Environmental Contaminants and Marine Mammal Health: Research Applications

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ENVIRONMENTAL CONTAMINANTS AND MARINE MAMMAL HEALTH:

RESEARCH APPLICATIONS

by

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TABLE OF CONTENTS

ABSTRACT

Ross, P.S., and S. De Guise. 1998. Environmental contaminants and marine mammal health: Research applications. Can. Tech. Rep. Fish. Aquat. Sci. 2255 v + 29 p.

Marine mammals often occupy elevated trophic levels in the world's oceans, and can therefore be exposed to high levels of persistent, fat-soluble chemicals. Elevated levels of these anthropogenic chemicals, including the DDT group, the polychlorinated-biphenyls (PCBs), -dibenzo-*p*-dioxins (PCDDs) and -dibenzofurans (PCDFs), are suspected to have caused adverse biological effects in certain marine mammal populations. These include immunotoxicity, endocrine disruption, developmental anomalies and reproductive impairment. However, establishing a causal link between exposure and effect in free-ranging marine mammals is confounded by the complexity of the contaminants to which they are exposed through the consumption of prey species, and also by the numerous other natural factors that affect physiology, including age, sex, condition, disease, diet, stress, diurnal rhythms and reproductive status. This report contains a summary of an international workshop held at the Institute of Ocean Sciences, Sidney, BC, Canada, where marine mammal toxicologists, researchers and veterinarians explored the relationship between contaminants and marine mammal health, and identified promising new directions for research.

RÉSUMÉ

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Les mammifères marins occupent souvent un niveau trophique élevé dans les océans du monde, et peuvent conséquemment être exposés a de hautes concentrations de composés chimiques liposolubles et persistants. Des concentrations élevées de ces composés anthropogéniques, incluant les dérivés du DDT, les biphényls polychlorés (BPCs), dibenzo-*p*-dioxines et dibenzofuranes, sont soupconnées d'avoir causé divers effets biologiques néfastes chez certaines populations de mammifères marins. Ces effets incluent immunotoxicité, dérèglement du système endocrinien, anomalies développementales et effets néfastes sur la reproduction. L'établissement d'un lien de causalité entre exposition et effets chez des mammifères marins sauvages est toutefois difficile à établir compte tenu de la complexité des contaminants auxquels ils sont exposés via l'alimentation, et de la multitude d'autres facteurs naturels qui affectent leur physiologie, tels l'âge, le sexe, la condition générale, les maladies, la diète, le stress, le rythme circadien, et l'état reproducteur. Ce rapport inclu le sommaire d'un atelier international tenu à l'Institut des Sciences de la Mer, Sidney, Colombie-britannique, Canada, au cours duquel des toxicologistes, chercheurs et vétérinaires ont exploré les relations entre les contaminants et la santé des mammifères marins, et identifié des avenues de recherche nouvelles et prometteuses.

INTRODUCTION

Peter S. Ross and Sylvain De Guise

Despite a wide interest in issues of pollution and marine mammal health, there is a distinct lack of conclusive evidence for contaminant-related effects in free-ranging marine mammals. While epidemiological, associative or observational evidence suggests that certain marine mammal populations in some parts of the world have suffered from contamination of the environment, the power of the studies published to date is limited by the lack of direct causal relationships. Studies of laboratory animals, captive harbour seals, and other wildlife species (fish-eating birds, in particular), have found that many fat-soluble contaminants cause immunotoxicity, endocrine disruption and reproductive impairment, providing important background information for those studying free-ranging marine mammal populations inhabiting contaminated coastal areas. However, numerous factors, including age, sex, condition, season, diet, climate, diurnal rhythms, reproductive status, genetics, stress and disease, can and do affect the often poorly understood physiology of marine mammals. Those confounding factors can impede the ability to detect effects of contaminants on physiological systems, often frustrating attempts made by wildlife toxicologists to study free-ranging marine mammals. On the other hand, those in the veterinary sciences have long attempted to assess the health of domestic animals, agricultural herds and wildlife populations, using notions such as "normal ranges". We therefore felt that wildlife toxicologists could benefit from a better understanding of what defines "health" in an individual or population, some of the veterinary approaches to characterizing "health", and some of the factors which might confound the measurement of the very physiological systems being studied for contaminant-related effects in marine mammal research.

The goal of the present workshop was to gather scientists from different disciplines to assess the present state of knowledge of the effects of environmental contaminants on marine mammal health, to identify important directions for future research, and to encourage multidisciplinary collaborations, with an emphasis on the Pacific coast of Canada and the USA.

Contaminants are ubiquitous in the marine environment and can accumulate to very high levels in marine mammals at the top of the food chain. However, establishing whether the contaminant levels observed in free-ranging marine mammals are adversely affecting their well-being represents a considerable challenge. Workshop presentations addressed a number of issues, including the spatial and temporal trends of different classes of chemicals found in marine mammals; the evidence collected to date that such contaminants are affecting marine mammals; the definitions of "health" used by veterinary specialists and epidemiologists when examining individuals, populations and species; and new tools, techniques and strategies which could improve the power of toxicology studies to be carried out in the future. In each of these areas, multidisciplinary approaches, consisting of a wide range of molecular, cellular, organismal, and ecological techniques were identified as important components of any

study being designed to address contaminant-related effects on marine mammal health. The lack of "normal" or baseline data for many species of marine mammal represents an impediment to interpreting physiological information from individuals being studied.

The interpretation of data collected during toxicological studies of marine mammals requires detailed information on the diet, natural history, distribution, population numbers and population dynamics of the species being studied, as well as information on changes in the ecosystem, temporal and spatial trends of contaminant concentrations in animal tissues and in the environment, and the identification of any factors which may confound the interpretation of contaminant-related impacts. It was also felt that controlled *in vitro*, *in vivo*, and surrogate laboratory animal studies are vital in demonstrating cause-and-effect and dose-response relationships, as well as mechanisms of action, for chemicals and chemical classes. Finally, multidisciplinary investigations with standardized methods of sample collection, archival and analysis will be required.

Despite the logistic, ethical and financial challenges inherent in working with marine mammals, as well as the multiple confounding factors encountered when working with wild populations, it was deemed important to pursue investigations on the effects of environmental contaminants on health of marine mammals. Reasons include the potential effects of the numerous new chemicals produced every year, many of which bioaccumulate in the marine food chain and in marine mammals; the social value attributed by much of society and many cultural groups to marine mammals; and the value of marine mammals as sentinels for evaluating the risks associated with chronic exposure to environmental contaminants on the health of wildlife and humans. It is imperative that good scientific studies are designed to assess the issue of contaminant-related effects, and that factors which might confound the outcome of such studies be identified and eliminated or minimized.

This two-day workshop was hosted by the Institute of Ocean Sciences (Canadian Department of Fisheries and Oceans), and supported by the Vancouver Aquarium, the Marine Mammal Center (Sausalito, California), and the Whale Watch Operators Association NW. Wendy Simms proved invaluable to the workshop, contributing her energies to the many logistical preparations.

ORGANOCHLORINES IN MARINE MAMMALS: AN OVERVIEW

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Marine mammals usually occupy high trophic levels in marine ecosystems; they therefore tend to accumulate high concentrations of persistent contaminants such as organochlorines (OC) and these have been of concern for the last 30 years. Most studies have focussed on the following questions:

1. What compounds are present, in what concentrations, and how do these vary spatially and temporally?
2. What effects of OC on marine mammals can be inferred or demonstrated from observational or experimental studies?
3. What is the significance to human consumers, usually indigenous groups, of high OC concentrations in some marine mammal populations?

Most data which have been gathered over the last three decades focus on Question 1. All the main OC (DDT-group, PCBs, HCHs, Toxaphenes, PCDD/F) are found in marine mammals, usually, though not exclusively, reported from the northern hemisphere. As far as it is possible to make spatial comparisons, biota from industrialised northern Europe are (or were) usually more contaminated by DDT-group and by PCBs than those from North America. In northern Canada, there appear to be both longitudinal and latitudinal variations in the extent of OC contamination. In terms of temporal variation, both DDT-group and PCB concentrations appear to be declining in northern Europe, eastern and Arctic Canada, though changes in analytical procedures confound some long- term comparisons. However, these temporal declines probably reflect the bans on DDT (in "western" countries) and PCB use (world-wide) introduced in the early 1970's.

Question 2 will be addressed in detail in other presentations at this meeting.

Question 3 is of particular importance to some indigenous groups. Concentrations of PCDD/F and PCBs in some Strait of Georgia harbour seals are high enough that Health Canada has advised only limited human consumption of seal tissues. In the Canadian eastern Arctic, where ringed seal is eaten frequently, the high (human) OC body burdens that result have raised the question of possible neuro-developmental and immune function effects.

ENDOCRINE DISRUPTING CHEMICALS IN THE MARINE ENVIRONMENT

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In the past several years a substantial body of research has pointed out the hazards posed to wildlife and human health by synthetic endocrine-disrupting chemicals (EDCs). Broadly defined, EDCs are synthetic and/or natural agents present in the environment that interfere in some way with normal endocrine function (i.e. the synthesis, secretion, transport, receptor binding, action and/or metabolism of hormones is altered leading to adverse effects on the normal operation of organisms and offspring). A number of industrial, municipal, agricultural, and natural compounds have been shown or are suspected to be estrogenic. Such compounds include polychlorinated dioxins, furans (PCDD/F) and biphenyls (PCBs); selected pesticides (DDT, methoxychlor, kepone); non-ionic surfactants such as nonylphenol (NP) and biodegradation products of alkylphenol ethoxylates (APEOs); other industrial chemicals such as phthalates and bisphenol-A, as well as natural and synthetic estrogens (17β-estradiol, diethylstilbesterol, β-sitosterol and flavones).

There is a large body of scientific literature associated with the fate, distribution and endocrine effects of classical organic contaminants such as PCDD/Fs, PCBs, DDT etc. The biological end-points used to assess endocrine disruption vary depending on the species examined and the exogenous chemical tested. Due to the fact that the examined organism may contain a large variety of endogenous and exogenous chemicals which may interact synergistically and/or antagonistically with each other, there is some uncertainty when trying to link a target chemical to a biological end point.

In this talk an overview on EDCs will be presented and examples will be given where tissues of marine organisms have been found to contain some ten different families of organochlorine contaminants at various concentrations; how effective the biological end-points are in such situations? In the second part of the talk data on the fate, distribution and the potential adverse effects of a "new" class of EDCs (non-ionic surfactants, APEOs) in the marine environment will be presented.

PCBs AND DDTs IN WASHINGTON STATE MARINE MAMMALS EXAMINED SINCE THE 1970s: TEMPORAL, SPATIAL, AND SPECIES-RELATED PATTERNS

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Environmental contaminants, primarily the chlorinated hydrocarbons PCBs and DDT, have been examined in a variety of marine mammals from Washington waters since the 1970s. These include both published and unpublished findings from studies conducted by the author and other researchers. Data on contaminant concentrations in marine mammals from the Puget Sound area are available primarily from four species: harbor seals, harbor porpoise, killer whales, and gray whales. Sample sizes for other species of pinnipeds and cetaceans are more limited. Highest contaminant concentrations have been found in harbor seals and killer whales and lowest concentrations in gray whales and other baleen whales. Regional patterns in contaminant concentrations have been most apparent in harbor seals, with highest concentrations in animals from southern Puget Sound and Hood Canal compared to animals from the Strait of Juan de Fuca and the outer coast areas. Regional differences were not apparent in the species that ranged widely. Temporal trends in contaminant concentrations are only available for harbor seals. Concentrations in harbor seal pups from sites in southern and northern Puget Sound have declined steadily from 1972 to 1990. Harbor seal pups have proved to be ideally suited for examining trends because they are readily available and show a high degree of consistency within each set of samples (low variation) which allows sensitive detection of trends. New data on contaminant concentrations from the mid-1990s for biospsied live harbor seals and dead neonates should allow continued evaluation of trends in some of these contaminants.

CONTAMINANT-ASSOCIATED IMMUNOTOXICITY AND ENDOCRINE-DISRUPTION IN MARINE MAMMALS

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Environmental contaminants including the polychlorinated- biphenyls (PCBs), -dibenzo-*p*-dioxins (PCDDs or dioxins), and -dibenzofurans (PCDFs or furans), bioaccumulate in the food chain. Levels of many of these and other fat-soluble contaminants have been found to be very high in many free-ranging marine mammal populations, particularly those inhabiting the industrial coastal waters of North America and Europe. While conclusive evidence that such contaminants are adversely affecting these populations is lacking, a considerable "weight of evidence" exists which implicates ambient pollutant levels in a number of bioeffects. This evidence comes from i) mechanistic, cause-and-effect laboratory animal studies using single and/or multiple chemical doses in one-time or chronic exposure models; ii) epidemiological, associative or descriptive studies of free-ranging marine mammals inhabiting contaminated waters; iii) semi-field (captive) studies of marine mammals, usually harbour seals, involving the feeding of diets containing different contaminant levels; and iv) parallel laboratory animal models, where subjects (e.g. rodents) are exposed to an extract of contaminants from the same herring used in captive harbour seal feeding studies. While studies of free-ranging marine mammals is fraught with logistical and ethical difficulties, the harbour seal has gained favour as an important wildlife sentinel in ecotoxicology. This pinniped is relatively small, reasonably abundant, and widely distributed in both uncontaminated and contaminated areas of the northern hemisphere. In addition, numerous publications have addressed topics of physiology, immunology, disease, and toxicology in harbour seals. Captive feeding studies have established that ambient levels of environmental contaminants in Baltic Sea herring can be both immunotoxic and endocrine-disrupting to harbour seals, affecting T-cell function and natural killer cell activity, as well as vitamin A and thyroid hormone levels. Such effects may lead to an increased susceptibility to infectious disease in the case of immunotoxicity, or developmental, neurological or reproductive impacts in the case of endocrine disruption. The "weight of evidence" gathered from these and the other lines of research described above suggests that free-ranging harbour seals in many parts of Europe and North America may be at risk to contaminant-related bioeffects. While inter-species extrapolations should be made with caution, such evidence may provide an indication of potential problems in other pinniped and cetacean species exposed to elevated contaminant levels in their diet.

ST. LAWRENCE BELUGAS: AT RISK TO TOXICS?

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A small isolated population of beluga whales (*Delphinapterus leucas*) resides in the St. Lawrence estuary, Québec, Canada. High concentrations of different pollutants, such as industrial PCBs or agricultural DDT, have been demonstrated in the tissues of those whales. Overhunting in the first half of the century was the probable cause for this population to dwindle from approximately 5000 animals to the current estimate of 500. The failure of the population to recover might be due to contamination by organochlorine compounds, which are known to lead to reproductive failure and immunosuppression in domestic and laboratory animals and seals. We investigated that hypothesis on the basis of pathology findings as well as in vitro and in vivo models. Functional and morphological changes have been demonstrated in thyroid gland and adrenal cortex in many species exposed to organochlorinated compounds, including seals. Morphological lesions, although different, were also found in St. Lawrence belugas. Functional evaluation of thyroid and adrenal glands of contaminated (St. Lawrence) versus much less contaminated (Arctic) belugas is currently under way. Necropsy of St. Lawrence belugas showed numerous severe and disseminated infections with rather mildly pathogenic bacteria, which suggests immunosuppression. Organochlorine compounds and other contaminants found in beluga whales cause immunosuppression in a variety of animal species including seals. In vitro assays with Arctic beluga lymphocytes showed the susceptibility of the species to environmentally relevant concentrations of heavy metals and organochlorines. Thirty-seven percent of all the tumors reported in cetaceans were observed in St. Lawrence beluga whales. This could be explained by two different mechanisms: high exposure to environmental carcinogens and suppression of immunosurveillance against tumors. Overall, St. Lawrence belugas might well represent the risk associated with long-term exposure to pollutants present in their environment and might be a good model to predict health problems that could emerge in highly exposed human populations over time.

LOW-LEVEL ORGANOCHLORINE EXPOSURE: ARE NEONATAL SEALS AT SPECIAL RISK?

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In adult harbor seals, low to moderate body burdens of PCBs have been shown to be responsible for significant immune impairment [1-4] and alterations of thyroid hormone and retinol (vitamin A) levels [5]. The threshold levels at which these effects may occur in young seals are virtually unknown. Since seal pups are exposed to organochlorines in appreciable quantities *in utero* and through mother's milk [6,7], and appear to be less capable of metabolizing these compounds than adults [8,9], they are likely to be more vulnerable to immune suppression, thyroid toxicity, and other effects of these chemicals. In order to examine this possibility, samples were collected from 22 neonatal harbor seal (HS) and 26 weanling elephant seal (ES) pups undergoing rehabilitation at the Marine Mammal Center, Sausalito, CA. 73 PCB congeners were detected in blubber, including 13 coplanar congeners. Cellular immune function was evaluated using a whole blood lymphoproliferative assay to mitogens. Endocrine parameters included thyroid hormones (T^4, T^3, FT^4, and FT^3) and retinol in serum. The HS pups (gestational exposure only) had significantly higher contaminant loads and different congener profiles than the ES pups (gestational + lactational exposure). ΣPCB levels were low compared to those reported from seal populations considered highly contaminated; however, the more bioactive, non-*ortho* coplanar PCBs in the HS pups were comparable to levels reported in the contaminated (Baltic) group of juvenile HS with immune impairment [10]. In the HS pups, significant inverse correlations were observed between ΣTEQ and lymphoproliferative responses to Con A and PWM; and between thyroid hormone levels and the coplanar congeners 77, 126, and 156. Fewer significant contaminant-effect links were observed in the ES pups. These results suggest the need for additional studies to further clarify the links between low-level PCB exposure and toxic effects in neonatal seals.

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ASSESSMENT OF POTENTIAL HEALTH EFFECTS FROM ORGANOCHLORINE CONTAMINANT EXPOSURE IN FREE-RANGING NORTHERN FUR SEAL (*Callorhinus ursinus*) PUPS FROM ST. GEORGE ISLAND, ALASKA

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The northern fur seal (*Callorhinus ursinus*) population that breeds on St. George Island, Alaska, declined at an annual rate of approximately 6% from 1980 to 1996. Previous studies found lower than expected return rates after initial 2 year post-weaning migrations. A study was initiated to survey the health status of accessible pre-weaned pups at risk of high at-sea post-weaning mortality. In order to elucidate the possible role of organochlorine contaminant-linked immunosuppression, two cohorts of pups and their dams were examined. Forty-two neonates were captured for blood sampling and re-sampled at 4-6 weeks later. In addition, matched dams of 33 were concurrently captured for blood and milk sampling. Organochlorine (OC) compounds were extracted from whole blood and milk then subjected to high-performance liquid chromatography to identify 14 selected individual polychlorinated biphenyl (PCB) congeners and DDT metabolites. Cellular immune function assays along with complete blood cell counts, retinol and thyroxine levels were used as indicators of potential effects of OC exposure on health status. The pup's blood parameters were then compared to their individual and maternal OC congener profiles. PCB congener profiles of pup blood were better correlated to the dam's milk than blood with variations due to age and other factors. Inter-annual differences in exposure levels and specific congener concentrations were apparent. Neonates of young dams (presumably primiparous) had significantly elevated levels of various congeners over neonates of older dams. Serum retinol and thyroxine levels, which decrease as part of the toxic effect of PCBs in laboratory animals, were negatively correlated to increasing toxic equivalency quotients (TEQs) and select congeners in pups. Functional lymphocyte proliferation responses to the mitogen ConA were decreased in correlation with increasing levels 9 PCB congeners. Additional studies of cellular and humoral immune function of pups followed from birth to weaning are planned to further delineate the correlation between OC exposure and immune functional development in fur seal pups.

ENVIRONMENTAL ENDOCRINE DISRUPTORS: EFFECTS AND POSSIBLE MECHANISM(S) IN YOUNG MALE RIVER OTTER

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A recently completed study (1994-95) on river otters (*Lutra canadensis*) from the lower Columbia River showed males less than one year old had significantly smaller baculum (penis bone) size and weight, and much smaller testes when compared to reference animals. This study was conducted on frozen carcasses of otter collected by trappers in both the Portland, Oregon metropolitan area and at various downstream sites along a 110 mile stretch of the lower Columbia River. No other body measurement appeared significantly different from those of reference animals. Furthermore, reproductive organs of adult male river otters collected on the lower Columbia River did not appear to be significantly different from reference animals. Reproductive organ hypoplasia of young male river otter was correlated with 6 organochlorine insecticides (OCs), 29 polychlorinated biphenyls (PCBs), 2 polychlorinated dibenzo-p-dioxins (PCDDs), and 4 polychlorinated dibenzofurans (PCDFs). The current study (1996 to date) broadens our data base (different contaminant concentrations and ratios) to further understand which contaminants might be responsible for the adverse effects observed and to gain a better understanding of baculum and testes growth and development. Further laboratory studies (determination of cytochrome P450 levels, steroid receptor numbers, steroid receptor binding, steroid metabolite levels) will be conducted using live-trapped river otter from the lower Columbia River system and reference sites, to establish the probable mode(s) of action or mechanism(s) by which the observed reproductive organ hypoplasia occurs, and to use feces as a biomonitoring tool to assess river otter contaminant body burdens (possible biomarker). Also, live-trapped otters will provide fresh tissue for histological samples, free of freezing artifacts. If endocrine disruptors are responsible for the observed reproductive effects of young male river otter, then continued study of these effects could provide important information concerning both wildlife and human health risk.

ENVIRONMENTAL CONTAMINANTS IN FISH-EATING BIRDS FROM THE STRAIT OF GEORGIA, BRITISH COLUMBIA

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Fish-eating birds such as the great blue heron, double-crested cormorant and bald eagle are among the most visible wildlife inhabitants of the BC coast. Since 1970, the Canadian Wildlife Service has collected samples of herons and cormorants to monitor environmental contaminants, such as organochlorine pesticides and PCBs, in wildlife food chains. More recently we have studied those species in context of exposure and effects of persistent pulp mill pollutants, in particular the polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), in wildlife. Over the period 1983-92, elevated PCDD/F levels were found in eggs of herons, cormorants and eagles foraging in areas impacted by pulp mill effluents. During that period we conducted a series of experiments, which documented the effects of 2,3,7,8-TCDD and related compounds on embryonic development in those species. We also conducted studies of reproduction and examined chicks for deformities. By the early 1990s, most B.C. pulp mills had implemented process changes to eliminate PCDD/Fs in effluents. Levels of those chemicals subsequently decreased significantly in eggs of herons and cormorants from the impacted areas. Chick health and reproductive success also improved. However, particularly around the pulp mill at Crofton on Vancouver Island, bald eagles continued to show poor reproduction. In 1996 and 1997, we conducted a study to examine the roles of contaminants and/or food stress on bald eagles breeding near Crofton and at a reference area in Barkley Sound on the west coast of Vancouver Island. Data were collected on prey availability, adult and eaglet behaviour and adult and eaglet blood contaminant levels at both the treatment and control sites. Results indicate that food stress and contaminants are likely acting together to cause reduced productivity at bald eagle breeding territories located south of Crofton mill.

THE NEED FOR A NEW PERSPECTIVE IN WILDLIFE TOXICOLOGY

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Some synthetic chemicals have the ability to interfere with the natural endocrine, neuroendocrine, and developmental chemical messengers that co-ordinate development and functioning in an individual. These chemicals are widespread, are detectable in biological tissues, and many are widely distributed in the environment and in the food web. As the literature that describes the effects in laboratory and field situations grows, there is an increasing concern that animals exposed to high concentrations of these chemicals may be at greater risk. We are at the point today where in order to more fully understand the risks involved we must adopt a new perspective of how these chemicals operate. We can no longer limit our concern to the quantities in tissue, but instead to subtle effects that may be associated with developmental and functional deficits arising out of exposure at critical points in the life of an animal. As new information is learned in the laboratory concerning the pathways along which these chemicals act, new biomarkers must be developed that can allow us to assay the health of probably some of the most difficult animals to study- the marine mammals. For marine mammals the task may appear hopeless, but we can not succumb to such emotions. We must form multidisciplinary partnerships to share experience, observations, and data, as well as to design the critical research programs that can answer critically important questions. It is only with such efforts can we begin to fully assess the role synthetic chemicals plays in the health of marine mammals.

STATUS OF CETACEANS IN BRITISH COLUMBIAN WATERS

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A total of 23 species of cetaceans has been recorded in coastal and offshore waters of British Columbia. The current status of the majority of these is poorly known. Knowledge of the population abundance, distribution and ecology of most cetacean species in BC waters is based on sightings collected incidentally during studies of other species, or from anecdotal sighting reports. Most sighting records are from nearshore waters during spring through fall. Historical data from commercial whaling operations shed light on the former occurrence of some baleen whale species in BC waters, but provide little useful information on current abundance or distribution. Eight of the 23 cetacean species recorded for BC can be considered common in the region, 2 species are uncommon, and the remaining 13 species are rare or accidental. By far the best known cetacean in BC waters is the killer whale. Photo-identification studies undertaken annually since the early 1970s have documented three distinct populations, resident, transient, and offshore, that differ to varying degrees in seasonal distribution, social structure, behaviour, diet, acoustics and genetics. The resident population totals approximately 320 and is increasing at 1-2% per annum. About 220 transient and 200 offshore killer whales have been identified to date. Other common odontocetes include the harbour porpoise, Dall's porpoise, Pacific white-sided dolphin and sperm whale. Harbour and Dall's porpoise are found year round throughout the area, but population abundance is unknown. Pacific white-sided dolphins have increased in abundance in nearshore BC waters during the past 15 years, but total population size is unknown. Sperm whales are found in deep waters off the continental shelf and are seldom sighted, but are likely fairly abundant. Three baleen whale species are common in BC. Gray whales are abundant migrants along the coast, but small numbers, perhaps less than 200 in total, can be found feeding at several nearshore locations throughout the year. Humpback whales are also common in certain feeding areas during spring through fall, and about 300 individuals has been documented to date. Minke whales are sighted regularly in small numbers throughout the coast, but no abundance estimates are available. The only recent information for the remaining 15 cetacean species are occasional sightings or strandings.

Systematic surveys to determine population abundance and seasonal distribution of cetaceans in BC are urgently needed, particularly in coastal areas with growing industrialization and for species which conflict with human fisheries. A good understanding of the species' life history and ecology is also needed, particularly for features that may affect the dynamics of contaminant accumulation. For example, large differences in life expectancy among species can affect accumulation of pollutants, as can population or individual differences in diet and fidelity to feeding locations. Behavioural factors, such as communal nursing, may also play a role in the distribution of contaminants within social groups.

THE ROLE OF DISEASE IN THE ECOLOGY OF WILDLIFE POPULATIONS

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Disease is an integral part of natural communities. Yet, we often only become aware of disease when it is manifested as dramatic event such as outbreaks, mass strandings, or die-offs. Ecology textbooks traditionally have only considered infectious and parasitic diseases when discussing wildlife and have often concluded that it only acts as a density-dependent population regulating factor. However, disease is more broadly defined and can affect animal productivity, distribution and abundance in a variety of ways on both a short and long-term. Reviews of infectious and non-infectious disease epidemiology reveals that disturbances in local ecosystems often precipitate health effects in animal populations. While it is difficult and perhaps undesirable to try to regulate endemic diseases in wildlife, managers and the public are becoming more interested in mitigating disease effects in species affected by human actions. Anthropogenic changes such as the movement of species, alteration of habitat or introduction of toxins have had profound disease effects on wild species. Examples will be given to illustrate these points. It is concluded that an improved understanding of the determinants of disease in wild populations and its role in undisturbed populations will be required to predict and prevent dramatic disease effects that can accompany environmental change.

MORTALITY IN FREE-RANGING MARINE MAMMALS IN BRITISH COLUMBIA (1993 - 1997)

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Tissues were submitted to the Animal Health Centre from ninety-three cetaceans including 54 harbour porpoises, 32 Dall's porpoises, 5 Pacific White-Sided dolphins, 1 grey whale and 1 common dolphin between 1992 and 1998. There appeared to be relatively equal representation of sexes in this group.

A submission may be either a whole animal or tissues collected from a whole animal. Histopathology was performed on tissues from seventy-two animals and 162 diagnoses were made from these tissues. Due to the limitations imposed by the nature of the submissions, laboratory diagnoses were not necessarily the cause of death. Autolysis was a diagnosis in 22 animals and 13 submissions demonstrated no microscopic abnormalities. Parasitism was included as a diagnosis in 43% of the submissions, 5% of the diagnoses were attributed to bacterial infections, and 22% were diagnosed as "other" (category consisted of a variety of findings, none of which were large in number).

Trace mineral (selenium, copper, manganese, iron, zinc, calcium, and magnesium) and heavy metal (lead, cadmium, mercury, and arsenic) analyses were performed on all 93 submissions from which fresh liver and kidney were submitted. Samples in which blubber, liver, and kidney were included were forwarded to other laboratories for screening for organochlorines. The levels of these agents were generally low and unremarkable. One sample, however, had high levels of OCs; interestingly, this animal also had a congenital foreshortening of the mandible, and died from erysipelas infection.

Unfortunately, in most cases when animals are discovered, post-mortem decomposition is advanced which significantly limits the types of testing (bacteriology, mycology, virology) that can be performed. As well, it is unusual that a veterinary pathologist attends which may limit the observations that might otherwise be made. The examination of tissues from free-ranging marine mammals, however, provides an insight into the conditions faced by these animals in their environment. It is through such evaluations that the importance of factors such as parasitism and background mineral levels can be assessed.

HEALTH ASSESSMENT IN STRANDED AND REHABILITATED PINNIPEDS

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Health, defined as freedom from defect, pain or disease (Webster's dictionary) is routinely assessed in humans and domestic animals by a variety of techniques including body morphometry, auscultation, palpation, ophthalmoscopy, radiology, electrocardiography, ultrasonography, endoscopy, organ biopsy, urinanalysis, hematology and serum biochemistry. Results are compared to known "normal" values, established by extensive longitudinal sampling of many healthy individuals. A number of these techniques have been used on pinnipeds, but few have been evaluated in sufficient individuals, or at a sufficient number of different time points, to determine the effects of developmental, physiological and environmental variables on measured parameters. In addition, the definition of "normal" health is unclear in pinnipeds. Data are available from captive animals fed diets that differ markedly from their natural one, and that are maintained in very different social groups and habitats from those of free-living conspecifics. Many data from wild animals are single time-point samples, and the fate of the animal post-sampling is unknown.

The most frequently used tools for assessing health are hematology and serum biochemistry. Many standard methodologies require modifications for use on pinniped blood due to differences in cell size and the lipemic nature of pinniped plasma after feeding. Sampling methods can also affect parameters due to stress responses and anatomical and physiological adaptations of pinnipeds to diving. Cell type and number are affected by disease states such as infection, inflammation and neoplasia, but may also be altered by age, sex, reproductive status, season, diet, hydration status and stress. Serum electrolytes are rapidly altered in many disease states, but are also affected by a number of physiological factors. Sodium and potassium balance in marine mammals appears to be extremely susceptible to stress, compared to terrestrial mammals. Serum enzyme levels can yield useful information on organ function, but little is known about the relative abundance of enzymes in different cell types in pinnipeds. Hormone levels, especially T3, T4, retinol and cortisol, are frequently used as indicators of organ function, although little is known about seasonal and developmental changes in these hormones in most pinniped species.

It is thus difficult to interpret results of single assays and relate them to health status of pinniped individuals and populations. More detailed laboratory studies are required, focusing on longitudinal changes in health parameters and how they are affected by developmental, physiological and environmental factors.

MONITORING THE HEALTH OF PUGET SOUND HARBOUR SEALS

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Puget Sound is an estuary of national significance encompassing the marine waters of Washington state including Admiralty Inlet, Hood Canal, Strait of Juan de Fuca, and Strait of Georgia. The Puget Sound Ambient Monitoring Program (PSAMP) was established in 1988 to assess the health of Puget Sound and its resources. PSAMP coordinates the collection of baseline and long-term information on water quality, sediments, biological resources, and habitats of the Puget Sound ecosystem. As part of this effort, the Washington Department of Fish and Wildlife (WDFW) is responsible for implementing studies to address the health of marine birds, waterfowl, and marine mammals. To address the health of Puget Sound marine mammals, harbour seals were selected as an indicator species. Harbour seals are considered a good indicator species because they are year-round, breeding residents of Puget Sound, as well as being abundant, widely distributed, and relatively easy to study. As upper trophic level predators, harbour seals also provide a mechanism for monitoring the potential effects of contaminants on other marine species as well as humans. In order to look at the overall health of Puget Sound harbour seals, WDFW has worked with researchers at the National Marine Mammal Laboratory and the Department of Fisheries and Oceans to conduct 1) annual assessments to determine status and trends in abundance, 2) annual capture and marking of cohorts to monitor vital parameters and reproductive success, 3) screening of exposure to calicivirus, influenza virus, morbillivirus (canine and phocine distemper), leptospirosis and brucellosis using serology, and 4) monitoring of temporal and spatial trends in selected contaminants (primarily PCBs). Based on a review of these monitoring efforts, evaluation of the "health" of Puget Sound harbour seals were equivocal, with the population increasing at 8-10% annually despite showing evidence of exposure to a number of potentially harmful diseases as well as having elevated levels of PCBs which could affect immune function.

MARINE MAMMAL HEALTH INVESTIGATIONS: LINKING BIOMONITORING, STRANDING RESPONSE AND SPECIMEN ARCHIVAL

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The trophic position of many marine mammal species increases their risk for bioaccumulation of persistent lipophilic organochlorine (OC) contaminants (e.g., PCB congeners, DDTs and other pesticides) and of certain trace elements (e.g., lead, mercury, cadmium). Consequently, marine mammals may serve as appropriate bioindicator species for detecting changes in environmental quality. Mass mortalities of marine mammals occur periodically, raising concern that degradation in environmental quality may have a role in these events. Whether mass mortalities are linked to changes in environmental quality, in general, or exposure to toxic contaminants, in particular, is contentious. What is more generally accepted is that quantifying the effects of anthropogenic contaminants on marine mammals, as well as other aquatic species, remains elusive and controversial. Reasons for this view vary from the practical difficulties in investigating marine mammals and the lack of appropriate bioanalytical tools to emerging ecological issues of multiple stressors that affect marine mammal health and the difficulty in assessing cumulative effects and the partitioning of effects from individual stressors. We advocate that ecotoxicologic investigations of cause-and-effect relationships in marine mammals will benefit from the integration of biomonitoring, stranding response, quality assurance, and specimen archival, and that such an integration can aid in facilitating multidisciplinary investigations. The US National Marine Fisheries Service's Marine Mammal Health and Stranding Response Program (MMHSRP) serves to integrate these components to improve collection of baseline data for marine mammals on exposure to toxic chemicals, disease occurrence and prevalence, and to implement epizootiological investigations of the relationship between deleterious biological effects and contaminant exposure. We will use recent findings to highlight approaches being used in the MMHSRP, including the inclusion of stranded and free-ranging animals in studies, as well as discuss both the successes and current limitations in facilitating a true multidisciplinary approach to ecotoxicologic investigations in marine mammals.

MOUSE TO MARINE MAMMAL: NEW APPROACHES IN COMPARATIVE TOXICOLOGY

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related planar halogenated aromatic hydrocarbons (PHAH), including PCBs, are ubiquitous contaminants of the global environment. PHAH are highly toxic to many vertebrate species, but there is substantial variability in sensitivity (up to 1000-fold) among species and classes. Some marine mammals are highly exposed to PHAH and these contaminants have been suggested to contribute to marine mammal mortality and morbidity. The magnitude of the risk that PHAH pose to the health of marine mammals is controversial, however, because there is little direct information on the sensitivity of these animals to PHAH and no data on the relative potencies of individual PHAH (i.e. toxic equivalency factors or TEFs). Because legal and ethical concerns preclude addressing these questions through direct testing of toxic chemicals on protected animals such as marine mammals, alternative approaches are needed for assessing the susceptibility of these species to effects of environmental contaminants.

Assessing the PHAH sensitivity of marine mammals requires an understanding of the molecular mechanisms by which these chemicals act. For PHAH, that mechanism involves the aryl hydrocarbon receptor (Ah receptor or AhR) and AhR nuclear translocator (ARNT), components of the AhR signal transduction pathway. Many, if not all, PHAH effects occur through this pathway. Studies in inbred mice and other species have shown that the expression and properties of the AhR can determine the sensitivity of animals to these compounds.

We are investigating the PHAH sensitivity of cetaceans using three approaches: 1) Characterization the structure and function of a cetacean AhR through RT-PCR cloning, expression, and biochemical analyses. 2) Determination of the responsiveness of cetacean cell lines to TCDD and related PHAH. 3) Characterization of AHR and ARNT expression and PHAH responsiveness in cetacean peripheral blood lymphocytes.

The results of these studies will be compared to existing data on PHAH responses and structure-activity relationships in cells from other mammals and other vertebrate groups. Application of these methods to cetaceans will serve as a model for a new approach to assessing the risk of environmental contaminants to protected species.

NEW REAGENTS AND TECHNIQUES FOR MARINE MAMMAL HEALTH ASSESSMENT

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The health and welfare of free-ranging and captive marine mammals has gained impressive visibility in recent years. The public has demanded that attention be given to the well-being of these animals and that steps be taken to determine the adverse effects of industrial and environmental pollution. Identification of an adverse relationship between animal health and the presence of potentially toxic contaminants, both in tissues and in the external environment, has been fraught with difficulty. While associations between large chemical spills and acute animal disease is easily recognized, the more subtle effects of long-term exposure to a low or moderate level of pollutants is more difficult to assess. Our laboratory was established to address issues of marine mammal health from an immunologic perspective. Such an approach would be supported by the fact that the immune system is easily influenced by minor perturbations in the external environment and the previous demonstration that many environmental contaminants are immunosuppressive. The ability to evaluate the immune system via non-invasive collection of a blood sample further lends immune system analysis to assessment of animal health.

While general features of the immune system are similar between mammalian species, only the classic assays capable of identifying dramatic immunologic dysfunction can be applied across species. Our laboratory has optomized and established baseline values for many of these classic assays in multiple marine mammal species. In addition, we have begun to establish species-specific reagents that will permit advanced analysis of the immune system of multiple species. These efforts have been directed at components of both the innate and adaptive immune system. For clarification, the innate system is not pathogen specific and represents the first line of immunologic defense including phagocytosis, inflammation, natural killer (NK) cell activity, etc. The adaptive immune system is antigen- (pathogen)-specific and provides memory (long-term immunity) via effector mechanisms that include antibody production, cellular cytotoxicity, T cell lymphokine-mediated immunity and the arming and/or amplification of multiple innate defenses. A number of these new techniques evaluating both arms of the immune system are outlined below.

The inflammatory response is indicative of disease, tissue damage or other trauma. A number of soluble mediators (acute phase proteins and cytokines/interleukins) of inflammation can potentially be identified in the peripheral blood of an animal and thus serve as a sensitive and early indicator of tissue damage. One such important mediator is interleukin-6 (IL-6) for which a murine bioassay has been adapted to detect

elevated levels in the sera of diseased marine mammals. Furthermore, development of monoclonal antibodies, specific for harbor seal C-reactive protein (CRP; an acute phase protein), has allowed the development of an enzyme-linked immunoassay to quantify this mediator in seal sera. Quantitative evaluation of these two inflammatory mediators has demonstrated excellent association with disease. In addition to identification of inflammation, assays have been adapted to address the relative functionality of cells of the innate immune system including neutrophil phagocytosis and NK cell activity.

The adaptive immune system has classically been divided into two branches, a humoral arm (antibody production by B cells) and a cellular arm (T cell-mediated immunity). Assays have been developed to qualitatively and quantitatively identify antibodies of multiple marine mammal species. These assays have been refined to varying degrees, depending on the species, and range from monoclonal-based antibody techniques for defining antibody isotypes to polyclonal antibody-based assays for identifying total immunoglobulin and some of their subclasses. These reagents have also been used to identify antigen-specific antibody, indicating current (rising titer in paired sera samples) or previous exposure to multiple pathogens.

Direct assessment of lymphocytes, which are the primary mediators of adaptive immunity, has been realized from both a functional and phenotypic approach. Assays have been developed to quantify mitogen-induced T cell activation via identification of interleukin-2 (IL-2) receptor expression on the cell surface using analytical flow cytometry. This technique has been used to augment lymphocyte functional data obtained from the classic mitogen-induced lymphocyte blastogenesis assay. These lymphocyte function assays are based upon rather non-specific stimuli of lymphocytes (mitogens) and are thus indicators of rather severe immunologic abnormalities. Assessment of minor perturbations in the immune system is best realized by application of reagents for qualitatively and quantitatively identifying lymphocyte surface proteins referred to as differentiation antigens. Such proteins are responsible for cell-cell communication and leukocyte traffic (ability of immune system cells to travel throughout the body). We have developed two panels of monoclonal antibodies specific for leukocyte differentiation antigens of cetaceans and phocid pinnipeds in order to evaluate the sub-populations of morphologically indistinguishable lymphocytes in clinical samples. Data is currently being collected from both apparently healthy, diseased and stressed animals such that perturbations can be identified.

Collectively, the information obtained from these assays contributes to the overall picture of an individuals health. These assays are currently being evaluated for use with wild and captive marine mammals.

QUANTITY VS QUALITY: ASSESSING THE IMMUNOCOMPETENCE OF MARINE MAMMALS

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Marine mammal immunology is a relatively new, rapidly evolving science. Some of the reasons for its recently recognized importance include the realization that the immune system plays a central role in general health, and the fact that immune functions can be influenced by several factors such as stress or pollution. Nevertheless, it is important to understand the complexity of the immune system and its functions, and the fact that no single assay can pretend to evaluate the immune system as a whole, but that different assays can evaluate different components, mechanisms or functions. In order to compare immune functions in individuals or populations, it is important to target study systems and to understand the functions looked at, their meaning, and the possible sources of variation. The handling of the samples is the critical initial potential source of variation. It is important to consider the planning of the experiment with limited number of samplings in order to realistically achieve standardized high quality collection of samples, storage and processing rather than higher number of samples of doubtful quality that will result in meaningless data. In order to minimize the assay variations, it is important to run proper controls concurrently with every assay. It is also important that the manipulations, conditions and reagents for the assay be standardized. Finally, the interpretation of the data must rely on thorough knowledge of the biological meaning of the function studied. In conclusion, in order to be able to compare immune functions of different individuals or populations of marine mammals, it is important to prioritize highly standardized procedures for: (1) the collection, handling, transport and processing of the samples; (2) performing the assays; and (3) the interpretation of the results. This will be achieved through thoroughly planned experiments and collaborations with experienced laboratories.

CONTAMINANTS AND HEALTH ASSESSMENT RESEARCH IN ARCTIC ALASKA WILDLIFE: BIOLOGISTS, VETERINARIANS, AND SUBSISTENCE HUNTERS TAKE ON THE CHALLENGE

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The Department of Wildlife Management (DWM) has ongoing and recently funded projects addressing contaminants and adverse effects in marine mammals and exposure of people consuming tissues. Projects were funded by the DWM and NOAA, currently Office of Naval Research (ONR), and recently the Cooperative Institute For Arctic Research (CIFAR) funded by NOAA. Progress has been made, but logistic constraints and a lack of basic biologic and health data compromises our assessment. Assessments begin with gross examination by collecting vital morphologic, body condition, parasite burden, suspect lesion, hunt-related and other data. Collection of a suite of samples for microscopic examination, aging, disease monitoring etc. will be discussed. Too many studies in arctic species report very little data with regards to the animals health and condition. Observations of the hunters on animal condition and aspects of the population they observe while hunting are integrated. Lesions, "normality" or suspect changes can be falsely associated with contaminants if other factors are not considered. How does one show "no effect" conclusively? This workshop will discuss progressive tools for the future. We agree strongly, but we stress the importance of establishing in arctic marine mammals what is now considered "basic data" in most domestic and some wildlife species. Evaluation of tissue and subcellular distributions of metals (autometallography) and intensive examination of suspect metal induced changes is underway. Recent studies address more intensive evaluations of bowhead whale health and the food chain (trophic transfer and "who's eating whom!"). This unique situation combines traditional knowledge and subsistence activities, with biology and biomedical professionals (in this case veterinarians) to form a local cooperative program to collect the data and samples. This workshop represents many of the best marine mammal contaminants health investigators and offers the chance to improve our studies and future collaboration.

DISCUSSION

Sylvain De Guise and Peter S. Ross

The goal of the present workshop was to gather scientists from different disciplines to assess the state of knowledge of the effects of environmental contaminants on marine mammal health, identify important areas for future research, and promote collaborations, with an emphasis on the Pacific coast of Canada and USA.

Only a short reminder at the outset of the workshop was necessary to establish that lipophilic contaminants are ubiquitous in the environment and make their way to the tissues of marine mammals at the top of the food chain, often reaching very high concentrations. However, demonstrating adverse contaminant-related effects on the health of free-ranging marine mammals represents a daunting task. Workshop presentations addressed i) spatial and temporal trends in different types of contaminants found in marine mammals; ii) the evidence that such contaminants are having adverse effects on free-ranging marine mammals; iii) approaches used by veterinary specialists and epidemiologists in defining and assessing health; and iv) new tools, techniques and approaches that are being developed to address the effects of contaminants on marine mammal health. For each of these issues, a broadly-based, multidisciplinary approach was encouraged, and it became evident that an understanding of effects from the molecular to the ecological level were essential to addressing the question of contaminants in wildlife. In this regard, it was noted that we lack "normal" or baseline data and background information on various aspects of marine mammal physiology and ecology.

A "perfect" study design to demonstrate the effects of environmental contaminants on marine mammal health does not exist. Nevertheless, the presentations and discussions of the workshop led to the following observations:

1- The "big picture" is required. Data on the natural history, distribution, population numbers and dynamics of the species studied, as well as changes in the ecosystem, are required for a proper evaluation of data on the potential effects of environmental contaminants on marine mammal health.

2- Monitoring of temporal and spatial trends of contaminant levels in tissues of different species of marine mammals would provide more useful information than single measurements.

3- Controlled *in vitro*, *in vivo,* and surrogate laboratory animal studies are necessary to understand cause-effect and dose-response relationships as well as mechanisms of action of environmental contaminants on different parameters of health in marine mammals.

4- Multidisciplinary investigations with standardized methods of sample collection, archival and analysis will be required.

5- While it will continue to be difficult to unequivocally demonstrate contaminant-associated effects in free-ranging marine mammals, a "weight of evidence" approach, which incorporates extrapolation between laboratory studies (cell lines, laboratory rodents, single chemical vs complex chemical mixtures), semi-field studies (e.g. examples of past studies of harbour seals fed herring from either "clean" or "contaminated" areas), and field studies (epidemiological studies of contaminated marine mammal populations), is encouraged.

6- The harbour seal was put forth as potentially useful "sentinel species" for temperature regions of the northern hemisphere, because of its wide distribution, and the reasonably large literature on contaminants and contaminant-related effects in this species.

Despite logistical and ethical problems, the large number of confounding variables, and the costs associated with working with marine mammals, the value of marine mammal toxicology was evident, and attributed to several key points:

1- Several thousand new chemicals are designed and introduced to the environment every year. Many of these have demonstrated effects on development, behaviour, reproduction, and endocrine, neurological, and immune function processes in laboratory animals. In addition, subtle effects with important repercussions have been observed in organisms exposed to extremely low concentrations of certain chemicals during particular developmental (e.g. fetal) "windows".

2- The aquatic ecosystem is the ultimate sink for industrial, domestic, and agricultural pollutants through atmospheric transport processes, drainage and sewage systems.

3- Marine mammals are often good integrators of ecosystem contamination because of their relatively high trophic level in the food chain.

4- In addition to their cultural and economic value in certain locations, an important social value is generally attributed to marine mammals. Many national laws and international treaties have been adopted for their protection, and they have acquired considerable public popularity. This gives them a high visibility, and facilitates the distribution of relevant scientific information to the general public.

5- Results obtained by researchers to date suggest that marine mammals are useful sentinels for demonstrating the adverse effects of environmental contaminants on the health of wildlife populations. In addition, because marine mammals share the top of the food chain with certain human consumer groups, they may represent a good model for the evaluation of risks associated with chronic exposure to environmentally-relevant concentrations of contaminants on human health.

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