine mammals in U.S. waters fall under the jurisdiction of the DOC's National Marine Fisheries Service (NMFS).

Two agencies within the DOC are most directly involved in research, monitoring, and assessment of contaminant levels and biological effects in marine mammals. These are the National Oceanic and Atmospheric Administration (NOAA) and the National Institute of Standards and Technology (NIST). NOAA has management responsibilities related to the status and protection of marine mammal populations and stocks as well as their habitats, under provisions in the U.S. Marine Mammal Protection Act of 1972 (MMPA) and subsequent amendments. NMFS is the principal entity in NOAA entrusted with scientific missions for assessing environmental effects, including anthropogenic activities, on these protected resources. NIST, in collaboration with NMFS, (1) provides analytical support and technical cooperation for contaminant quality assurance analyses through the National Marine Analytical Quality Assurance Program, and (2) archives marine mammal tissues in the National Marine Mammal Tissue Bank as part of the National Biomonitoring Specimen Bank (NBSB). NOAA's National Ocean Services complements NMFS missions through trustee-related damage assessments or other analyses associated with its coastal monitoring and

benthic surveillance.

In response to monitoring requirements specified in the MMPA, NMFS initiated a long-term, multidisciplinary program called the Marine Mammal Health and Stranding Response Program (MMH&SRP). It is a national program that involves partnerships within NOAA and other federal agencies and with numerous state and local governments and non-government organizations. A major purpose of the MMH&SRP is to investigate biological linkages between chemical contaminants in the marine environment and specific population health indices or parameters in key populations. Information about the bioaccumulation and distribution of toxic contaminants is derived from samples obtained from marine mammal strandings, subsistence hunts, bycatch, die-offs, live capture-release programs, biopsy programs, and disease investigations. Quality assurance is emphasized in the MMH&SRP, and participation in the program requires adherence to standard protocols in the field and state-of-the-art analytical techniques in the laboratory. Research collaborations typically include projects where complementary biological observations on individual and population health are available with tissue collections.

Two bureaus within the DOI conduct research and monitoring with specific objectives for study of environmental contaminants in marine mammals. Under the MMPA, FWS has management authority for certain marine mammals and conducts species-specific or site-specific studies through its Marine Mammal Management and Ecological Services offices. As an example, FWS is presently studying contaminants in polar bears and sea otters in Alaska. The FWS projects tend to be issue-oriented surveys to establish baseline conditions. The U.S. Geological Survey, through the Biological Resources Division (BRD), is involved in a variety of research projects that address DOI and other partner needs regarding managed populations, their habitats, and ecosystems of concern. The BRD research projects tend to be population-oriented and three to five years in duration. An exception is the Arctic Marine Monitoring and Trends Assessment Program, or AMMTAP (formerly the Alaska Marine Mammal Tissue Archival Project) which was initiated in 1987 to develop collection protocols for sampling and to provide long-term archiving of marine mammal tissues. The AMMTAP has a long history of research and administrative collaboration with NOAA, NIST, FWS, the DOI's Minerals Management Service, the State of Alaska, and various non-government organizations.

Over the last two decades, the EPA has performed chemical analytical studies on tissues from marine mammals and other marine organisms for specific studies or specific sites. As an example, the EPA participated in the investigation of die-offs of bottlenose dolphins along the Atlantic coast of the United States in 1987–1988. In related follow-up investigations, the EPA has completed several additional studies on bottlenose dolphins, particularly in the coastal zone of the eastern United States. The agency is presently administering several food-web and subsistence-oriented studies in Alaska.

Numerous non-government organizations and international agencies have funded, are currently funding, or are planning relevant contaminant research on marine mammal populations of interest to U.S. management and tribal authorities or international management bodies. These include such organizations as the Arctic Monitoring and Assessment Project (AMAP), the International Whaling Commission, the United Nations Environment Programme, the North Slope Borough, and the World Wide Fund for Nature. Other international organizations also perform specific contaminant studies and have long-term biomonitoring and specimen-banking programs. Within the United States, major universities have established cooperative research programs (e.g., University of Alaska's Center for Arctic Research) and sponsor directed research to answer specific questions about contaminant pathways, processes, and probable effects.

A common objective of many research and monitoring programs has been to determine baseline levels of heavy metals and persistent organic pollutants in marine mammal populations. Few

programs have adequately addressed the numerous experimental design issues that inevitably arise in studies of contaminant trends. For example, quality control and assurance are usually handled at the laboratory or study level. This has resulted in numerous problems with data comparability and repeatability, both ingredients of good science. As federal and state agencies adopt the precepts of ecosystem management, there is a growing need to coordinate, wherever possible, research, monitoring, and quality-assurance programs at national and international scales to better address questions about contaminant origins, transfer, biological fate, and impacts on marine mammals. The coordination among NOAA, NIST, and USGS is illustrated by the respective agency efforts in the MMH&SRP, NBSB, and AMMTAP in Alaska and by their contributions to the AMAP. In the future, government and non-government organizations should work collaboratively to address issues related to contaminants in marine mammals.

Overview of Health, Ecological, and Economic Dimensions of Global Change: Tracking Marine Disturbance and Disease

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We introduce the concept and discuss the potential of a world-wide system for reporting and documenting marine disease and mortality events. This information can be useful for determining causes, trends, impacts, costs, and environmental conditions. It is generally recognized that morbidity and mortality of marine organisms occur frequently, from both natural and anthropogenic causes, and that reported events represent only a fraction of the number that occur. Nevertheless, investigation of such events in the United States is an important function of the Marine Mammal Stranding Network, the Sea Turtle Stranding Network, and resource agencies responsible for managing fish, shellfish, corals, waterfowl, and other coastal organisms. Regardless of its limitations, this work is expected to generate valuable information related to public health, resource management, and environmental conditions.

Several important questions are triggered by marine mortality events, especially those that involve large numbers of organisms or a "sensitive" (e.g., endangered or threatened) species. Chief among these are the following: What was the cause? Was it related to disease, lethal exposure to a contaminant, physical trauma, or some other factor? There is also interest in knowing about aggravating factors, or contributing stressors. For example, did rapid changes in temperature or salinity, high contaminant burdens, or sublethal levels of a biotoxin contribute to the situation? And there are immediate concerns for human health. Have coastal residents been exposed to contaminants, toxins, or pathogens, either directly through air and water or indirectly through the food chain? Other questions raised by marine mortality events are broader in scope. Are such events becoming more frequent, and are more organisms being affected (trend over time)? Are events becoming more frequent or more serious in certain regions (trend over space)? Has the event or series of events had a significant, long-term impact on population abundance or viability? Does the event represent a deterioration in ecological conditions? What was the economic cost of the event — to fisheries, tourism, and local economies? And if ecological deterioration is a factor, what future costs might be expected?

Obviously, many of these questions cannot be answered by a forensic examination of individual organisms. Few investigations of marine mortality include anything more than such an examination, which is used primarily to determine cause. Perhaps

we are predisposed to this individual-oriented approach because so many of the disciplines represented in the investigations are based in the science and technology of human health (e.g., pathology, microbiology, immunology, etc.), where the well-being of individuals are a primary focus. For non-human species, however, concern for individuals is more often superceded by population issues, as reflected in the questions above. Measurements at the population level fall within the domains of epidemiology (human) and epizootiology (non-human organisms), applied scientific disciplines that examine the range, extent, rate, frequency, and duration of disease or mortality within a defined population.

We believe that a comprehensive epizootiological approach is needed to extract some of the most important information from marine morbidity and mortality events. To be successful, this approach should be tailored to provide comparisons of mortality or morbidity events with other major marine events (such as harmful algal blooms or mortalities of other species) and anomalies (such as climate change, weather, and freshwater intrusions). Most often, events are investigated as though all relevant stressors were generated at the local level, even though they may have originated from, or at least been influenced by, regional or global conditions. To ascertain which conditions are most closely linked to marine mortality events will require that data from local sources be compiled and set in a regional or global context. Thus, our approach must be able to accommodate relevant time scales (decades or longer) and spatial scales (ecosystem to global).

This proposition carries with it several challenging requirements, foremost of which are the collection of high-quality data (from a consistent reporting effort and with standardized reporting criteria) and a global framework for storing and relating complex information. Potential prototypes for meeting these requirements are provided by the two regional programs described below.

High-Quality Data: The Gulf of Mexico Aquatic Mortality Network

High-quality data are critical for interpreting marine morbidity and mortality, yet reporting efforts vary dramatically from place to place and over time. The lack of consistency undermines the credibility and usefulness of the data, particularly when the times and places of non-events (i.e., when and where events do not occur) are so important to interpretation. Additionally, those who report, document, or respond to mortality events often have differing standards, credentials, and motives. These difficulties have been recognized and confronted by a group of resource managers in the northern Gulf of Mexico. The Gulf of Mexico Aquatic Mortality Network (GMNET) is a collaboration among U.S. state and federal agencies to improve reporting and interstate communication regarding mortality events, to standardize response approaches and documentation, and to promote research in forensic pathology across the five states bordering the Gulf of Mexico. A long-term goal is to develop indicators of ecological conditions for the Gulf of Mexico based on mortality and disease monitoring data. Information on current mortality events and items of recent interest such as the 1996 manatee die-off associated with red tide can be found at the GMNET web site (http://pelican.gmpo.gov/gmnet/)

Several attributes of GMNET are instructive with respect to obtaining high-quality data for a global epizootiological approach. Consistency and quality are facilitated by the involvement and accountability of professionals employed by each state to investigate mortality events. These resource managers have adopted standard investigative procedures. Consistency is fostered through periodic interstate training exercises. In addition, a three-tiered data-entry format provides essential information (Tier 1: what, when, where, how many, and why); environmental information related to probable cause (Tier 2: water quality, lesions, weather conditions, etc.); and state-privileged information (Tier 3: chain of custody, litigation information, etc.). Only the first two tiers are shared in the common database, and the states maintain control of their own data. The three tiers

allow all states to use the same format while retaining the flexibility to control some types of information. The regional format of GMNET is another attribute that might serve a larger program. GMNET has been organized and implemented by group members because of their shared interests and their recognition of the value of neighboring-state interactions. The GMNET program was introduced to resource managers in 12 additional states along the east coast of the United States, and they overwhelmingly accepted the concept and its future expansion to their states. A global program might similarly be initiated in selected regions to overcome the greatest obstacles, and then be expanded afterward.

Documentation Framework: The Health, Ecological, and Economic Dimensions of Global Change Program

The value of morbidity and mortality data generated from a global reporting network cannot be estimated until it is examined in an epizootiological framework, which requires a relational database capable of holding large and complex datasets. Existing data archives are limited in extent (spatial and temporal), limited in coverage (e.g., marine mammals only), not always systematically indexed, and not always available to the public. Current databases have limited potential for comparisons with other databases and limited potential to integrate co-occurrences, trends, and models.

A relational database for marine disease and mortality events will be most effective if constructed using time and space (place) as indices, or cataloging tools. All events have these two factors in common. Such a system will demonstrate co-occurrences with other events and anomalies in the same database, reveal trends across time and space, and allow comparisons with other datasets that are cataloged by time and space, including population models (stock assessments, migration patterns), ecosystem models (habitat suitability, trophodynamic interactions, climate), and economic models (marketing, tourism). In addition, the use of time and space to catalog marine events makes it possible to apply the relatively new and powerful tool of Geographic Information Systems (GIS). These systems employ time and space indices to facilitate the management and analysis of large and complex datasets. A GIS provides a visual display of nearness and potential association among occurrences and anomalies (hypothesis generation), provides organizational structure to complex datasets through a point-and-click ability to retrieve complete event records from a map, and ultimately will provide analytical and statistical tools for epizootiological research. Once digitized, GIS can examine data for counts per area, frequency, distance from point sources, proximity, clustering, trends, and a variety of current and future mathematical and interpolative analyses.

The Health, Ecological, and Economic Dimensions of Global Change Program (HEED Program) (http://heed.harvard.edu) has recently developed a relational database to retrospectively compare major marine ecological disturbances, including infectious disease epizootics, mass mortality events, harmful algal blooms, and anomalous changes in marine species abundance and composition. The objectives of the program include detection of declines in ecosystem health, achieving a better understanding of the causes and consequences of environmental change, evaluating the ultimate impact on human health, and estimating market-related costs. The HEED Program links data from disparate ecological, climatic, and economic datasets to identify co-occurrences of recognized anomalies. Datasets include (1) biological events, e.g., diseases and deaths of marine organisms, harmful algal blooms, and marine-related human diseases, (2) climate anomalies, and (3) biophysical characteristics (e.g., water quality, hydrology). Data from the last 40 years, ranging geographically from Labrador (Canada) to Venezuela and including the Caribbean and the Gulf of Mexico, were obtained from science journal articles, mass media, government reports, and interviews with scientists and resource managers. This retrospective database includes event data for marine mammals, corals, seagrasses, bivalves, sea turtles, marine birds, fish, invertebrates, algae, and human health (related to

marine events). These have been integrated into a GIS that assists in hypothesis generation and supports ecosystem and economic models.

The relational framework for documentation of events supports an epizootiological approach, and that is the primary attribute of the HEED Program that can be applied to a global mortality monitoring system. The ability to compare with related factors in the same database and with external databases using a GIS format is highly desirable. Although currently holding only historical data, the framework is a promising prototype for documentation of future events. It may also be scaled from a regional framework to a global framework.

A Convergence of Prototypes

The GMNET and HEED programs offer potential resolutions to the foremost challenges of developing a global marine morbidity and mortality database that can be applied in an epizootiological perspective. We believe that the concept of having trained, accountable personnel use standardized procedures and data-entry formats, as in the GMNET program, can be duplicated at a larger scale (continent or large marine ecosystem) to provide consistent, high-quality data. A tiered data-entry format can provide continuity in reporting while retaining flexibility for participating countries. Reporting in a global system would have to be limited to major marine events and not include all of those reported in the GMNET program. A global network might be attained through sequential additions of regions, such as those defined by the Large Marine Ecosystem (LME) Program. The HEED documentation framework was designed as a hypothesis-generating epizootiological tool, and it should be easy to expand it to a global scale. As LME's are added to a global network, retrospective data searches could be used to establish a historical baseline.

We propose a convergence of concepts identified in these two programs for investigating, documenting, and tracking marine disease and mortality in a global, epizootiological context. Just as epidemiologists track insect and rodent vectors and microorganisms that infect humans, we will be able to track indicators, such as harmful algae and marine pathogens that respond rapidly to environmental change, to predict public health, ecosystem, and economic threats.

A Framework for Analyzing the Origins of Contaminants in Marine Mammals

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Introduction

This paper proposes a model process for determining the probable origins of contaminants in marine mammals. The process begins as an intellectual effort to analyze the problem from two perspectives — contaminants and their sources, and marine mammals that accumulate contaminants in their body tissues. The next steps are to identify key components of the model and to establish a framework for obtaining, organizing, and analyzing the required data. The desired outcome is to determine the origins of specific contaminants found in selected marine mammal populations.

Initial Considerations

There are many contaminants of interest, but for simplicity we consider four groups: lipophilic organics, toxic metals, agricultural contaminants, and plasticizers and industrial solvents. Lipophilic organics are important because they biomagnify as they pass through the food web. PCBs and DDT are primary examples of this group. Toxic metals occur in both inorganic and organic forms, with the organic forms typically being more biologically active. Mercury and tin are primary examples of this group. Agricultural contaminants include pesticides, herbicides, and fungicides, some of which can be active at very low doses. Plasticizers and industrial solvents are known carcinogens.

There are more than 100 species of marine mammals in six taxonomic groups: pinnipeds, baleen whales, toothed whales, sirenians, the sea otter, and the polar bear. Each group is fundamentally different with respect to life history and behavior. Within the groups, further differences exist between species and often between populations.

Contaminants enter the environment as waterborne pollutants from point sources (e.g., pipes or spills) or non-point sources (e.g., runoff), or as aerosols and particulates (e.g., dust from agricultural activities, vapors from pesticide applications, and industrial emissions). Transport is affected by estuarine, oceanic, and wind circulation as well as by the migratory behavior of contaminated prey.

Contaminants can enter a marine mammal through any of four pathways: mouth, skin, lungs, or transfer from mother to fetus. The two most important pathways are thought to be those involving food consumption (including mother's milk) and prenatal mother-offspring transfer. A substantial amount of a mother's contaminant burden is passed directly to her offspring, especially the first-born, via nursing. This is beneficial to the mother as it very effectively decreases her burden of contaminants. After weaning, consumption of contaminated food is probably the most important pathway for contaminants to enter marine mammals.

Generating a Framework

A framework for determining the origins of contaminants in marine mammals is shown in Figure 8. The framework's key components are

- Opportunistic sampling of stranded and by-caught animals, sampling from directed hunts, and non-destructive biopsy sampling from free-ranging animals.
- Field and controlled experimental data to develop information on the uptake and degradation of contaminants.
- Data on life history and seasonal distribution of marine mammals and their prey.
- Environmental climatology, which supplies information on contaminant movements and on stresses that can exacerbate the effects of contaminants or alter uptake pathways.
- Contaminant sources (i.e., where contaminants are manufactured and used, and how they are disposed).
- Marine mammal prey and their life histories to elucidate trophic pathways.Knowledge of the wider scientific community, especially for extrapolating information between species and between contaminants. Contributors would involve not only the extended marine mammalogy and contaminants communities represented at this workshop, but also chemists, physical oceanographers, and meteorologists.
- Tools to perform relatively simple analyses, such as spatial coincidence and likelihood inferences.

These components can be grouped functionally into four types of activity: (1) collection of information, especially updating and adding new data to the existing knowledge base; (2) assembly of key data into accessible and operable sets; (3) hypothesis framing with simple analytical tools; and (4) analyses in time and space (overlays), which lead to joint exposure analyses, which in turn lead to likelihood statements relating sources and destinations.

Discussion of the Components

Opportunistic Sampling of Marine Mammals and Controlled Experiments. Given the political climate and the endangered or protected status of marine mammals in much of the world, deliberately lethal sampling is generally impractical. Likewise, it is impractical to expect that sufficient and appropriate data on contaminants and physiology will become available from strandings alone. Every opportunity to obtain data needs to be pursued, including sampling from by-caught, live-stranded, and dead but fresh stranded animals, animals in rehabilitation programs, and free-living animals (through collection of non-destructive biopsies). Controlled experiments on captive animals are also necessary to elucidate effects and the mechanisms that cause them. A critical aspect of this data collection is that the data be made readily available to multiple users.

Data on Life History and Distribution. Many marine mammal populations can be assigned to one of three categories based on movement patterns: local populations, coastal migrators, and pelagic species. The life histories and distributions of local populations are relatively well known because they are readily available for observation and study. Less well known are the populations that migrate along the coasts. Most of what is known about these populations comes from observations made as they pass along a coastline and are, therefore, accessible. Least well known are the highly migratory pelagic species which spend most of their lives well away from the coast.

Environmental Climatology. With the advent in 1978 of satellite sensors collecting marine environmental data, we have a good understanding of "average" ocean circulation, wind patterns, and sea surface temperature distributions at scales approaching a few tens of miles. Because these data are collected routinely, we can also detect monthly or seasonal deviations from the average conditions over decades or longer time periods. This offers the potential to identify stresses which might confound analyses of animal responses to contaminants (e.g.,

by altering normal supplies of prey or causing migration patterns to vary). Available datasets include the 11 CD-ROMs describing the World Ocean Circulation Experiment. Global Data version 1.0, published by NOAA's National Oceanographic Data Center in 1998, and monthly summaries.

Contaminant Sources. All of the contaminants of interest continue to be manufactured and used. Also, substantial quantities are stored either in sediments and industrial stockpiles or as trophic burdens in food webs. Because of increased regulation in developed countries, much of the production has moved to other areas where the details of production and disposal are unavailable. Environmental non-government organizations and inter-government organizations such as the World Bank and the World Heath Organization are the main sources of data on current production and discharge levels of persistent contaminants.

Trophic Pathways. A major pathway of contaminants into marine mammals is food-chain biomagnification. Data on distributions and contaminant burdens of some marine mammal prey species are available, particularly for commercial fish and shellfish. Such data are rarely available, however, for non-commercial species. The two aspects of this component are the identification of which prey species are relevant, and then the collection of information on their life histories and distributions. The latter is most likely to come from the fisheries literature. Contaminant burdens of the prey species also need to be measured or estimated.

Knowledge of the Scientific Community. Probably the most important component of the framework is the knowledge and expertise of the broad-based scientific community. It will never be possible to answer all relevant questions definitively, and many extrapolations and inferences will be necessary. Making these will require multidisciplinary studies and novel types of collaboration. The proposed framework begins with the formulation of hypotheses relating marine mammals to their prey, contaminants, and environmental interactions. These largely biological models need to be tested using deterministic and statistical techniques with both target and surrogate species.

Tools and Analyses. Exposure analysis and likelihood inferences will rely on both simple and sophisticated tools and analyses. Simple spatial overlays of marine mammal populations, prey, and contaminants can be used to infer likelihood of hypothesized pathways and interactions. Estimates of contact time (co-occurrence) can also be derived from these overlays. Joint exposure analyses are the next analytical step: quantifying where and for how long each life stage is exposed to contaminated prey, and quantifying the exposure of prey to contaminant sources. The biological-process models can be tested using inferred and/or extrapolated uptakes and efficiencies, and probabilistic statements can be made about cause and effect. These models can also be used to set limits on uptake models and identify gaps in the data.

Considerations for Initial Steps

Implementing a framework such as the one proposed here will be difficult and will require considerable input from the scientific community. It is important to start with simple approaches and to add complexity in an incremental manner. The framework should be designed to grow and expand. People who will use the data and results need to be involved at all stages of implementation. Those who supply data and information must receive value for their time and effort, so feedback is essential.

Based on these considerations, implementation might focus initially on data collection from opportunistic sampling, using a form-based data-collection system. The data can be entered using hardcopy, softcopy, or on-line tools. Suppliers of data could receive feedback (added value) via a built-in mapping system, using either a host-resident mapper or a user-based desktop system, such as the no-cost ArcExplorer from Environmental Sciences Research Institute. In this way, maps could be delivered showing where data have been collected. Candidate forms for data collection already exist and are published in the recently released CD-ROM

"Marine Mammals Ashore: A Field Guide for Strandings" (available from the National Aquarium in Baltimore). Future expansion of the system could include mail relay to facilitate information exchange and an on-line encyclopedia of knowledge about life histories and spatial distributions of marine mammals.

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Appendix B: Workshop Agenda

Monday Evening, 12 October 1998

7:00 PM	Welcome, introductions, and review of workshop objectives and format (Conveners)
7:20 PM	Plenary Address 1. *Environmental Contaminants and Marine Mammals: A Retrospective Overview*. Thomas J. O'Shea and Shinsuke Tanabe
8:00 PM	Discussion (Working Group Leaders)
8:30 PM	Mixer (cash bar)

Tuesday, 13 October 1998

7:45 AM	Announcements
8:00 - 8:30	Plenary Address 2. *Reproductive and Developmental Effects of Endocrine Disrupting Chemicals in Marine Mammals*. Peter J. H. Reijnders
8:30 - 9:00	Discussion. Panelists: Richard Addison, Pierre Béland, Anders Bergman, Robert DeLong (Leader)
9:00- 9:30	Plenary Address 3. *Contaminants and Marine Mammal Immunotoxicology and Pathology*. Sylvain De Guise, Steven D. Holladay, and Kimberlee B. Beckmen
9:30 - 10:00	Discussion. Panelists: Pierre Béland, Michel Fournier, Ailsa Hall, Steven Holladay, Nancy Thomas (Leader)
10:00- 10:30	Break
10:30-11:00	Plenary Address 4. *Assessing the Risks to Marine Mammal Populations from Exposure to Contaminants*. John Harwood, Ailsa Hall, and Bryan Grenfell
11:00-11:30	Discussion. Panelists: Theo Colborn, Doug DeMaster, John Reif (Leader) Peter Ross, Kees van Leeuwen.
11:30-12:00	Plenary Address 5. *Overview of Federal and International Programs on Contaminants in Marine Mammals.* Teri K. Rowles and Lyman Thorsteinson.
12:00-12:30	Discussion. Panelists: Paul Jones, John Kucklick, Mary Matta, Derek Muir (Leader), Peter Reijnders
12:30-1:45	Lunch
1:45-2:15	Plenary Address 6. *Overview of Health, Ecological and Economic Dimensions of Global Change: Tracking Marine Disturbance and Disease*. William S. Fisher, Paul R. Epstein, and Benjamin H. Sherman.
2:15-2:45	Discussion. Panelists: Alonso Aguirre, Frances Gulland, Thomas Lipscomb, Teri Rowles (Leader)
2:45-3:15	Plenary Address 7. *A Framework For Analyzing The Origin of Contaminants in Marine Mammals*. Daniel J. Basta, Thomas F. La Pointe, and Peter L. Grose (Presenter)
3:15-3:45	Discussion. Panelists: R. F. Addison, Joseph Geraci (Leader), Ross Norstrom, Shinsuke Tanabe
3:45-4:15	Break
4:15-5:30	Review of Terms of Reference and Initial Meetings of Working Groups
5:30-7:00	Break (Dinner)
7:00 PM	Continuation of Working Group Organizational Meetings

Wednesday, 14 October 1998

8:00-10:00 AM	Working Groups and Subgroups in Session
10:00-10:30	Break
10:30-12:00	Working Groups and Subgroups in Session
12:00-1:30	Lunch
1:30-3:30	Working Groups and Subgroups in Session
3:30-4:00	Break
4:00-6:00	Working Groups in Session to Discuss and Consolidate Subgroup Reports
6:00-8:00	Dinner
8:00 PM	Working Groups in Session to Discuss and Consolidate Subgroup Reports

Thursday, 15 October 1998

7:30-10:00 AM	All Participants Convene and Main Working Groups Report to Floor
7:30	Report of Working Group on Endocrinology and Reproduction
8:00	Report of Working Group on Immunotoxicity, Pathology and Disease
8:30	Report of Working Group on Risk Assessment
9:00	Report of Working Group on Future Trends
9:30	General Workshop Discussion
10:00-10:30	Break
10:30-12:00	Working Groups and Subgroups in Session to Complete Final Report
12:00 - 1:30	Lunch
1:30-3:30	Working Groups and Subgroups in Session to Complete Final Report
3:30-4:00	Break
5:00	Written Working Group Reports Delivered to Workshop Report Editor

Appendix C:

Overview of Federal Programs That Address Issues Related to Contaminants in Marine Mammals

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Introduction

Many marine mammals bioaccumulate high levels of contaminants as a consequence of their position at or near the apex of marine food webs and their geographic range. All marine mammals have had some degree of exposure to chemical pollution. The uptake and accumulation of potentially toxic substances (e.g., pesticides, industrial chemicals, and trace elements) is of concern because adverse effects have been observed in many wildlife species. In addition, high levels of contaminants have been implicated in several die-offs of large numbers of marine mammals. Thus, there is a need to establish baseline health and contaminant loads in marine mammals (Becker et al. 1994). The presence of contaminants often reflects degradation in marine environmental quality and long-term threats to ecosystems. In response to these concerns, management authorities (federal and state governments), private organizations, and international consortia have worked independently and in concert to identify exposure pathways and to assess biological effects of contaminants in marine ecosystems. Many studies have been short-lived, tactical responses intended to meet the specific need for mitigative or preventive measures (Waring 1998). Other short-term efforts have focused on human health and risk assessments associated with consumptive pathways. Longer-term, strategic programs are rare and expensive, and they require extensive scientific cooperation. Long-term multidisciplinary approaches are needed to understand regional contaminant trends, ecosystem conditions, and animal health.

U.S. Federal Programs

U.S. research and management responsibilities for the health and protection of marine mammals reside in several governmental agencies. Most prominently, these include the Departments of Commerce (DOC) and the Interior (DOI) and the Environmental Protection Agency (EPA). The DOC and DOI have trust responsibilities that include marine mammal research, management, and enforcement activities (e.g., DOI 1997, DOC, 1998). Manatees *(Trichechus manatus)*, sea otters *(Enhydra lutris)*, walruses *(Odobenus rosmarus)*, and polar bears *(Ursus maritimus)* are managed by the DOI's Fish and Wildlife Service (FWS); all other marine mammal species are managed by the DOC's National Oceanic and Atmospheric Administration's (NOAA's) National Marine Fisheries Service (NMFS). The EPA mission is broader and gives the agency regulatory authority to protect the natural environment and human health. Occasionally, the Department of Health and Human Services conducts relevant studies to better understand contaminant exposure pathways (e.g., subsistence use) and risks to human health (e.g., Egeland et al. 1998).

Two agencies within the DOC — NOAA and the National Institute of Standards and Technology (NIST) — are directly involved in marine mammal research, monitoring, and assessment of contaminant-related effects. NOAA's research and management responsibilities are described in the

Marine Mammal Protection Act of 1972 (MMPA). Within NOAA, NMFS is the principal scientific entity entrusted with developing information to assess the effects of environmental change, natural and anthropogenic, on protected marine mammal species and habitats. The NIST, in collaboration with NMFS and others, provides (1) chemical analytical support and cooperation for quality assurance analyses through the National Marine Analytical Quality Assurance Program, and (2) archiving of marine mammal tissues in the National Marine Mammal Tissue Bank as part of the National Biomonitoring Specimen Bank (NBSB). NOAA's National Ocean Service (NOS) complements NMFS missions through benthic monitoring efforts (e.g., mussels and sediments) within the Coastal Oceans Program and through its resource damage assessments conducted following oil spills or other contamination events.

NMFS initiated a long-term, multidisciplinary program — the Marine Mammal Health and Stranding Response Program (MMHSRP, see Becker et al. 1994) — following amendment of the the MMPA in 1992. Today, the MMHSRP provides scientific oversight and coordination for most of the DOC's health and effects research on marine mammals. It is a national program that depends on partnerships within NOAA, with other state and federal agencies, and with numerous local governments, non-governmental organizations, and volunteers. The MMPA-mandated goals for the MMHSRP are to (1) facilitate the collection and dissemination of baseline data on the health and health trends in marine mammal populations; (2) correlate the health of individuals and, wherever possible, populations with available environmental information; and (3) coordinate effective emergency responses to strandings and unusual mortality events. One emphasis of the program is to describe contaminant transport pathways through marine food webs and, ultimately, to provide an understanding of the biological effects of contaminants on marine mammal populations. A veterinary–ecotoxicological design approach has evolved where physical, biological, and chemical measurements are being related to a suite of life history features (e.g., age, sex, parity, genetics) and animal health condition factors (e.g., histology, pathology, and serology) (DOC 1998).

The main administrative functions of the MMHS RP include (1) coordination and management of stranding networks and emergency responses; (2) biomonitoring, including research and assessments; (3) cryogenic banking for trend monitoring; (4) chemical quality assurance and control; and (5) information management and outreach. Tissue sources in the biomonitoring include subsistence hunts, strandings and unusual mortality events, fishery bycatch, live capture and release programs, and opportunistic sampling (e.g., ongoing research and monitoring programs).

Contaminant issues have played a major role in guiding the planning, program development, and conduct of the MMHSRP. Obtaining adequately large and representative samples is always a challenge in a program that depends so much on opportunistic sampling. Thus, some of the research questions being addressed within the MMHSRP concern monitoring protocols, statistical approaches, and methods development as they pertain to field collections, specimen banking, and analytical chemistry. Additional, more focused, species-oriented studies involving controlled experiments are underway, or are planned, to develop baseline physiological profiles of healthy captive animals as references for similar observations from the wild.

NMFS's Northwest Fisheries Science Center (NWFSC) is the NOAA lead for analytical chemistry and contaminants monitoring in the MMHSRP. Other NMFS science centers, field personnel, and NOAA partners are actively involved in other aspects of the program. The MMHSRP components and partnership roles are briefly summarized below.

Marine Mammal Stranding Networks: Stranding networks are an integral part of the MMHSRP and have contributed significantly to scientific understanding of species life history, marine mammal diseases, contaminant processes, and population health and mortality. In addition, they play a crucial role in the detection, investigation, and scientific response to strandings and unusual mortality events. In the last decade, and especially

through the MMHSRP, NMFS has enhanced its overall capability through coordination, training (NMFS personnel, participating scientists, and volunteers), and public outreach in support of stranding response. A national coordinator and five regional coordinators oversee, coordinate, and authorize all network activities. The quick response of NMFS and other network participants to stranding events is critical to the quantity and quality of information obtained. During each mortality event, a MMHSRP-designated on-site coordinator and research team assess the event, which may include toxicant exposure and effects, and prepare a final report. Response guidance to this team is developed by NMFS's Working Group on Marine Mammal Unusual Mortality Events (Wilkinson 1996). Since 1990 there have been 16 marine mammal mass mortality events internationally, 13 of which were in the United States (Vidal and Gallo-Reynoso 1996).

Biomonitoring Program: This program attempts to integrate medical and ecological disciplines to provide a holistic investigative approach to health assessment. The goal is to develop reliable, consistent methods for assessing individual and population health within the broader contexts of population ecology and environmental change. The biomonitori ng elements address biological effects, analytical methodology, case study research, and quality assurance and control (Becker et al. 1994). Initially, new techniques, quality assurance, and standardization (e.g., collection, analysis, and reporting) were major concerns. With respect to contaminant trends or effects detection, chemical analyses are conducted on target organs and tissues that are known or suspected to be important sites for bioaccumulatio n.

Necropsies are performed on as many animals as circumstances allow (e.g., Dierauf 1994). In some instances, ambient environmental conditions, an animal's physical state, or other logistical problems preclude necropsies and collection of high quality, non-degraded tissues. Under the best field conditions, a suite of biological measurements (e.g., size, sex, reproductive status), and observations (general health and condition) are obtained for every animal sampled. In addition, trained field workers collect soft tissue or other biological samples (e.g., teeth) for specific laboratory purposes (e.g., age estimation, contaminants, gross pathology, histopatholog y, serology, genetics, biomarkers). Analyses of contaminants are performed by the NWFSC, and in some instances, by other MMHRSP partners such as the NIST. Recent case-specific research has included contaminant exposure studies on killer whales in Prince William Sound, Hawaiian monk seals on the Leeward Islands, and bottlenose dolphins off California, in the Gulf of Mexico, and along the mid-Atlantic states of the United States. Efforts to correlate contaminant residues with observed biomedical effects include MMHSRP-funded studies of transitional cell carcinomas in California sea lions, immunological profiles of northern fur seals, and cardiovascular and renal disease in rough-toothed dolphins.

Scientific partnerships and coordination within the MMHSRP allow NOAA to address priorities in areas where the agency's own capabilities and expertise are limited, lacking, or unavailable due to other commitments. For instance, specialized necropsies are conducted and biopsies are analyzed for NOAA by scientists from the Armed Forces Institute of Pathology and other veterinary pathologists. Similarly, serological studies are conducted by the U.S. Department of Agriculture's (USDA's) National Veterinary Services Laboratory and other clinical laboratories possessing specialized knowledge about infectious diseases in wildlife. These investigations have identified causative agents through cell cultures, molecular approaches (e.g., polymerase chain reaction DNA), and morphological examinations. Current research is addressing the prevalence of morbillivirus, herpesvirus, and brucella in marine mammal populations (e.g., northern sea lions). The combination of veterinary medical, toxicological, and other environmental objectives in conjunction with existing population research (e.g., status and trends) provides the mechanism for integrated assessments of population health status and evaluations of threats from contaminants and other stressors.

Specimen Banking: The requirement for a well-organized scientific specimen bank for marine

mammals was recognized by NOAA and others in the mid-1980s (e.g., Lauenstein and Calder 1988). Initially, the need was to preserve reference materials for contaminant trend analyses. This led eventually to the formation of NOAA's National Marine Mammal Tissue Bank (NMMTB). Long-term banking objectives meant that methodologies had to be developed for field sampling and preservation of tissue specimens in a state that is representative of the animal's condition at the time of death (i.e., minimal autolytic alteration). Today, NMMTB samples are archived as a major component of the National Biomonitoring Specimen Bank at NIST environmental chemistry facilities in Gaithersburg, Maryland, and Charleston, South Carolina.

The DOC (NOAA and NIST) and DOI (FWS, U.S. Geological Survey [USGS], and Minerals Management Service [MMS]) have been involved in collaborative research on and monitoring of Alaska marine mammals for more than a decade. In 1986 NOAA and the MMS initiated the Alaska Marine Mammal Tissue Archival Project (AMMTAP) to address Alaska Native concerns about the effects of various industrial proposals for the outer continental shelf (e.g. oil and gas, offshore mining) on subsistence resources. By 1992 AMMTAP had become an integral component of the MMHSRP specimen banking effort, especially with respect to NOAA trust resources in Alaska (Becker et al. 1993, Becker et al. 1997). The creation of a Biological Resources Division (BRD) within the USGS modified the existing DOC-DOI relationship in AMMTAP to provide greater scientific participation by DOI in MMHSRP planning and research activities.

The BRD's participation in the MMHSRP has resulted in a longer list of indicator species that is more representative of DOC and DOI responsibilities and information needs. Importantly, it has brought additional biological and field and laboratory capability to Alaska efforts. The inclusion of additional expertise in population and arctic ecology is leading to novel approaches to delineate contaminant pathways. As an example, a special emphasis by NMFS and BRD investigators will focus on polar bear–ice seal food chain interactions and contaminant transfers. The FWS's Marine Mammal Management in Alaska, MMS, State of Alaska, and several tribal entities (e.g., the North Slope Borough) work closely with the BRD and NOAA to set AMMTAP priorities and assist with the collection of biological materials from subsistence hunting.

The selection of DOC and DOI target species and geographic areas of concern (Table 11) is based on resource management needs and priorities. In general, these include (1) access — available source at a regular time and location; (2) representation of marine mammal species and U.S. ecosystems (e.g., pelagic vs. coastal); (3) potential contaminant bioaccumulation to levels of health concern; (4) representative trophic level and transport pathways; and (5) human use and economic values (e.g., subsistence). The principal MMHSRP tissues for archiving include liver, kidney, and blubber. Rigorous sampling protocols, developed by NOAA and NIST through the AMMTAP, are adhered to by MMHSRP participants in the field (Becker et al. 1991). As the program evolves, new protocols are being developed for blood collection and banking with contaminant analyses in mind.

Since 1987 the AMMTAP and MMHSRP programs have archived 1,173 tissue specimens from 27 species and 391 individuals (Figure 9). On a regional basis, this includes 72 cetaceans and 20 pinnipeds from the Atlantic Ocean; 17 cetaceans

Table 11. "Indicator Species" for the National Marine Mammal Tissue Bank

Species	Region	Habitat	Source
Harbor Porpoise, *Phocoena phocoena*	Northeast U.S. Pacific U.S.	Coastal	Incidental

Dall's Porpoise, *Phocoenoides dalli*	Pacific U.S.	Pelagic	Incidental
Bottlenose Dolphin, *Tursiops truncatus*	Southeast U.S. including Gulf of Mexico	Coastal	Strandings
Atlantic White-sided Dolphin, *Lagenorhynchus acutus*	Northeast U.S.	Coastal & Pelagic	Strandings
Beluga Whale, *Delphinapterus leucas*	Bering Sea and Arctic Ocean	Coastal	Subsistence
Pilot Whales, *Globicephala* spp.	Northeast U.S. Southeast U.S., including Gulf of Mexico	Pelagic	Strandings
Bowhead Whale, *Balaena mysticetus*	Bering Sea and Arctic Ocean	Coastal	Subsistence
Harbor Seal, *Phoca vitulina*	Northeast U.S. Pacific U.S. including Gulf of Alaska	Coastal	Incidental Subsistence
Ringed Seal, *Phoca hispida*	Bering Sea and Arctic Ocean	Coastal	Subsistence
Northern Fur Seal, *Callorhinus ursinus*	Pacific U.S. including Gulf of Alaska	Pelagic	Subsistence
California Sea Lion *Zalophus californianus*	Pacific U.S.	Coastal	Strandings Incidental
Polar Bear, *Ursus maritimus*	Bering Sea and Arctic Ocean	Coastal & Pelagic	Subsistence

from the Gulf of Mexico; 26 cetaceans, 33 pinnipeds, and 3 sea otters from the Pacific Ocean (including southeastern Alaska); and 76 cetaceans, 116 pinnipeds, and 28 fissipeds (sea otters and polar bears combined) from the Arctic (includes Gulf of Alaska, Bering Sea, and Arctic Ocean). Periodically, chemical analyses for specified contaminants are conducted on a selection of NMMTB blubber and liver samples. These analyses provide baseline information to resource managers, in some instances the only such information available. They also allow NIST investigators to monitor tissue stability and the effects of cryogenic storage. Importantly, they also provide an analytical comparison for NWFSC investigators.

In view of the high costs associated with the collection and archiving of MMHSRP specimens, and their increasing value with time, the MMHSRP managers and major partners have formalized a policy for access to samples. Access refers to the availability of archived samples to the scientific community and the contributor for environmental baseline analyses. In brief, this policy addresses the ownership and disposition of banked tissues, procedures for requesting banked samples including scientific justification, review and approval processes, and analytical and data sharing requirements (Wise and Koster 1995). To date, only a very limited number of samples have been shared. In every case, DOC and DOI information needs and MMHSRP goals have been addressed by collaborative studies with shared resources (Table 12).

Figure 9. Tissues in National Marine Mammal Tissue Bank, cumulative by year.

Examples include contaminant, genetics, and proximate composition analyses performed on tissues from beluga whales, ringed seals, polar bears, and other marine mammals (e.g., HCHs, PCBs, and DDT in northern fur seals [Moessner et al. 1992, 1994]; organoarsenic compounds in livers [Goessler et al. 1998]; methyl mercury in belugas [Behlke et al. 1996]).

The NMMTB is not intended to preclude other biomonitoring programs conducted independently. The specimen bank functions as a research tool that can complement existing efforts by preserving and providing biological reference materials. In addition, analytical laboratories that participate regularly in the MMHSRP are able to conduct quality analyses on reference or newly collected materials in support of a program's particular objectives.

Quality Assurance Program (QAP): One role of the QAP is to ensure the accuracy, precision, level of detection, and intercomparison of analytical data resulting from analyses of MMHSRP samples (Wise 1993). In this role, the QAP provides federal and non-federal partner laboratories with biological collection information, control materials, standard reference materials, and interlaboratory comparisons (Wise and Koster 1995, Becker et al. 1997). In support of the MMHSRP's biomonitoring function, the NIST conducts the Marine Mammal

Table 12. Interlaboratory comparison exercises conducted for the Marine Mammal Health and Stranding Response Program, 1991–1997

Dates	Analytes	Matrix	Objective	Participants
1991–92	PCBs Cl pesticides[1]	whale blubber	lab comparability	NIST; NWC; DFO Canada; GERG; Univ. of Ulm
	Elements	whale liver CM[2]	analytical control	NIST; NWC
1992–93	PCBs Cl pesticides[1]	whale blubber; whale blubber CM[2]	lab comparability	NIST; DFO Canada; Univ. of Ulm
1993–94	PCBs Cl pesticides[1]	whale blubber; whale blubber CM[2]	SRM development	NIST; NWC; DFO Canada; GERG; Arthur D. Little; NW Aquatic Sciences; Univ. of Ulm
1993–97	Elements PCBs Cl pesticides[1]	whale & seal liver whale & seal blubber	sample comparability	NIST; NWC
	Elements	whale liver CM[2]	analytical control	NIST; NWC
1997–98	Elements	whale liver	lab comparability & control material development	NIST; NWC; Texas A&M Univ.

[1] Chlorinated Pesticides
[2] Control Material

Quality Assurance Project (MMQAP) for organic and inorganic analyses. A primary focus is the quality assurance associated with NMMTB samples. The MMQAP is co-managed by NMFS and NIST. NIST has developed blubber and liver control materials and standard reference materials (blubber) for interlaboratory comparisons. These materials have become important resources for quality testing by other analytical facilities in the United States and elsewhere.

NIST conducts interlaboratory comparison exercises among the analytical laboratories participating in the MMHSRP and other facilities engaged in marine mammal studies on an annual basis. Interlaboratory comparisons address techniques and data quality and identify potential sources of procedural and measurement variability. All NMFS-sponsored marine mammal studies with objectives related to contaminants are required to participate in the MMQAP. Blood serum protocols (i.e., procedures for collection and storage of serum) are being developed that make quality assurance part of the research.

In addition to the DOI's collaboration with the MMHSRP, two bureaus within the DOI conduct ecological research and monitoring to investigate contaminant trends in marine mammals. Within its management prerogative, the FWS may conduct species-specific biomonitoring or site-specific surveys through its Marine Mammal Management and Ecological Services offices (e.g., polar bears; Norstrom et al. 1998). FWS projects tend to be issue-oriented surveys to establish baseline conditions, assess damage, and develop mitigative or preventive measures. For example, the FWS's Environmental Contaminants Program explores wildlife contaminant issues and threats at more than 75 locations nationwide (see www/http:/fws.gov). These efforts have provided important baseline data and, in several instances, highlighted the need for additional species- or habitat-related studies. To illustrate, cadmium concentrations in kidney tissues of Pacific walruses from the Bering and Chukchi Seas were reported to be high (147 to 204 ug/g dw; Warburton and Seagars 1993). The levels were high enough to raise concerns about animal health and about cadmium sources and pathways. These concerns are topics of current BRD research in Alaska.

A primary FWS mission is to maintain the health and stability of the marine ecosystems and populations for which it is entrusted. A major thrust of FWS research and surveys has been to delineate important habitats and migratory pathways and to determine the status and trends of managed populations. With respect to marine mammals, the most extensive contaminant surveys have targeted Pacific walruses and polar bears in the Bering, Chukchi, and Beaufort Seas and sea otters along the west coast and Alaska. In Alaska, the FWS shares its data with public health organizations. It has also provided tissue samples for archiving through the AMMTAP. With respect to the MMHSRP national goals, the DOI needs to focus more attention on manatees in Florida and the Caribbean Sea.

The USGS's BRD is the responsible scientific entity for biological research on marine mammals within the DOI. The BRD conducts a variety of research projects to meet the need for information on marine mammal populations, habitats, and ecosystems of concern. The development of scientifically reliable methods to enumerate populations and stocks, describe seasonal habitat use, and assess environmental impacts are major program areas. Thus, BRD research projects tend to be population-oriented and often last 3 to 5 years. Longer-term goals of BRD marine mammal research are to improve understanding of ecological relationships and the effects of environmental change. BRD studies generally are not directed contaminant studies, *per se*. One exception is the division's participation in the MMHSRP through the AMMTAP. Another is a study of sea otters throughout their North American range (e.g., Estes et al. 1997). Special research emphasis has focused on the effects of the *Exxon Valdez* oil spill on sea otters in south-central Alaska. In particular, near-shore predator-prey relationships and population recovery have been investigated. Continuing efforts to assess radionuclide contaminant levels in polar bears involve international coordination with circumpolar nations.

In addition to DOI and DOC contaminant studies, the EPA has performed chemical analyses on tissues from marine mammals and other marine organisms in support of specific studies or at specific sites. As an example, the EPA participated in the investigation of die-offs of bottlenose dolphins along the U.S. Atlantic coast in 1987 and 1988. It also conducted several related follow-up studies of Atlantic bottlenose dolphins. The EPA is presently administering several subsistence-oriented studies in Alaska, through the University of Alaska, which involve marine mammal contaminant analyses and database development.

Non-governmental and International Studies

Numerous non-governmental and international organizations have funded, are currently funding, or are planning relevant contaminants research on marine mammal populations of interest to U.S. management and tribal authorities. Most noteworthy of these are the Arctic Monitoring and Assessment Program (AMAP), the International Whaling Commission (IWC), the United Nations Environment Programme, the North Slope Borough Wildlife Department, and the World Wildlife Fund International. The AMAP, a circumpolar program, was established under the Arctic Environmental Protection Strategy in the early 1990s and is a continuing effort under the Arctic Council (AMAP 1998). The AMAP plan for 1998-2002 includes marine mammal contaminant monitoring (persistent organic pollutants, petroleum hydrocarbons, heavy metals, and trace elements) in key indicator species including polar bears, ringed seals, and beluga whales. The IWC considers potential pollutant effects relevant to the management of cetacean populations. An IWC-sponsored workshop was held in 1995 to assess contaminant effects (Reijnders et al. 1999). This workshop concluded that multidisciplinary and multinational research planning and execution are needed to understand the impacts of contaminants on marine mammal populations. Tribal governments, such as the North Slope Borough, are conducting biological research and monitoring contaminants and, in Alaska, work in close association with AMMTAP and MMHSRP investigators. Tribal concerns are focused primarily on the health of wildlife populations hunted for subsistence and on the potential risks to indigenous people from consuming marine mammal products (e.g., cadmium in beluga whales) and from other changes in rural communities. A major concern of Alaska natives is that traditional knowledge be incorporated in future scientific planning. Finally, within the United States, many universities and national and international foundations and organizations have established cooperative research programs and sponsored directed research to answer specific questions about contaminant pathways, processes, and probable effects on marine mammals.

Conclusion

A common focus of many research and monitoring programs has been to determine baseline levels of heavy metals and persistent organic pollutants in marine mammal populations. Few programs have adequately addressed issues of experimental or study design. These issues must be addressed, however, for information on contaminant trends and effects to be scientifically defensible. For example, quality assurance and quality control are most frequently addressed at the laboratory or study level. The lack of standardization in field and laboratory approaches has resulted in numerous problems with comparability and repeatability — essential ingredients of quality science. Similarly, few studies have incorporated a health perspective. Other problems that arise particularly in monitoring efforts, such as sample size, frequency, and intervals, require pilot research and must be addressed with practical (logistics and cost) and statistical objectives in mind (e.g., Jensen and Cheng 1987, Bignert et al. 1993).

As federal and state agencies attempt to make the concept of ecosystem management operational, there is a growing need for coordination of research, monitoring, and quality-control programs at regional, national, and international scales. The coordination among NOAA, NIST, and the USGS through their respective roles in MMHSRP, NMMTB-AMMTAP, and AMAP provides an example. By working together, and with others, these agencies are engaged in efforts to conserve valued species, ensure ecosystem health, and protect

rural lifestyles. Coordination, synthesis, and cross-fertilization of multidisciplinary interests are essential. The integrated assessment approach requires that a large cross-section of the scientific community shares data and information and works cooperatively to produce a synthesis. This synthesis combines information on contaminants, the status and trends of marine mammal populations, population biology and ecology, and animal health.

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Appendix D:

Prevalence of Lesions Associated with a Disease Complex in the Baltic Gray Seal (*Halichoerus grypus*) during 1977–1996

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Introduction and Summary

The investigation was initiated due to a serious reduction of the Baltic gray and ringed seal populations. Earlier, impaired reproductive ability had been reported, with occlusions and stenoses of the uterus in the two species as well as a high frequency of uterine smooth muscle cell tumours (leiomyomas). The lesions of the female sex organs were found to be part of a disease complex in adults of both sexes of these species. Besides lesions of the uterus, the disease complex comprises lesions of

1. Integument, especially claws;
2. Skull bones, especially masticatory bones;
3. Intestine (colonic ulcers);
4. Kidneys (glomerulopathy, tubular cell proliferations);
5. Arteries (sclerosis); and
6. Adrenals (cortical hyperplasia, cortical adenomas).

Here I focus on the results of a time-trend study of the two decades 1977–1986 and 1987–1996 (Bergman 1999). During 1977–1996, necropsies were performed on 159 Baltic gray seals.The animals were grouped by sex and the following age classes: subadults 1–3 years old and adults 4–15, 16–25, and >25 years old. Seals 4 years and older were considered sexually mature. Regarding upper age limits, some females were around 40, and the oldest male was 25 years old. A semi-quantitative evaluation was made of the degree and extent of lesions in the non-reproductive organs, using the following scale: no change apparent (0), slight (1), moderate (2), severe (3), and fatal (4).

Prevalences (%) were compared between the two decades in "moderate" to "severe" lesions of claws, intestine, arteries, and adrenals. Similar comparisons were made for lesions of the female reproductive organs and rates of pregnancy. Using the same material, animals born before 1980 were compared with those born after 1979 to investigate a possible relationship between seal health and the evident decrease of PCB and DDT pollution of the Baltic during the 1970s.

In summary, there was a positive trend in gynecological health over time, with a decrease from 42% to 11% in the prevalence of uterine obstructions, and an increase from 9% to 60% in pregnancies. The incidence of uterine tumors (leiomyomas) seems to have decreased slowly, from 53% to 43% between the two decades.

Of special interest is that the prevalence of colonic ulcers has tended to increase in young gray

seals. This finding indicates that their food may contain "new" (previously unidentified) toxins or increased amounts of "old" ones, affecting the seals' immune system.

Favorable Tendencies

Some important favorable tendencies were noted with regard to the lesions examined (Bergman 1999). In particular, the changes in the prevalence of uterine obstructions and the rate of pregnancy were statistically confirmed, while the change in the incidence of leiomyomas was not. A slight improvement was also found in the incidence of adrenocortical hyperplasia, but it was not statistically significant. A reduced incidence of bone lesions has also been found (Bergman et al., in prep.). Arteriosclerotic lesions were strongly correlated with increasing age, but no difference in prevalence was discerned between the two periods (Bergman 1999). The apparently favorable tendencies are probably related to the decreases of DDT and PCBs in Baltic biota recorded during the 1970s (Olsson and Reutergård 1986, Bignert et al.1995).

As discussed in previous and forthcoming reports on ringed seals (e.g., Helle 1980 a,b; Bergman et al., in prep.) and gray seals (Bergman and Olsson 1985) of the Baltic, uterine occlusions and stenoses are related to pregnancy. Some cases have shown retained fetal membranes containing fetuses, aligned to stenosed parts of the horns. The obstructions probably develop secondarily as a result of interrupted pregnancies (Bergman and Olsson 1985). The lesions may be associated with hormonal imbalance and immune suppression, probably due to interference of organochlorines with steroid metabolism. The lesions may also result from a disturbed post-parturient puerperium, or from fetal death due to the influence of organochlorines (Bergman et al., in prep.).

The prevalence of uterine leiomyomas in gray seals seems to have been very high in both collection periods. According to Kennedy and Miller (1993), leiomyoma is a common tumor of the female tubular genitalia in the dog, but it is uncommon in other domestic species. It rarely occurs in female dog genitalia before middle age and is often associated with ovarian follicular cysts or estrogen-secreting tumors, endometrial and mammary hyperplasia, and mammary neoplasia. When bitches are castrated early in life, leiomyomas do not develop. If leiomyomas are already present, they regress following castration. These circumstances implicate a background of endocrine disorder as being linked to the development of leiomyomas.

Uterine leiomyoma is a common tumor in humans. It affects 20 to 25% of women during their reproductive years. There is strong evidence that the growth of leiomyomas is estrogen-dependent; they generally regress after the menopause (Cotran et al.1989). The clinical use in women of gonadotropin-releasing hormone agonists has improved knowledge about the nature of leiomyoma. Treatments are intended to reduce circulating levels of uterine tissue hormones, particularly estradiol (Ueki et al.1995), resulting in reduced leiomyoma size,

The continued high incidence of uterine leiomyoma in Baltic seals is a matter of concern. This tumor's "natural" frequency of occurrence in the seal populations is unknown. There is reason to believe, however, that the high prevalence observed in gray seals is due to the influence of one or more factors in the environment which cause hyper-estrogenic states. This belief is supported by the results of examining large samples of kidney tissue from Baltic seals with light microscopy (Bergman et al. 1999) and electron microscopy (Bergman and Bergstrand, in prep.). Besides the presence of glomerular lesions, a high frequency and degree of tubular-cell proliferations were observed in these investigations. These observations indicate a background of hormonal disturbance, probably excess estrogen. Renal tubular-cell proliferations, with the character of squamous metaplasia and with localization similar to that found in the Baltic seals, have been described in a male dog with Sertoli-cell tumors in the testes and feminization (Lindberg et al.1976) and in a bitch with an ovarian granulosa-cell tumor (Wouda et al. 1978).

Unfavorable Tendencies

Seals 4 to 15 years old born after 1979 had a higher incidence of lesions in their digits and claws than this same age class born before 1980, but this difference was not statistically significant. Of more consequence, however, is the evidently increasing prevalence of colonic ulcers, which are often fatal. This increase was statistically significant for young animals (1 to 3 years old). Colonic ulcers in Baltic seals seem to be associated with hookworm *(Corynosoma* sp.*)* infection, which is suspected to initiate the primary lesion of the intestinal mucous membrane (Bergman and Olsson 1985). Compared to the findings in Baltic gray seals, British grey seals have a much lower incidence of intestinal lesions in combination with *Corynosoma* sp. infection. Baker (1980) examined two juvenile and two adult British gray seals and found ulceration of the ileum in just one animal, an 18-year-old female heavily infested with *Corynosoma* sp. In a survey of pathology in 34 juvenile and 12 adult British gray seals, Baker (1987) reported that 81% of the animals were infested with *Corynosoma* sp., but all of them lacked signs of intestinal ulcers. Judging by the literature, Baker (1987) concluded that *Corynosoma* sp. usually do not cause gross intestinal changes in seals.

Apart from drowning in fishing gear (72%), severe colonic ulcers, with perforation of the intestinal wall, was the most common cause of death (7%) for gray seals in the Baltic. Fatal colonic ulcers seem to most seriously afflict middle-aged and aged males (16 to 25 years old). Such ulcers were found in three out of six animals in this age group, all of them collected between 1987 and 1996.

The increasing prevalence of colonic ulcers in young Baltic seals indicates an increasingly compromised immune system during recent years. In recent Dutch experiments, when groups of captive harbor seals *(Phoca vitulina)* were fed Baltic or Atlantic herring, suppression of various cellular and antibody responses of the immune system was observed in the seals fed the Baltic herring (De Swart et al. 1994, 1995; Ross et al. 1995, 1996). These findings indicate that the food available to Baltic seals is still sufficiently contaminated to affect health. The fact that the incidence of colonic ulcers has continued to increase in Baltic seals, despite the reduced concentrations of known organochlorines, might indicate that prey resources contain hitherto-unidentified toxic contaminants, or at least increased amounts of such contaminants. Several industrial contaminants have been detected recently that were previously not known to be present in Baltic biota, including seals. These include polybrominated diphenyl ethers (PBDE) (Sellström et al. 1993), used as flame retardants in polymeric materials, and bis (4-chlorophenyl) sulfone (BCPS) (Olsson and Bergman 1995), a compound used in the production of high-temperature polymers. Little is known about the ecotoxicological effects of these compounds.

Autopsies of Baltic seals indicate that the endocrine system has been compromised in some way, with probable involvement of the hypothalamic-pituitary-gonadal and adrenal axis (Bergman and Olsson 1985). Strong uptake of PCBs in corpora lutea, adrenal cortex, and the pituitary has been demonstrated in mice by using whole-body autoradiography (Brandt 1975). Accumulation of certain PCB congeners in the pituitary and/or hypothalamus may lead to effects on gonadotropin release and gonadal function. The ability of PCBs to influence pituitary function has been demonstrated experimentally. When studying the effect of Aroclor 1242, a commercial PCB product, on cultured anterior pituitary cells from female immature rats, Jansen et al. (1993) found that these cells exhibited increased gonadotropin responses.

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Appendix E:

What Experiments Might Have Told Us

PART ONE OF A DIALOGUE

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The Workshop on Marine Mammals and Persistent Ocean Contaminants has provided an opportunity to review what we now know about the impacts of synthetic organic contaminants on individual marine mammals and on marine mammal populations. It was gratifying to learn that the population of harbor seals in the North Sea has recovered and that the situation of seals in the Baltic appears to have improved in a number of respects. Globally, with the possible exception of the belugas of the St. Lawrence River, no marine mammal population appears now to be threatened by contaminants[1], and organic contaminant levels in marine ecosystems appear to have stabilized, or to be changing only very slowly.

Yet there are suggestions of continuing problems in the form of a continuing high incidence of uterine smooth muscle cell tumors and an increase in colon ulcers in gray seals in the Baltic, at a time when concentrations of the "conventional" pollutants have declined. In the 1960s, the Baltic Sea provided many of the "early warnings" of contaminant effects on natural ecosystems. It is certain that human populations will continue to expand through at least the next century, and that their chemical technologies will continue to expand indefinitely. Some effects of new chemical technologies on humankind and our fellow species cannot be predicted at this time.

What, however, have we learned from the past that might provide some guidance in developing conservation strategies for the protection of marine mammals from any effects of synthetic chemicals in the future?

Thirty years ago, a number of bird populations were in serious decline; the peregrine falcon was almost extinct as a breeding species in the lower 48 of the United States. In 1968 and 1969, the Wisconsin Department of Natural Resources conducted hearings to determine whether the continuing use of DDT was in violation of a state law that prohibited the use of any substance that, in consequence of its use, entered the waters of the state and harmed fish or wildlife. I was there, both as a scientific witness and as an advisor to the Environmental Defense Fund.

Eggshell thinning was a symptom common to all of the species undergoing population declines. Joseph Hickey and Daniel Anderson of the University of Wisconsin had just demonstrated that herring gull eggs with higher amounts of the DDT metabolite DDE had thinner shells than eggs with lower amounts of DDE. But the eggs with higher amounts of DDE also had higher concentrations of several other pesticides and higher concentrations of the PCBs. It was impossible to tell which one of them was actually responsible for the eggshell thinning. The pesticide industry stressed that there was no compelling evidence that DDE was the cause of the problem.

[1]Editors' note: Some marine mammal populations that inhabit extremely polluted waters (e.g., river dolphins, finless porpoises, and coastal marine dolphins in parts of Asia) have not been studied as intensively as belugas in the St. Lawrence, and the role of contaminants in their declines has not been adequately assessed.

The most dramatic moment during the hearings was probably the testimony of Dr. Lucille F. Stickel, Director of the U.S. Fish and Wildlife Service's Patuxent Wildlife Research Center. She presented the results of a controlled experiment that showed that ducks on a diet containing DDE laid thin-shelled eggs. The DDE content of the food was comparable to DDE concentrations found in the environment, and DDE concentrations in the eggs were similar to those in the thin-shelled eggs of wild birds. Immediately, we had a winning case in Wisconsin; in the hearings held shortly afterward in Washington, DC, by the newly formed EPA to consider whether the use of DDT should continue or end at the national level, these experimental data provided critical support for the testimony on shell thinning of eggs of the brown pelican, bald eagle, and peregrine falcon. Follow-up experiments confirmed that the other pesticides and the PCBs had not contributed to the thinning.

Wildlife benefited enormously from the decisions to end DDT use in the USA; without the scientifically sound, scientifically clean experimental results, the outcome could have been very different. The bald eagles would surely have followed the peregrines into extinction as breeding species in the lower 48 states, and high rates of reproductive failure of fish-eating birds would have continued.

We still do not understand exactly how DDE thins eggshells. In some species, at least, the number of organic sites at which shell crystallization starts is lower in birds with higher levels of DDE contamination; DDE may therefore block a receptor involved in shell synthesis. Understanding the molecular mechanisms of eggshell thinning, however, is no longer a conservation priority for the species of birds that were affected. Moreover, the physiology of falcons and pelicans is different from that of marine mammals, and also from the physiology of those families of birds in which DDE does not produce shell thinning. But any increase in our understanding of how DDE causes shell thinning in even a few species would provide insight into possible modes of action of other synthetic organic contaminants in other biological systems. Follow-up research on the mechanisms of shell thinning would therefore be worthwhile.

In the early 1970s, I was privileged to work with Bob DeLong, Bill Gilmartin, John Martin, and a number of other colleagues in an attempt to determine the cause of the high incidence of premature births in California sea lions. Females giving birth prematurely had higher concentrations of DDE and PCBs than females with normal births, suggesting a cause-effect relationship. But there were other differences between the two groups. The females giving birth prematurely were younger and had contaminant profiles typical of the Southern California Bight; older females had contaminant profiles typical of central California to the north. Disease vectors endemic in the population were known to cause reproductive disorders, although of a different nature. Under the El Niño conditions of 1997–199 8, with a much-reduced food supply, there was an increase in the incidence of premature births, indicating that nutrition was a probable factor, and that it could also have been a factor in the earlier cases.

Do DDE, PCBs, a combination of DDE and PCBs, or a combination of these with other contaminants contribute to the premature births? No one knows. Because the California sea lion population increased substantially in southern California, this question no longer has a conservation priority. But a scientifically sound answer would be immensely valuable in assessing the potential risk of future contaminants to marine mammals, other wildlife species, and humans. Again, any increase in our understanding of the molecular mechanisms whereby synthetic chemicals interact with biological systems is of potential benefit in developing conservation strategies.

The political climate of the early 1970s (and even more so in recent times) was unfavorable to the temporary captivity of any marine mammal for research purposes, even if undertaken for the long-te rm benefit of the species. Surely it is essential to know what factor or factors are harming a marine mammal population. There was not and there has not been, however, any kind of dialogue and communication among all groups and individuals interested in the welfare of the sea lions and other

species of marine mammals. If there had been, it is likely that an acceptable protocol would have been drawn up and implemented. Two comparable groups of sea lions, one fed wild-caught fish from the coastal waters near Los Angeles with higher DDE levels, the other fed fish from central California with lower DDE levels, could have been followed through the pregnancy cycle to answer the key question of whether DDE was contributing to the premature births. If it was, the knowledge gained would have been beneficial; if not, other causes, particularly one or more disease vectors, could have been looked for and the study might have provided some clues.

The population of harbor seals in the western portion of the Dutch Wadden Sea dropped sharply between 1960 and 1975 as a result of a decrease in the production of young. Dr. Peter Reijnders fed a captive group of 12 female seals fish from an area of the North Sea that received waters from the river Rhine River; another group of 12 seals was fed less contaminated fish from the Atlantic Ocean. Reproduction was significantly reduced in the group fed the more contaminated fish.

Chemical analyses indicated that PCB concentrations were significantly higher in the North Sea fish, suggesting that PCBs were the probable cause. This was sufficient to persuade the government of The Netherlands to support a European Community ban on the production and use of PCBs. PCB contamination in the area subsequently declined and the harbor seals have recovered. But like the eggs of herring gulls in Lake Michigan, the fish from the North Sea contained a complex soup of chemical contaminants, some of which might not be detectable by analytical methodologies current then or now. A chemical or group of chemicals that covari ed with the PCBs could have been the real cause of the reproductive failures. As in the United States, the political climate was unfavorable to what might be perceived as an experiment with marine mammals that involved toxic compounds, even if the amounts of the toxic compounds were equivalent to those in fish consumed by both people and wildlife. If, however, a third group of harbor seals had been fed fish containing an equivalent amount of PCBs, without the other components of the soup, the results would have been much more definitive. If the PCB group had not shown the depressed reproduction, one or more of the other contaminants must have been responsible. An effort to track them down would be the next immediate conservation priority.

Many environmentalists are not aware that the final decision to ban DDT in the United States was not based primarily on its effects on pelicans and peregrine falcons but on its potential harm to human health; this concern, however, was based largely on the results of the wildlife studies. Nevertheless, the end result was the same; the peregrines have returned. Next to, or with DDT, the highly toxic organochlorine pesticide dieldrin has probably been the most detrimental to wildlife. Millions of birds have been killed by dieldrin. For our participation in the hearings that addressed the question of wheth er the use of dieldrin should end, we analyzed samples of mothers' milk; a friend came with her infant daughter and sat in a conspicuous place to ensure eye contact with the judge. If the argument that dieldrin was harmful to human health would stop the killing of bald eagles and egrets, let that be the argument. Wildlife will benefit. Similarly, an argument that a proposed experiment will benefit humans should not be rejected out-of-hand by an environmentalist more concerned with the welfare of wildlife. Interests frequently overlap and sometimes even coincide.

If more people see the broader picture the next time a proposal is made to use captive marine mammals to address a conservation-related problem, the proposal is much more likely to be approved. But communication and dialogue are critical. During the exciting days of the DDT hearings, when I depleted my own lifetime supply of adrenaline, there was a very close collaboration between the environmental groups and the environmental scientists. Now, there is frequently little or no communication. A greater appreciation of scientific considerations by environmentalists and a greater effort by environmental scientists to provide information could only benefit the cause to which both groups are dedicated.

Appendix F:

Abbreviations and Acronyms Used in This Report

Abbreviations for Chemical Names, Physiological Terms, and Related Items

ACHE	acetylcholinesterase
ACTH	adrenocorticotropic hormone
Ah	arylhydrocarbon
AR	androgen receptor
BPA	bisphenol A
BCPS	(bis (4-chlorophenyl) sulfone), a compound used in the production of polymers
CYP1A	subfamily of monoxygenases
DAS	delayed anovulatory syndrome
DBCP	(dibromochloropropane), a fungicide
DDT	(22,-bis-(*p*-chlorophenyl)-1,1-trichloroethane), a widely used organochlorine pesticide
DDTs	the sum of concentrations or amounts of DDT and all its isomers and metabolites
3DDT	the sum of concentrations or amounts of DDT and all its isomers and metabolites
DDD	(2,2,-bis-(*p*-chlorophenyl)-1,1-dichloroethane), metabolite of DDT
DDE	(2,2,-bis-(*p*-chlorophenyl)-1,1-dichloroethylene), metabolite of DDT
EROD	(7-ethoxyresorufin o-deethylase), a mixed function oxidase
FSH	follicle stimulating hormone
GH	growth hormone
HCG	human chorionic gonadotropin
DES	diethylstilbestrol
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
EDCs	endocrine disrupting compounds
FSH	follicle stimulating hormone
GnRH	gonadotropic releasing hormone
HAHs	halogenated aromatic hydrocarbons
HCHs	hexachlorocyclohexanes
$HgCL_2$	mercuric chloride
LH	luteinizing hormone
MT	metallothionein
NK	natural killer cell
OP	organophosphate
P450	(cytochrome P450), a family of monooxygenase enzymes that are induced by certain toxic substances. The name is based on an absorption spectrum of 450 nm.
PACs	polycyclic aromatic compounds
PAHs	polycyclic aromatic hydrocarbons
PBDE	polybrominated diphenyl ethers
PBPK	physiologically based pharmacokinetic models
PCBs	polychlorinated biphenyls
PCB-MSFs	polychlorinated biphenyl methyl sulfones
PCDDs	polychlorinated dibenzo-p-dioxins
PCDFs	polychlorinated dibenzofurans

PCNs	polychlorinated napthalenes
PFC	plaque-forming cells
POPs	persistent organic pollutants
PROD	(pentoxyresorufin o-dependentylase), a mixed function oxidase
PTBS	persistent, toxic, and bioaccumulative substances
PVC	polyvinyl chloride
QA	quality assurance
QC	quality control
SCID	Severe Combined Immune Deficient
SRMs	standard reference materials
TBT	tributyltin
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TEF	toxic equivalency factor
TEQs	toxic equivalents
TSH	thyroid stimulating hormone
TTR	transthyretin

Acronyms for Organizations, Agencies and Programs

AMAP	Arctic Monitoring and Assessment Program
AMMTAP	Alaska Marine Mammal Tissue Archival Project
BRD	Biological Resources Division, U.S. Geological Survey
CDC	Centers for Disease Control and Prevention, U.S. Department of Health and Human Services
DOC	Department of Commerce
DOI	Department of the Interior
EPA	U.S. Environmental Protection Agency
IPCS	International Program on Chemical Safety
IWC	International Whaling Commission
MMHSRP	Marine Mammal Health and Stranding Response Program
MMPA	U.S. Marine Mammal Protection Act of 1972
MMS	Minerals Management Service
NIST	National Institute of Standards and Technology
NMFS	National Marine Fisheries Service
NMMTB	National Marine Mammal Tissue Bank
NOAA	National Oceanic and Atmospheric Administration
NWFSC	Northwest Fisheries Science Center, National Marine Fisheries Service
OECD	Organization for Economic Cooperation and Development.
UNEP	United Nations Environment Programme
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey

Appendix G:

Common and Scientific Names of Vertebrate Animals Used in This Report

Amazon River dolphin	*Inia geoffrensis*
Anchovy	*Engraulis mordax*
Bald eagle	*Haliaeetus leucocephalus*
Beluga (whale)	*Delphinapterus leucas*
Bottlenose dolphin	*Tursiops truncatus*
Bowhead whale	*Balaena mysticetus*
California sea lion	*Zalophus californianus*
Common dolphin	*Delphinus* spp.
Dall's porpoise	*Phocoenoides dalli*
Dover sole	*Microstomus pacificus*
Dugong	*Dugong dugon*
Elephant seals	*Mirounga* spp.
Fin whale	*Balaenoptera physalus*
Florida manatee	*Trichechus manatus latirostris*
Gray seal	*Halichoerus grypus*
Gray whale	*Eschrichtius robustus*
Hake	*Merluccius* spp.
Harbor seal	*Phoca vitulina*
Harbor porpoise	*Phocoena phocoena*
Humpback whale	*Megaptera novaeangliae*
Killer whale	*Orcinus orca*
Long-finned pilot whale	*Globicephala melas*
Marine otter	*Lutra felina*
Mink	*Mustela vison*
Minke whale	*Balaenoptera acutorostrata*
Northern elephant seal	*Mirounga angustirostris*
Northern fur seal	*Callorhinus ursinus*
Northern right whale	*Eubalaena glacialis*
Northern (Steller's) sea lion	*Eumetopias jubatus*
Peregrine falcon	*Falco peregrinus*
Polar bear	*Ursus maritimus*
Ringed seal	*Phoca hispida*
Rockfish	*Sebastes* spp.
Rough-toothed dolphin	*Steno bredanensis*
Sardine	Family Clupeidae
Sea otter	*Enhydra lutris*
Southern (California) sea otter	*Enhydra lutris nereis*
Sperm whale	*Physeter macrocephalus*
Striped dolphin	*Stenella coeruleoalba*
Walrus	*Odobenus rosmarus*
Weddell seal	*Leptonychotes weddellii*
West Indian manatee	*Trichechus manatus*
Brown pelican	*Pelecanus occidentalis*
Hamster	*Mesocricetus auratus*
Herring gull	*Larus argentatus*
Rhesus monkey	*Macaca mulatta*
White croaker	*Genyonemus lineatus*