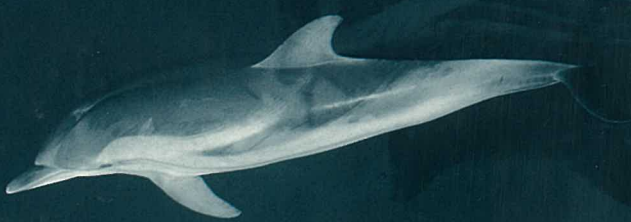
The Journal of

CETACEAN RESEARCH AND MANAGEMENT

Special Issue 1

Chemical Pollutants and Cetaceans

P. J. H. Reijnders, A. Aguilar and G. P. Donovan



Chemical Pollutants and Cetaceans

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INCORPORATING THE REPORTS LEADING TO THE DEVELOPMENT OF THE POLLUTION 2000+ PROGRAMME

Series Editor

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Journal of Cetacean Research and Management (Special Issue 1)

Cambridge 1999

The Journal of Cetacean Research and Management

ISSN 1561-0713

The Journal is published thrice yearly by the International Whaling Commission (IWC), with at least one supplement that will contain the full report of the Scientific Committee of the IWC.

The Journal has been established to publish papers on those matters of most importance to the conservation and management of whales, dolphins and porpoises, and in particular papers that are relevant to the tasks of the IWC Scientific Committee.

Special Issues on particular subjects will be published from time to time (*ISSN 1561-073X*).

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Editorial

Welcome to this, the first Special Issue of the *Journal of Cetacean Research and Management*. From time to time the *Journal* will publish Special Issues on particular topics. Although these will follow the same Editorial standards and procedures they will often involve guest editors and be hardbound. They will be additional to the standard *Journal* subscription and will be charged for separately; they also have a different ISSN number (ISSN 1561-073X).

Cover photographs: Greg Donovan (humpback whale) and Jaume Forcada (striped dolphin)

Introduction

This volume, the first special issue of the *Journal of Cetacean Research and Management*, traces the work of the IWC (International Whaling Commission) Scientific Committee on the issue of chemical pollutants and cetaceans. It culminates in the major research initiative, POLLUTION 2000+, agreed by the Committee and the Commission at the 1999 Annual Meeting held in Grenada.

The involvement of the IWC in the issue of pollutants and cetaceans goes back to 1981 when, in response to work by the Scientific Committee, the Commission passed a Resolution noting the potential threats to whale species (particularly sperm whales) raised by heavy metals, PCBs and other organochlorines and calling for governments to initiate research on the subject (IWC, 1982).

The issue was placed as a standing item on the Committee's agenda and in 1984, the Committee adopted a standard approach for the collection of samples and presentation of results for organochlorine analyses (IWC, 1985). The following year, a Working Group was established to consider the question of whale habitats and in particular, chemical pollution (IWC, 1986). It was noted that although there had been a number of studies measuring levels of pollutants in cetacean tissues, not enough emphasis had been placed on (a) ensuring consistency of methodology and reporting of the variables that affect interpretation of levels (e.g. age, sex, reproductive condition, health) and (b) on studies to examine mechanisms and effects. The Working Group also considered the question of whether cetaceans could be considered as useful indicators of the 'health of the ocean environment'. It agreed that, while cetaceans were not suitable for monitoring global ocean pollution in a strict sense, it may be possible to obtain some information on trends in certain pollutants. Finally, the Working Group considered the question of the suitability of samples from stranded animals for pollutant analysis. There are problems in considering stranded animals and their tissue levels as being representative of those characteristic of the true population and the Working Group recommended that, where alternatives exist, these should be used. However, in some areas and for some species there may be no alternative; in these circumstances, such samples may provide an insight into the magnitude of the species' exposure to pollutants, although the representativeness of the sampled animal should be carefully considered taking into account its particular characteristics (e.g. estimated post-mortem time, likely cause of death, nutritive condition, pathology). The Committee adopted the report of the Working Group.

Despite these initiatives, pollutant studies subsequently received relatively little attention in the Committee, largely due to efforts being concentrated on the development of the Revised Management Procedure (e.g. Donovan, 1995; IWC, 1999) as part of the Comprehensive Assessment (Donovan, 1989) arising out of the decision for a pause in commercial whaling. In 1993, work on pollutants was again considered but this time in the broader context of the overall effects of environmental change on cetaceans (IWC, 1994). This was partly due to some concern that environmental factors had not been taken sufficiently into account in the development of the RMP. In response, the Committee drew attention to the work it had already carried out in this regard as well as the results of some additional simulations. Environmental threats affect all species of cetaceans, not merely those subject to direct capture. Indeed, the most vulnerable species to such threats would be those species already reduced in numbers - in the context of the RMP these would be populations for which zero catch limits would be set even if the RMP was to be applied. The Committee stressed that the Commission would have to contemplate response strategies outside the direct management of whaling activities if it wished identified threats to be

alleviated. These may include local measures in terms of habitat protection or much wider action with respect to global threats.

In the context of environmental change, the Committee noted several areas that would require consideration, including: global warming; ozone depletion; pollution (e.g. chemical and noise); direct and indirect effects of fisheries; coastal development and tourism. It recognised, of course, that the question of synergistic and cumulative effects would need to be addressed. Given the broad nature of the subject, it was agreed to focus initially on two subjects: chemical pollution; and environmental change and ozone depletion. Subsequently, two workshops have been held. The first, on chemical pollution, forms the basis of this volume. The report of the second workshop is given in IWC (1997).

An important factor in the decision to hold a workshop on chemical pollutants was the fact that in modern times man has introduced over 200,000 synthetic chemicals into the environment and has profoundly altered the availability to living organisms of naturally occurring elements (e.g. mercury). An undetermined but significant portion of these chemicals are not rapidly degradable, have been incorporated into food webs and have a demonstrable or suspected detrimental impact on living organisms. Chemical pollutants are widely recognised as perhaps having one of the most potentially pervasive impacts on wildlife.

As cetaceans are long-lived, have extensive fat stores and are often top predators, some species carry tissue pollutant levels that are among the highest recorded. This has obviously raised concern over the potential impact of these chemicals on the long-term survival of the affected species and populations. Responding to this concern, in the last three decades a substantial effort has been made in establishing the levels of exposure, tissue levels and dynamics of the main chemical pollutants in marine mammals. However, the complexity of the processes involved in the storage, detoxification and physiological action of the wide variety of chemicals currently present in the environment has impeded the establishment of a clear link between observed tissue concentrations and their actual effects on cetacean individuals and hence populations.

The Workshop on Chemical Pollutants and Cetaceans was held in Bergen in March 1995 with over 40 participants from 10 countries. The primary aim of the Workshop was to carry out a multidisciplinary assessment of the significance of chemical pollutants for cetaceans. The specific objectives of the Workshop were therefore to: (i) critically review and synthesise current knowledge on pollutants in marine mammals; (ii) to identify tools to investigate cause-effect relationships; and (iii) to develop initiatives aimed at determining the actual impact of pollutants on cetacean populations and facilitate the design of a monitoring scheme.

Given the different disciplines represented at the Workshop, it was agreed to concentrate on those areas where the necessary expertise was available. The Workshop did not address the following subjects, which were referred to a later workshop or workshops: (1) oil pollution; (2) marine debris; (3) sewage related pathogens; (4) nutrient related environmental alterations; and (5) radionuclides.

The Workshop was structured around three major items: key-note presentations to provide an overview of the disciplines represented; a review of direct and indirect effects of chemical pollutants on cetaceans including research implications of the review; and implications of the findings of the Workshop - including recommendations - for the future work of the Scientific Committee and the Commission.

The key-note speakers addressed factors affecting variability of persistent pollutant levels; metabolisation of organochlorines in mammals; incidence of cancer in cetaceans; epidemiology/epizootics and contaminants; and the significance and potential of biomarkers in marine mammal toxicology.

Two categories of effects of chemical pollution on cetaceans were considered: direct effects, either lethal or sublethal; and indirect effects. There is no indication of acute poisoning of cetaceans. Sublethal effects considered included: (a) increased susceptibility to disease; (b) impairment of reproduction and early development; (c) immune suppression; (d) cancer induction and mutagenic effects; (e) changes in behaviour; and (f) occurrence and extent of epizootics.

In its review on indirect effects, the Workshop primarily discussed effects of pollutants on cetacean prey species and the role of prey as a source of pollutant exposure to cetaceans. In the absence of significant data the workshop's review on effects culminated in a series of research recommendations. In addition, the Workshop devoted considerable effort to considering future research on a number of topics including: synergistic/cumulative effects; exotic compounds; adequacy of present monitoring; further evaluation of the relationship between toxic burdens and impacts; risk and hazard assessment techniques; and trends in global pollution.

The Workshop developed a comprehensive list of recommendations, which addressed further research as well as implications for the work and involvement of the Scientific Committee and the Commission. Given the relative inertia after its initial examination of this question in the mid-1980s, the Workshop stressed the need to ensure that the impetus generated by its report and recommendations should not be lost.

The main conclusions from the Workshop were that: (1) there are sufficient data on adverse effects of pollutants on other marine mammals and terrestrial species to warrant concern for cetaceans; (2) a considerable amount of fundamental research is needed to adequately address the question of the effects of pollutants on all cetaceans; and (3) if any progress is to be made within a reasonable time frame, a multidisciplinary, multinational, focussed programme of research is required that concentrates on those species where there is most chance of success.

In order to forward this work, the editors of this volume and the co-convenor of the Workshop developed a proposal for future work and submitted this to the Scientific Committee and the Commission (Aguilar *et al.*, 1998). That proposal was reviewed and accepted by the IWC and is reprinted in this volume. However, we would like to stress that by concentrating on the Workshop's primary recommendation and focal species, it was not implied that work on other pollution related matters and other species should be discontinued. For example, the work in progress on North Pacific minke whales appears promising.

It was clear from the Workshop (and indeed from the discussion in the mid-1980s) that establishing a relationship between the tissue pollutant levels observed and their potential harmful effects at the individual and population level is extremely difficult for cetaceans. In other taxa, such studies have commonly required experimentation with live animals. For both ethical considerations and the practical impossibility of keeping in captivity the number of individuals necessary to produce the desired statistical certitude, this has not been possible for cetaceans. The research proposal of Aguilar *et al.* (1998) tries to overcome this difficulty. The rationale behind the proposal was to study a number of variables indicative of chemical impact in selected cetacean populations of the same species subject to a gradient of pollutant exposure. The proposal focused on pollutants for which there is already extensive information both on levels and potential effects. The species selected were the three considered by the Workshop: bottlenose dolphins, harbour porpoises and white whales, plus an additional species, the Amazon river dolphin, chosen to specifically address the possible impact of heavy metals. These species all include populations subject to pollutant gradients and from which adequate sampling is feasible. The ultimate aim is to try to produce a predictive model that, with the necessary caution, may be applied to other cetacean species.

Given the acceptance of the outline proposal, the Committee agreed to hold a Workshop to develop a more detailed and costed proposal for the Commission. That Workshop was held in Barcelona in March 1999 and its report is the final report included in this volume.

At the Barcelona Workshop, the following short-term objectives were identified for POLLUTION 2000+:

(a) to select and examine a number of biomarkers of exposure to and/or effect of PCBs and try to determine whether a predictive and quantitative relationship with PCB levels in certain tissues exists;

(b) to validate/calibrate sampling and analytical techniques to address such questions for cetaceans, specifically

  (i) determination of changes in concentrations of variables with post-mortem times;
  (ii) examination of relationships between concentrations of variables obtained from biopsy sampling with those of concentrations in other tissues that can only be obtained from fresh carcasses.

Given these objectives and the levels of resources and effort necessary to examine them, it was agreed that the work should be divided into two phases - information from Phase 1 is important in providing the calibration/validation tools necessary to better focus and design Phase 2. Data from Phase 1 will provide information not only essential for completing Phase 2 of POLLUTION 2000+ but also of fundamental importance to many research programmes examining issues of chemical pollutants and cetaceans. Phase 1 concentrates largely on Objective (b) above and comprises two sub-projects: (1) effect of post-mortem time; and (2) relationship between information obtained from biopsy samples with that obtained from live-captured animals or carcasses (either from bycaught or freshly stranded animals).

Highest priority will be accorded to sub-project 1. Changes in levels of contaminants and indicators of exposure are known to occur after death due to the inevitable physiological changes and breakdown of tissue. It is essential that these changes are quantified to determine the effect of post-mortem time on levels in the various tissues if the implications of measured levels of these in animals whose time to death is uncertain are to be correctly interpreted with respect to concentrations in the living animal.

The initial focus of POLLUTION 2000+ will now be on the harbour porpoise and the bottlenose dolphin. Sample size considerations precluded the inclusion of the white whale and the Amazon river dolphin as had been planned earlier, but studies on these species (and indeed others) are important and may be included in future phases of this iterative project. Interested groups are encouraged to undertake such studies.

Production of this volume has occurred over a number of years but we believe that its value is considerably enhanced now that it includes the full development of a focused research programme arising out of the valuable review developed at Bergen. It is vital that such fundamental research is carried out if we are to expand pollutant studies of cetaceans from merely documenting levels of pollutants in various tissues. We hope that the relevant governments and institutions co-operate financially and scientifically to ensure that this work is completed as soon as possible.

Thanks are due to a number of people involved in the production of this volume. In particular, thanks are due to the many scientists from many nations who have participated in the development of the POLLUTION 2000+ programme over the last five years. A number of these (including A. Aguilar, A. Bergman, A. Borrell, A. Brouwer, G.P. Donovan, C. Kemper, D. Martineau, D. Muir, D.W. Kuehl, J. Boon, J. Everaarts, J. St David, L.R. Shugart, M. Moore, M. Theo, N. Kannan, P. Best, P.J.H. Reijnders, R. Tatsukawa, R.J. Norstrom, S. Kennedy, S. Tanabe and T. O'Shea) have also acted as referees to the papers

included here. The editors would particularly like to thank Mark Simmonds for his enthusiasm in ensuring that this issue has become an important topic in the context of the IWC.

Several individuals have played an important part in the organising of the Workshops and they are included in the Acknowledgements at the end of the relevant reports. Finally thanks are due to the administrative staff of the IWC (especially Martin Harvey) who have dealt with the mundane but vital financial and logistical aspects of the development of the POLLUTION 2000+ programme.

P.J.H. Reijnders
A. Aguilar
G.P. Donovan

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Photograph courtesy of Jaume Forcada

J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 1–42, 1999

Report of the workshop on chemical pollution and cetaceans

EDITED BY P.J.H. REIJNDERS, G.P. DONOVAN, A. AGUILAR AND A. BJØRGE

1. INTRODUCTION (including arrangements for Workshop)

The Workshop was held from 27-29 March 1995 in Bergen, Norway, under the Chairmanship of Peter Reijnders.

Bjørge welcomed the participants to Bergen and summarised the practical arrangements for the Workshop. The list of participants is given in Annex A. Participants acting as rapporteurs and assisting with the Report included Aguilar, Bjørge, Boon, Donovan, Reijnders, Simmonds and Stein. Final editing was carried out by Reijnders, Donovan, Aguilar and Bjørge, including updating before inclusion in this volume.

Donovan thanked the Norwegian Government and the Environmental Investigation Agency Charitable Trust for their financial support, welcomed the participants on behalf of the IWC and described the background to the Workshop.

In 1993 the Commission had stated that the Scientific Committee should

> 'give priority to research on the effects of environmental changes on cetaceans in order to provide the best scientific advice for the Commission to determine appropriate response strategies to these new challenges' (IWC, 1994a).

At the 1994 Scientific Committee meeting (IWC, 1995a), the Committee had examined this question further and had identified a number of areas that would need to be addressed including: (1) global warming; (2) ozone depletion; (3) pollution; (4) direct (intentional and incidental mortality) effects of fisheries and indirect (ecological ramifications) effects of fisheries; (5) noise; and (6) other human activities (e.g. tourism, coastal developments). Implicit in this is the question of the synergistic and cumulative effects of all of these factors.

The Committee had noted that it was not feasible to address all these topics simultaneously; this would be a longer-term iterative project. Given this, it agreed that initially two specialised Workshops should be held, one relating to chemical pollutants and cetaceans, and the other on the potential ecological effects on cetaceans of climate change and ozone depletion. The latter Workshop was held in 1996 (IWC, 1997) after the publication of the Report of the Intergovernmental Panel on Climate Change (IPCC, 1996). A draft agenda for the present Workshop was drawn up and a Steering Group comprising Reijnders, Bjørge, Aguilar and Stein worked throughout the intervening period to organise the Workshop and ensure that a broad range of expertise was available.

The Scientific Committee (IWC, 1994b) had drawn the Commission's attention to the fact that environmental threats affect all species of cetaceans, not only those subject to direct capture. In fact, the most vulnerable species to such threats might well be those species that are already reduced in numbers, i.e. those for which the Revised Management Procedure would set zero catch limits even if it were applied (Donovan, 1995). It stressed that the IWC may have to contemplate response strategies outside the direct management of whaling

operations if it wishes any identified threats to be alleviated. This may include local measures in terms of habitat protection or much wider considerations concerning pollutants and greenhouse gas emissions.

Reijnders thanked the participants for their acceptance of the invitation to attend the Workshop and emphasised that the objective of the Workshop was to carry out a full multi-disciplinary assessment of the significance of chemical contaminants for cetaceans.

Relevant documents submitted to the 1994 Scientific Committee meeting were distributed to participants along with those submitted to the Workshop. The list of documents is given as Annex C.

2. ADOPTION OF AGENDA

The Agenda adopted is given as Annex B. It was noted that it was important to recognise those biological differences between odontocetes and mysticetes that might affect their susceptibility to certain chemical pollutants. It was also agreed that the Workshop did not have the expertise necessary to discuss the effects of oil spills and oil pollution on cetaceans. However, it draws this subject to the attention of the Scientific Committee as one that warrants consideration in the future (e.g. see review in SC/46/O 8, SC/46/O 14 and Item 5.7). A glossary of terms used and abbreviations included in this Report is given in Annex D.

3. KEYNOTE PRESENTATIONS

This section contains summaries by the keynote speakers of their presentations and a summary of the comments made by the 'opponent'. Important points arising from general discussions of these presentations are dealt with under the relevant Agenda Items. Readers are referred to the literature cited in the keynote papers for details of references in this section.

3.1 Individual variation in contaminant levels

Aguilar presented a review of factors affecting variability of persistent pollutant levels in cetaceans (SC/M95/P6[1]).

(1) *Diet* is significant because many pollutants are concentrated through food webs. This explains most interspecific differences in pollutant levels and it may also contribute to variation among populations of the same species or even among different components of the same population when diet is subject to age-related or sex-related variations.

(2) The effect of *body size* is complex. Excretion rate and activity of detoxifying enzymes decrease as body weight increases, processes which tend towards higher pollutant concentrations in larger animals. By contrast, a high metabolic rate - which is inversely correlated to body size - is associated with high pollutant concentrations. These opposite effects usually result in higher residue levels in smaller individuals.

(3) *Body composition* affects the contribution of each body compartment to the overall pollutant load. Therefore, the body load of lipophilic pollutants will strongly depend on the relative mass of blubber, a variable that can show a threefold variation among cetacean species or, in seasonal feeders, among individuals.

(4) *Nutritive condition* also affects the dynamics of lipophilic pollutants. Lipid mobilisation results in an increase in residue levels, in lipid as well as other tissues and blood. However, this variation is not as large as a purely concentrative model would suggest

[1] A revised version of this paper is published in this volume.

because of the enhancement of detoxification processes following a rise in tissue pollutant concentrations.

(5) *Disease* affects pollutant levels in different ways: impoverishing nutritive condition; altering normal physiological functions; and depressing reproduction and therefore reducing reproductive transfer in females. The combined result of these processes is usually an increase in pollutant levels and a redistribution in tissues of diseased individuals.

(6) The concentrations of most lipophilic pollutants and several trace elements increase with age in males because input exceeds the ability of the organism to excrete these compounds. Variable proportions of the pollutant load are transferred to offspring during gestation and lactation, for which reason tissue concentrations in adult females increase more slowly, stabilise or decrease, thus producing lower residue levels in adult females than in adult males. However, because not all compounds are transferred at the same rate, their relative abundance varies with *age* and *sex*. Intensity of reproductive transfer is also associated with the reproductive traits of the species, particularly the length of lactation. For most trace elements these differences between males and females may not be apparent or may even be reversed, with concentrations in females being higher than those in males.

The significance of these factors of variation should be taken into account when designing sampling programmes, comparing sample groups or evaluating toxicological impact.

Skåre (opponent) re-emphasised the need to take into account biological factors when interpreting pollutant levels. She presented data on ΣDDT, ΣPCB and ΣChlordane for different mammal species from the northern North Atlantic and Arctic regions. These showed a heterogeneous distribution of residue levels among species, with higher values in polar bears and harbour porpoises. This variation was attributed to differences in diet and other biological traits. Patterns of variation of the relative abundance of the various compounds among species and age-related trends in harbour porpoises were discussed. She concluded that the effort devoted in recent years to improving analytical ability should now focus on improving sampling techniques and identifying sources of variability (e.g. nutritional status, tissue location) - aspects that have been frequently neglected in the past.

3.2 Xenobiotics and metabolism

Brouwer presented a review of metabolisation of organochlorines in different mammal species with particular reference to the MFO (Mixed Function Oxidase) system (SC/M95/P8[1]).

He noted that persistent induction of biotransformation enzymes may have the following consequences:

(a) increased oxidative stress to the organs/cells experiencing it;
(b) enhanced formation of bioactive metabolites;
(c) increased elimination of endogenous substrates, such as vitamins and hormones;
(d) increased formation of mutagenic, relative intermediates from other xenobiotics, such as PAHs present in the same exposure matrix.

With regard to the formation of bioactive metabolites, he described the metabolism of PCBs and reached a number of conclusions.

(1) Several PCB congeners are readily metabolised to hydroxylated-PCB derivatives. Seals and cetaceans are able to metabolise PCB congeners with adjacent H atoms at the *ortho*, *meta* position, in combination with at most one *ortho* chlorine atom.

[1] A revised version of this paper is published in this volume.

(2) There is a clear correlation between EROD (ethoxyresorufin-O-deethylase) induction and metabolism of PCBs (e.g. 3,3',4,4'-TeCB [OH-PCB-77] as model congener) indicating an important role for cytochrome P450-1A1/2 as a catalyst.
(3) Although in general there is a good correlation between EROD induction and OH-PCB-77 formation, there are some exceptions: (i) fish and fish-eating birds show a low OH-PCB-77 metabolite formation capacity even though EROD is considerably induced, in contrast to liver microsomes from harbour porpoises and harbour seals which do show a considerable potency of OH-PCB formation; (ii) the pattern of OH-metabolites formed from PCB-77 may differ with different species.
(4) High levels of OH-PCB metabolites have been shown to accumulate in the late foetal and early neonatal rats born to mothers perinatally exposed to Arochlor 1254, or PCB-77 (Morse *et al.*, 1995).

OH-PCBs show a metabolic-specific range of bioactivities, including: T_4-binding competition on TTR; inhibition of type 1-deiodinase; uncoupling of mitochondrial respiration; oestrogen receptor binding; Ah-receptor binding (rat liver cytosol); IC inhibition (Hepa1c1c7); embryo mortality (chick, experimental); EROD induction (Hepa1c1c7).

The high levels of foetal accumulation of OH-PCBs may, therefore, be particularly relevant for the observed developmental neurotoxic and reproductive effects in laboratory animals and may thus also pose a risk for adverse developmental effects in wildlife species.

There is a high risk of synergistic effects on the depletion of thyroid hormone and vitamin A by complex mixture exposures, since these mixtures contain parent compound inducing CYP-1A1/2, metabolisable PCB congeners and inducers of UDP-GT isozymes (both 3-MC and Pb-types) that enhance hepatic elimination of thyroxine and vitamin A derivatives. Studies with several fish-eating birds (cormorants, eider ducks, common terns) and harbour seals have shown reductions in plasma retinol and thyroid hormones associated with EROD inductions and PCB dietary intake levels. The observed PCB-dependent changes in biochemical parameters suggest that they can be useful as biomarkers in wildlife species.

In addition to the possible adverse effects associated with persistent CYP-1A1/2 induction, this parameter itself is nowadays frequently used as a biomarker for exposure, along with assays for CYP-1A activity, in particular the de-ethylation of ethoxyresorufin (EROD). A close correlation has been shown between CYP-1A content and EROD activity in the liver, and non-*ortho*/mono-*ortho* PCB content in the blubber of white whales[1] *Delphinapterus leucas* (White *et al.*, 1994). This is a clear correlation between toxic chemical exposure and a biological effect known to be central to the mechanisms involved in diverse forms of chemical toxicity, as summarised above.

Moore (opponent) reported that recent techniques are available to determine CYP-1A1/2 protein *in situ* by immunohistochemical studies (SC/M95/P2). In these studies it was observed that there is also a considerable expression of CYP-1A1/2 protein in dermal endothelial cells.

Correlations between this protein content and hepatic and blubber contaminant load suggest that flux of inducing compounds is complex, greater in females than males and probably affected by other compounds in addition to those measured. Determining the significance of these observations against standards is critical for interpretation of CYP-1A/EROD data in dermal biopsy samples.

[1] The accepted IWC common name for this species is the white whale. It is also often referred to as the beluga, belukha or beluga whale.

The latter observations pave the way for the development of immunohistochemical biomarkers of CYP-1A1/2, using relatively small pieces of dermal tissue, which can be collected in a non-invasive way.

3.3 Cancer in cetaceans, a potential biomarker of environmental contamination

Martineau presented an overview (SC/M95/P10[1]) of cancer cases that have been observed in the small (*ca* 500 animals) population of white whales found inhabiting the St Lawrence Estuary (SLB) in Quebec, Canada, over a 12 year period, 1983-1994. A total of 175 white whales was reported stranded on the shoreline, of which 73 carcasses were examined. Of these, 14 were affected by a malignant tumour (cancer). This represents 40% of the 35 cancer cases reported worldwide in cetaceans (although this figure is difficult to interpret directly as it is only in this population that rigorous post-mortems are routinely carried out). The annual incidence rate (AIR) of cancer in that population (a minimum of 233/100,000 and a more realistic figure closer to 750/100,000) is much higher than that reported for any other population of cetaceans and is similar to that of man and hospitalised domestic animals. More specifically, the AIR of small intestinal cancers (minimum 67/100,000) is much higher than that observed in all animals and man, except sheep in certain parts of the world where an environmental carcinogen is believed to be aetiologically related to this condition. Considering: (1) the demonstrated exposure of SLB to PAHs of industrial origin; (2) the contamination of sediments of the SLB habitat by PAH; and (3) that among odontocetes, this is the only species that feeds predominantly on bottom invertebrates, Martineau suggested that these compounds should be included in any list of possible causes for the high rate of cancers observed in that population.

Clausen (opponent) drew attention to the fact that one should be cautious in comparing AIR values between species because of likely differences in confounding factors.

3.4 Epidemiology/epizootics and contaminants

Kennedy presented an overview of the morbillivirus epizootics that had occurred in aquatic mammals in recent years (SC/M95/P5[1]). Morbillivirus infections were unknown in these species until thousands of Baikal seals (*Phoca sibirica*) died as a result of morbillivirus infection in Lake Baikal in 1987 (Grachev *et al.*, 1989). Approximately 18,000 harbour seals (*Phoca vitulina*) and a few hundred grey seals (*Halichoerus grypus*) died as a result of morbillivirus infection in northwestern Europe in 1988 (Kennedy *et al.*, 1988b; Osterhaus and Vedder, 1988; Dietz *et al.*, 1989) and a similar infection killed thousands of striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea in 1990-92 (Aguilar and Raga, 1993). Morbillivirus infection has been shown to be the cause of death of bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico in 1993-94 (Lipscomb *et al.*, 1994a). Recent studies have established morbillivirus infection as the cause of a major epizootic among bottlenose dolphins along the eastern seaboard of the United States in 1987-88 (Lipscomb *et al.*, 1994b). A previous investigation had implicated brevetoxin as the cause of the die-off (Geraci, 1989).

Clinical signs and lesions in the affected cetacean populations were generally similar to those of morbilliviral diseases such as canine distemper and rinderpest in terrestrial mammals (Kennedy *et al.*, 1988b; 1989; Domingo *et al.*, 1990; 1992). Immunosuppression as a direct result of viral damage to lymphoid tissues was the likely cause of the many secondary bacterial, fungal and protozoal infections seen in morbillivirus-infected animals (Kennedy *et al.*, 1988a; b; Domingo *et al.*, 1992; Lipscomb *et al.*, 1994a; b). Large lysosomal inclusions, apparently not directly caused by morbillivirus infection, were seen in

[1] A revised version of this paper is published in this volume.

hepatocytes of a high proportion of striped dolphins during the Mediterranean epizootic (Kennedy *et al.*, 1993).

Comparative genomic analysis and reactivity patterns to monoclonal antibodies, indicate that a strain of canine distemper virus was responsible for the morbillivirus epizootic in Lake Baikal seals (Osterhaus *et al.*, 1989) whereas a newly recognised virus (phocine distemper virus; PDV) caused the European pinniped epizootic (Cosby *et al.*, 1988; Blixenkrone-Moller *et al.*, 1992; Curran *et al.*, 1992). Similar studies indicate that another newly recognised virus, distinct from PDV, was the cause of the epizootic among striped dolphins in the Mediterranean Sea (Visser *et al.*, 1993; Blixenkrone-Moller *et al.*, 1994). This virus is similar but not identical to that isolated from morbillivirus-infected harbour porpoises in northwestern Europe (Kennedy *et al.*, 1988b; Visser *et al.*, 1993). The relationship of the virus or viruses that infected bottlenose dolphins along the eastern coast of the United States in 1987-88 and in the Gulf of Mexico in 1993-94 to the other known morbilliviruses has not been established.

There is evidence that organochlorine contaminants have biological effects, including alterations of *in vitro* and *in vivo* indices of immune function in pinnipeds (De Swart *et al.*, 1994; Ross *et al.*, 1995), and it has been suggested that these substances contributed to the mortality of morbillivirus-infected marine mammals. However, such an effect on mortality could not be demonstrated in a study by Harder *et al.* (1992). Furthermore, morbilliviruses are highly virulent pathogens frequently producing high mortality in susceptible populations and epizootics in terrestrial animals pre-date anthropogenic contaminants. At this stage it is unclear whether contaminants had a role in morbillivirus epizootics in marine mammals. On the other hand, no explanation has been found for the unusually high concentrations of organochlorines found in tissues of striped dolphins during the 1990-92 epizootic in the Mediterranean Sea (Kannan *et al.*, 1993; Aguilar and Borrell, 1994). Likewise, there is no explanation for the occurrence of the hepatocytic lysosomal inclusions in a high proportion of these animals (Kennedy *et al.*, 1993). The possibility that contaminants may have increased the susceptibility of cetaceans to morbillivirus infection, or had an aetiological role in the hepatocytic damage cannot therefore be excluded.

Simmonds (opponent) made reference to the fact that although several large die-offs had been reported in pinnipeds before the recent morbillivirus epizootics (Dietz *et al.*, 1989), mass mortality was an apparently new phenomenon in cetacean populations. He suggested that although morbillivirus infection was the primary cause of these epizootics, they should be regarded as multifactorial events (Eis, 1989). With the possible exception of the Lake Baikal epizootic, all the recent morbillivirus epizootics had been adjacent to heavily industrialised and, therefore, polluted coasts. Some of these events had been associated with relatively high tissue contaminant concentrations (Dietz *et al.*, 1989; Kannan *et al.*, 1993; Aguilar and Borrell, 1994). There was some indication that seal mortality was apparently lower in some relatively unpolluted areas (Hall *et al.*, 1992). He noted that PCBs had been demonstrated to have immunosuppressive effects in laboratory animals and suggested that the hypothesis that morbillivirus epizootics have been exacerbated by contaminants will probably not be proven because of the difficulty in establishing simple cause and effect relationships for many environmental issues.

During the discussion it was agreed that morbilliviruses were capable of causing high mortality in susceptible (i.e. previously unexposed) populations and were the primary cause of the recent epizootics. However, the possibility of PCB and other contaminants playing a significant role in the mass mortalities of striped dolphins in the Mediterranean and other epizootics cannot be excluded because of the immunosuppressive capacity of such compounds, the uniquely high concentrations of organochlorines in the tissues of these animals and the occurrence of lysosomal inclusions in hepatocytes (see Item 4.1.2.6).

3.5 Significance and potential of biomarkers in marine mammal toxicology

Peakall (SC/M95/P9[1]) presented a review of biomarkers. These were defined (Peakall and Walker, 1994) as 'a biological response to a chemical or chemicals that give a measure of exposure and sometimes, also of toxic effect'. While the term 'biological response' is broad, it is usually taken to be any response up to the organisation level of the intact animal, although there has been an emphasis on biochemical and physiological effects.

There are two main difficulties with using biomarkers in marine mammals.

(1) The difficulty in collecting suitable samples. Non-destructive collection is desirable both on ethical and scientific grounds. Samples must be fresh and stranded animals, even if alive, may not be representative (see Item 5.3.1).

(2) The lack of experimental work on cetaceans that would allow the establishment of cause-and-effect relationships between biomarkers and chemicals. There is one study on bottlenose dolphins, which shows *in vitro* effects on immune function related to contaminants (Lahvis *et al.*, 1993; 1995). More extensive data are available on pinnipeds which show effects on vitamin A, thyroid and immune function in harbour seals fed fish from contaminated areas (Brouwer *et al.*, 1989; De Swart *et al.*, 1994; Ross *et al.*, 1995). However, it must be pointed out that pinnipeds are *not* closely related to cetaceans and therefore the observed cause-and-effect relationship in seals may be different in cetaceans.

He summarised the limited data available on biomarkers in cetaceans.

(1) *Formation of DNA adducts.* The presence of benzo(*a*)pyrene adducts has been demonstrated by Shugart *et al.* (1990) in the St Lawrence white whale population (a polluted area) but they are absent from Arctic specimens (low or less polluted areas). However, no difference in the total number of polyaromatic hydrocarbon (PAH) adducts with DNA was found between animals from these areas (Ray *et al.*, 1991). These studies were carried out on brain tissue, but it was pointed out that skin measurements are also possible.

(2) *Induction of Mixed Function Oxidase (MFOs).* MFO induction has been demonstrated in cetaceans and, in some cases, it has been correlated with PCB concentrations (Watanabe *et al.*, 1989; White *et al.*, 1994). While the liver is the main target organ, skin biopsies have also been successfully used (Fossi *et al.*, 1992).

(3) *Plasma hormone levels.* Some studies have been made on plasma hormone levels and a rather weak correlation found between testosterone and DDE (Subramanian *et al.*, 1987).

(4) *Immune responses.* Detailed studies are underway for the bottlenose dolphins (Lahvis *et al.*, 1993; 1995) and the white whale. With regard to the latter, the background studies in the Arctic have been completed but studies on animals from the St Lawrence population have not yet been carried out (Béland, pers. comm.).

Peakall and Stein (opponent) suggested that hazard assessment could be aided by biomarker data since this gives information on effects rather than merely documenting residue levels. As an ideal, they considered that cetaceans in the open ocean should be physiologically normal, i.e. that human activities should not result in their physiological functions being outside normal limits. The advantages of using physiological normality as a criterion are that it is:

(i) independent of the pollutants involved and thus avoids the problems of mixtures and unknown compounds;

[1] A revised version of this paper is published in this volume.

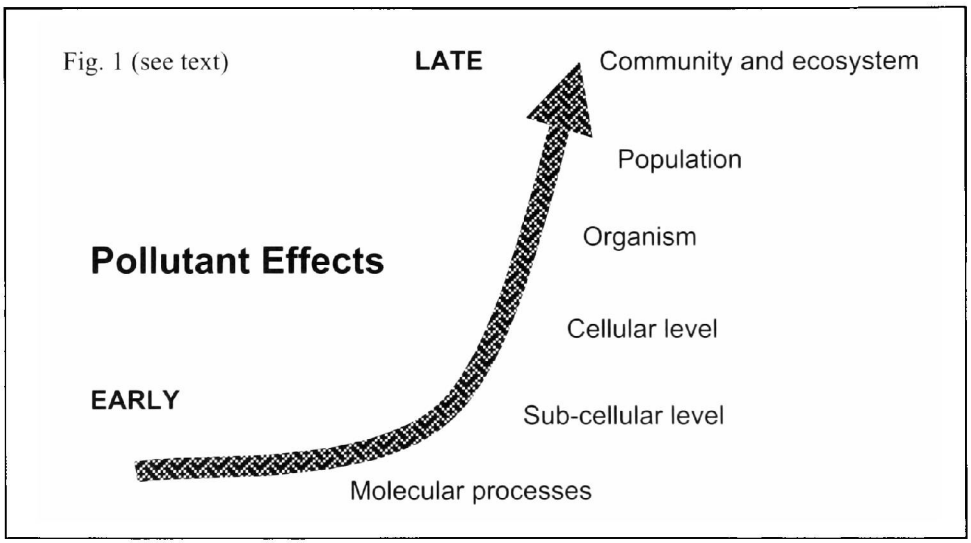
(ii) philosophically defensible - although it accepts the presence of some level of pollutants (analytical chemistry is too good for zero to be an objective) the levels are such that the physiological function of animals living in the area can be considered normal.

The disadvantages are that we need:

(i) to be able to *define* physiological normality and, because zero concentrations of pollutants do not exist, this means examining physiological parameters along gradients of pollution;
(ii) to be able to do a comprehensive battery of tests to be confident that physiology is normal and this battery of tests must cover all the major classes of pollutants.

4. EFFECTS OF CHEMICAL POLLUTION ON CETACEANS

This section of the Report considers the significance of pollutants to cetaceans with respect to both direct and indirect effects. Effects may be apparent at the molecular level, in an individual or up to the level of the ecosystem (Fig. 1).



4.1 Direct effects

4.1.1 Lethal effects

There is no indication in the literature of any acute chemical poisoning events (i.e. resulting in rapid death) affecting cetaceans. It was noted, however, that the detailed necropsies that could identify such events are rarely conducted on stranded animals; indeed in such an event, corpses might well not be recovered. Although, Koeman *et al.* (1971) reported that seals had been acutely poisoned by an accidental discharge of mercury-contaminated disinfectant, most cetaceans (apart from some river dolphins) live far from the point sources of pollution and thus a similar situation is unlikely.

4.1.2 Sub-lethal effects

The major chemical threat to cetaceans is through the continuous uptake of persistent lipophilic contaminants through the food chain; for more water soluble organic compounds and trace elements, uptake through water may also be significant. This, over time, may induce sub-lethal effects or, exceptionally, lead to the death of the animal.

4.1.2.1 DISEASES

Of course, animals suffered from diseases long before the advent of organochlorines and other pollutants. However, the ability of organochlorines (OCs) and other toxic chemicals to cause immunosuppression, secondary diseases and tumours, has also been demonstrated in many laboratory, domestic and free living animals that have been experimentally or accidentally intoxicated with them. They may also by themselves cause morphological changes that are relatively consistent among various animal species. For instance, squamous metaplasia of certain glands, mucous metaplasia of gastric glands, gastric erosions, hepatic lesions and lysis of the adrenal cortex have all been observed after accidental and experimental chronic intoxication.

Although high levels of tissular OCs have been found in a number of cetacean populations, only in a few instances have in-depth pathological and toxicological analyses been performed concurrently. As noted under Item 3.3, the small population of white whales, highly contaminated with OCs, that inhabits the St Lawrence Estuary has been studied over a 12 year period. Only by comparing this population with a 'clean' population has it been possible to demonstrate lesions and bacterial and viral infections consistent with OC chronic toxicity. In order to investigate whether similar patterns of pathological changes exist in contaminated populations of other cetacean species, the Workshop **recommends** that other cetacean species that are currently the object of toxicological analysis also be examined with regard to detailed pathology.

A range of pathological changes have been associated with contaminants in other species and these may provide some guidance in the examination of cetaceans. The Workshop noted that in order to investigate any potential relationship between contaminant levels and disease in cetaceans, a long term programme is required, with detailed pathology being combined with consideration of chemical burdens.

A priority list of pathological items to consider is given in Annex E. More detailed instructions for cetacean autopsies have recently been produced (e.g. Kuiken and García Hartmann, 1993; Law, 1994) but may not always be practical in the field. The establishment of a histological collection of normal tissue, might be helpful in the preparation of pathological diagnosis.

The presence of pox-like and other external skin lesions (e.g. Thompson and Hammond, 1992) and their potential relationship to pollutants has been noted in cetaceans and might be monitored as part of other studies.

4.1.2.2 REPRODUCTION AND EARLY DEVELOPMENT

A large number of the man-made chemicals that have been released into the environment, as well as a few natural ones, have the potential to disrupt the endocrine system of animals, including humans (SC/M95/P4). Among these are the persistent, bioaccumulative, organohalogen (OH) compounds that include some pesticides, fungicides, herbicides, industrial chemicals, other synthetic products and some metals.

Studies in terrestrial mammals, reptiles, birds and humans (reviewed in SC/M95/P4) have led to a hypothesis that persistent halogenated compounds can disrupt endocrine function. Evidence has come from studies of chemical residues, steroidal receptor number and function, enzyme induction, plasma steroid concentration, retinoid and thyroid hormones, reproductive micro- and macro-anatomy, reproductive physiology, behaviour and populations.

These effects were generally observed in offspring and were the result of hormonally-responsive tissues being affected during development. For many organochlorines there is no natural barrier to the brain or the womb. The determination of sexual development may be affected in the womb, during the period of time between

conception and the point when sex-related tissue differentiation normally begins. How far into gestation this normally occurs in cetacean species is unknown. The levels of hormone involved in this differentiation are extremely low - even 'minute' changes in concentrations may, therefore, affect sexual development.

A worldwide reduction in human sperm count has been reported (e.g. Auger *et al.*, 1995) and it has been hypothesised that this is linked to environmental factors, specifically exposure to oestrogen-like compounds *in utero* and in the early postnatal period. Similarly, sperm quality and mobility are declining for apparently similar reasons (Auger *et al.*, 1995). It was noted that cetaceans (like humans) would be exposed over relatively long periods of time to low doses of endocrine-disrupting chemicals; it is possible, therefore that their early development and reproductive capability may be affected.

There are few published data for cetaceans that allow testing of the hypothesis that there are comparable effects in odontocetes and/or mysticetes. True hermaphroditism in cetaceans (e.g. white whales - De Guise *et al.*, 1994a) is rare but pseudohermaphroditism has been reported in a number of species (e.g. bowhead whales - Koski *et al.*, 1993; fin whales - Bannister, 1962; sperm whales - Anon., 1960; striped dolphins - Nishiwaki, 1953). However, it should be noted that whenever endocrine disrupting chemicals have been sought in cetacean tissue they have been found. Effective doses in other mammals including humans are often extremely low; concentration levels in the blubber of some marine mammals exceed those. Odontocetes, especially males, have a higher blubber burden of suspect compounds than mysticetes from the same geographic region (O'Shea and Brownell, 1994). However, many large mysticetes undergo an extended annual non-feeding migration to their tropical or sub-tropical breeding areas. It has been suggested that their annual exposure to chemicals released during consumption of fat reserves during migration may therefore give an increased flux and exposure to persistent chemicals for adults, foetuses and sucklings (SC/M95/P4).

The Workshop considered whether priority species/areas could be identified to facilitate the examination of questions relating to pollutants and reproduction for cetaceans. Where possible, studies of bycaught, stranded and harvested animals should include interdisciplinary projects to analyse molecular, endocrine, enzymatic, histological and gross morphological indices designed to test the hypothesis that accumulation of complex mixtures of organohalogens disrupts normal reproductive form and function in odontocetes and mysticetes.

The Workshop stressed the need to learn more about the differentiation and development of embryos, foetuses and early postnatal to prepubertal whales and the dynamics of contaminant transfer to the offspring via gestation and milk. The Workshop **recommends** that such studies be developed. They should focus either on the analysis of data from bycaught animals in one area with a great range of exposure, or data from a single species in both relatively clean and contaminated areas, carefully measuring other possible confounding variables (e.g. food supply, density-dependent effects on reproduction).

The Workshop noted that measurement technology has already been established in the field of early development and endocrine disruption with laboratory animals. It **recommends** that samples be collected from neonatal and/or juvenile cetaceans (and other marine mammals) as part of a designed study to develop protocols in order to:

(i) assay for thyroid hormone, vitamin A and steroid hormone levels in blood plasma;
(ii) assay brain and liver samples for thyroid hormones and vitamin A;
(iii) assay oestrogen receptor levels in accompanying ovaries, brain and liver;
(iv) measure glial fibrillary acidic protein (marker of glial cells) and synaptophysins (marker of neuronal development) in various brain regions;

(v) analyse CYP-1A induction in appropriate organs.

In addition, the level of persistent organic contaminants in cetacean blubber and milk and hydrolysable metabolites in blood and brain should be analysed. The Workshop **recommends** that the accumulation of bioactive phenolic PCB metabolites in late gestational foetuses and neonates be further investigated in relation to potential developmental neurotoxic and reproductive effects. It also **recommends** that detailed pathological analysis of gonads, ano-genital axis and other determinants of sexual development be carried out.

The Scientific Committee's attention is drawn to a Workshop held in April 1995 by the US Environmental Protection Agency to develop research strategies for endocrine disrupters in the environment (Kavlock *et al.*, 1996).

It was also noted that functional changes are not always tied to histological changes and that, even within populations of the same species, individual variation in susceptibility is apparent (Langston, 1990). The uterine stenosis and occlusions and other major pathological changes reported from some seal populations (e.g. those in the Baltic and the Wadden Sea; Helle, 1980; Stede and Stede, 1990; Olsson *et al.*, 1994) have not been reported in any cetaceans, despite the fact that, for example, relatively large numbers of harbour porpoises in the North Sea region have been examined. The opportunities for investigations on bycaught cetaceans in other regions were also noted.

An analysis of a long time series of data on Barents Sea harp seal ovaries revealed changes in both the age of maturity and pregnancy rates (Kjellqvist *et al.*, 1995). The availability of suitable material to allow similar analyses for cetacean populations is rare and the difficulties in estimating such trends have been extensively discussed by the Scientific Committee (Cooke, 1985; Kato and Sakuramoto, 1991). However, it was noted that Norwegian scientists are presently analysing data from minke whale ovaries collected over a period of 40+ years. The results from such studies could be used in further studies to test for correlation with changes in demography and environmental factors, although the difficulty in determining any causal relationships is acknowledged.

4.1.2.3 IMMUNE SUPPRESSION

A relationship between certain chemicals and immune suppression has been well established in laboratory trials and was discussed under Item 3. Ross *et al.* (1995) and De Swart *et al.* (1994) have shown impairment of immunological functions in harbour seals fed on fish taken from polluted waters.

Immunosuppression is defined as a measurable alteration in any component(s) of the immune system that is likely to result in increased susceptibility to disease. Alterations in indices of immune function have the potential to act as important biomarkers of exposure to (alterations in *in vitro* indices but no clinical consequences) or effects of (increased susceptibility to disease) contaminants. A large number of tests are available to measure immune function in domestic and laboratory animals, and in humans. These tests include fluorescent antibody cell sorting (FACS) analysis of immune cells, lymphocyte stimulation tests, cytokine assays, responses to antigens and assessment of natural killer cell activity and phagocytosis.

There are no theoretical reasons why these tests could not be applied to cetaceans but, in practice, only some can be applied immediately; preparation of antibody reagents and workup of tests is required before many of these assays can be used. The Workshop identified the testing of existing antibody markers on cetacean immune cells and preparation of antibodies specific for cetaceans, where required, as priorities for research in this area.

As for other species, interpretation of the results obtained from the application of tests of immune function to cetaceans is likely to present major problems. Potential variables that

must be considered include sex, age, nutritive state, trace element and vitamin status, individual variation, daily variation, stage of lactation and gestation, and the stress associated with blood sampling. Qualitative and quantitative differences in contaminant burdens may affect the results of assays of immune function and would make it more difficult to establish a dose-response relationship unless there are relatively large changes in immune parameters. Assessment of contaminant burden (tissue concentrations or biomarkers, e.g. CYP-1A) in animals to which tests of immune function were applied, is essential in order to determine cause-effect relationships.

Because of the many confounding factors requiring consideration, establishment of 'normal' values for most assays of immune function would probably be difficult and require a large sample size. A realistic chance of success would only be obtained from the examination of a small number of carefully defined cetacean populations from which this type of sampling is feasible. Examples of these include Arctic and St Lawrence white whales; isolated populations of harbour porpoises along the US coast; and bottlenose dolphin populations along the central west coast of Florida (e.g. Scott *et al.*, 1990; Wells and Scott, 1990). Highly contaminated and relatively uncontaminated individuals are required to enable a comparison of immune function. Although controlled experimental studies would facilitate interpretation of results, such studies on cetaceans are probably impracticable.

In summary, the Workshop agreed that because of the potential importance of tests of immune function in cetaceans, possible difficulties should not delay the development of assays. The adverse effects of organochlorines on immune function and reduced resistance to infectious disease have already been demonstrated in laboratory species. The Workshop **recommends** that studies to determine reliable markers of immune function in cetaceans are given high priority. The need for increased numbers of detailed necropsies on cetaceans was also emphasised in order to detect infectious or neoplastic diseases that might result from immunosuppression.

The Workshop **recommends** that priority be given to the development of the following immunological markers:

(1) markers for CD 4 /CD 8 T-cell surface antigens;
(2) monocyte markers;
(3) B-cell markers;
(4) an *in vivo* model of grafting lymphocytes from e.g. cetaceans and mice for study of their functioning towards immunological challenges;
(5) further development of *in vitro* immuno-functional tests using white blood cells from cetaceans;
(6) more general markers that indicate structural components of immune system, such as blood cell counts, histopathology on thymus and lymph nodes, and immune globulins;
(7) markers that indicate susceptibility to infections that are not part of the structural integrity of immune system;
(8) vitamin A levels; and
(9) steroid/thyroid hormone levels.

Work is in progress on immune functions and *in vitro* exposure assays of immune cells of Arctic white whales that are only lightly contaminated with OCs (De Guise *et al.*, 1996a; b). Results from these studies may provide a unique opportunity to determine a dose-response relationship between alteration of immune functions and OCs in the St Lawrence population, since these whales show a wide range of tissular OC concentrations. Similar work has been initiated on bottlenose dolphins (Lahvis *et al.*, 1993; 1995) and collaboration between the two research groups is planned.

The Workshop noted that if blood samples are to be used in immunological studies, they must be obtained from living animals (or within a few minutes of death) that have not been subjected to excessive stress.

4.1.2.4 CANCER INDUCTION AND MUTAGENIC EFFECTS

Cancer generally results from a succession of separate injuries to the genome over a long period of time. Therefore, it is a rare event in wildlife and its detection requires the thorough examination of large numbers of individuals over an extended period. As in humans, there may be situations where exposure to specific and potent carcinogenic substances may lead to an unusually high prevalence of tumours. This has been observed in benthic fish feeding in areas contaminated with PAH/HAH. The high prevalence of cancer in St Lawrence white whales (De Guise *et al.*, 1994b and see Item 3.3) suggests a possible causal relationship through exposure to a carcinogen. The question of genetic susceptibility to cancer was raised; preliminary studies of white whale populations suggest that animals in the St Lawrence Estuary are more closely related to each other than animals within the other populations (Béland, pers. comm.).

However, the very low number of tumours reported from other cetaceans examined over decades, therefore remaining undetected (SC/M95/P10), indicates that cancer may not be a main cause of death for members of this taxa. However, there are numerous histological and molecular markers that precede the development of clear neoplasia. These include induction of cytochrome P4501A, the presence of mutant oncogenes and quantification of DNA adducts. These biomarkers have been used extensively in hazard assessment for chemical exposure in marine organisms and fish in particular (Hugget *et al.*, 1989; McCarthy and Shugart, 1990).

4.1.2.5 BEHAVIOUR

Behavioural toxicology is an area of considerable research involving laboratory animals and a number of studies have shown impacts of contaminants on various aspects of laboratory animal behaviour (Daly *et al.*, 1989; Daly, 1990; 1993). However, it is difficult to assess the relevance of these studies to animals in the wild. The difficulty in measuring behavioural changes in free-ranging cetaceans and attempting to relate this to contaminant levels is apparent. Although such changes cannot be precluded, the Workshop believes that logistical problems mean that this is not a promising area for future work compared with the others it had identified.

4.1.2.6 EPIZOOTICS

During the 1987-1988 North Sea harbour seal morbillivirus epizootic, high mortality rates were observed in the colonies in which pollutant levels were higher (Simmonds *et al.*, 1993). In addition, in the 1990-1992 Mediterranean striped dolphin epizootic, PCB levels were found to be significantly higher in individuals that died than in those sampled before the event or in those that survived (Aguilar and Borrell, 1994). These apparent associations between organochlorine levels and intensity of mortalities have only been observed in these two cases, although no proper analyses to determine the existence of this association were carried out in other epizootics. While a cause-effect relationship cannot be demonstrated (see Item 3.4), the Workshop **recommends** that further investigations are carried out to elucidate the involvement of pollutants in epizootics, and in particular, in cetacean immune competence (see Item 4.1.2.3).

4.2 Indirect effects

Attention was focused on the main effects of pollutants on fish and the role of fish in pollutant transmission to cetaceans. Fish are excellent vehicles of lipophilic pollutants such as organochlorine compounds. In addition, they are unable to metabolise many of these and thus are carriers of persistent chemicals. Recognised effects of pollutants in fish and other prey consumed by cetaceans include: (i) effects at the molecular level such as genotoxicity, activation of oncogenes, disturbance of molecular regulators, induction of biotransformation enzymes and increased activation of mutagens; (ii) effects at the subcellular and cellular level, such as proliferation of organelles, reduced stability of lysosomes and increased cellular transformations and necrosis; (iii) effects at the tissue (e.g. necroses) or systemic level, such as development of immunosuppression and tumours and impairment of reproduction; (iv) effects at the individual level, such as alterations of metabolism, homeostasis and behaviour, lowered fitness, increased susceptibility to pathogens and overall decrease in lifespan; (v) effects at the population level, such as lowered quality of genetic pool, population susceptibility to disease, depression of recovery rates and increases in mortality rates, all factors leading to a decrease in prey population size. Moreover, they may ultimately also induce changes in the structure of the community or even the ecosystem.

The discussion centred on the different mechanisms by which fish and invertebrate abundance and quality may be reduced by chemical agents. It was agreed that, in the long term, the anomalies observed have the potential for reducing prey population numbers and therefore cetacean food availability. ICES (the International Council for the Exploration of the Sea) has considerable experience and expertise in this field but was unable to send a representative to the Workshop. The Workshop **recommends** that ICES be approached for an evaluation of the effects of pollutants on the abundance and quality of cetacean prey (mainly crustaceans, cephalopods and fish). It also **recommends** that the IWC Workshop on climate change should give particular consideration to changes in overall productivity and prey species, and how this might be evaluated through predictive modelling.

5. RESEARCH IMPLICATIONS

5.1 Consideration of synergistic/cumulative effects

No information on synergistic effects of contaminants in cetaceans was presented at the Workshop and no examples exist in the published literature. However, the Workshop recognised that, although such effects are difficult to identify, they have been documented for some other species (e.g. Friend and Trainer, 1970). The Workshop considered that cumulative effects are more likely to occur in cetaceans.

5.2 Exotic compounds

Laboratories tend to routinely analyse concentrations of well-known compounds such as PCBs, DDTs and trace elements. However, information is available about the presence of many other contaminants in the marine environment. Because of the sheer numbers of these contaminants, a set of criteria needs to be developed to select priority compounds with regard to identifying effects on cetaceans. These criteria should include the levels of production, bioaccumulation potency and toxicity of these compounds.

One example considered was the insecticide toxaphene. Although not routinely analysed, it has a similar global production to PCBs and the bioaccumulation from mackerel to cetaceans in the North Sea is at least one order of magnitude (Andersson and Wartanian, 1992; De Boer and Wester, 1993). It is also a class of mutagenic and carcinogenic

compounds. The transport mechanism is evaporation from the tropics (where it is still produced and used) followed by atmospheric transport and subsequent condensation in polar regions.

Additional examples are tris(4-chlorophenyl)methane and -methanol, compounds of uncertain origin which occur in marine mammals; one possible source is from dye production.

Table 1 presents an overview of three categories of compounds: one that is already being monitored on a routine basis; one that at present can only be analysed by specialised laboratories; and one for which only limited information is available but for which it is suggested that bioassays should be carried out to determine if further action on them should be taken. Laboratories are therefore encouraged to measure these compounds to obtain a more complete picture of contaminants in cetaceans.

One approach to direct the selection of compounds for closer study is to screen on effects first e.g. by toxicological assays. Carcinogenicity, cytochrome P450 induction and sex hormone interference may be suitable criteria for this purpose. The calculation of QSARs (an index of similarity of structure and function) may also work well for chemicals with the same mode of action (e.g. Ah-responsive compounds).

The continuous input of non-bioaccumulative compounds into the marine environment may also present a hazard to marine mammals. An estimate of the exposure to these compounds could be obtained from the analyses of stomach contents (e.g. for PAHs) when bile (which is traditionally analysed) is not available.

In summary, a choice must be made whether to start from a consideration of the concentrations of compounds or from the consideration of effects. In principle, it is preferable to start with screening tests on effects, to be followed by a search for the responsible compounds when an effect is seen. However, immunoassay kits for total PCBs and some pesticides are currently being developed, and they may provide cost-effective analytical screening tools.

Table 1

Priority list of compounds to be tested for in cetaceans.

Routine	Non-routine[1]	Unknown[1]
PCBs[2]	Toxaphene[3]	TCP/TCPMe
DDT/DDD/DDE	Chlordane metabolites	Tetrabrombisphenol
HCB	PBDEs	CPs
HCHs	PCNs	Bromocyclene
Dieldrin	PBBs	Nitromusk compounds
PCDDs/PCDFs	PCTs	
Hg, Pb, Zn, Cd	PCDEs	
Cu, Se	PCBs metabolites (MSF and OH, blood only)	
Chlordanes	PAH-metabolites[4]	
	TBT/TPT	
	Chlorostyrenes	
	Radionuclides (Po-210, Cs-137)	

[1]Further information from bioassays is requested. [2]Including planar CBs. [3]Based on toxicity data and concentrations found, laboratories are encouraged to analyse toxaphene on a routine basis. [4]An alternative may be determining PAHs in the stomach contents of cetaceans not exclusively feeding on fish.

5.3 Adequacy of present monitoring

The main objective for monitoring pollutants in cetaceans is to determine whether levels at which adverse effects occur can be demonstrated, and to use these to infer the health of individuals or populations. This must include an understanding of temporal and geographical

trends in pollutant levels. Monitoring programmes should be carefully designed to achieve this objective.

Most past monitoring programmes for cetaceans merely measured concentrations of chemicals in tissues. This information alone is insufficient to determine the effects (if any) of pollutants on populations. The Workshop **recommends** that monitoring of tissue concentrations is accompanied by monitoring of appropriate biomarkers, pathological examinations and evaluation of incidence of alterations in reproductive biology and early development, as well as collection of appropriate biological data.

Given the present lack of knowledge on cause-effect relationships between cetacean health and chemical pollutants, the Workshop **recommends** that priority is given to carefully defined and designed studies on species and in areas where success is more likely, i.e. populations which are in highly polluted areas that are likely to exhibit adverse affects and those from the same species which are in pristine or relatively unpolluted areas that may be used to infer baseline biological parameters or physiological 'normality'. Potential species/areas include: bottlenose dolphins in areas where longitudinal studies have been established, such as central west Florida, which can be compared to captive animals and to those inhabiting less polluted areas; white whales from the well-studied population in the polluted St Lawrence River and those from other less/low polluted areas in the high Arctic; harbour porpoises from differently contaminated areas on both sides of the North Atlantic.

5.3.1 Sources of samples

Tissue samples for pollution studies in cetaceans may originate from three basic sources: (i) strandings; (ii) direct or incidental catches; and (iii) biopsies from free-ranging individuals. Each sample source has advantages and limitations.

Strandings may be divided into single and mass events. In the first case, the analytical results obtained are particularly prone to bias and should be carefully interpreted (see e.g. IWC, 1986). Single stranded cetaceans are likely to have suffered from a disease and, if this has been severe and long term, they are also likely to be in poor nutritional status. The sex- and age-composition of stranded individuals will probably not correspond with that of the 'healthy' population. In addition, stranded corpses are usually collected at unknown post-mortem times and some loss of pollutants is likely to have occurred. As discussed under Item 3.1, these variables strongly influence tissue pollutant levels, for which reason stranded individuals are likely to carry abnormal pollutant levels in their tissues. SC/M95/P6 compared pollutant concentrations found in stranded striped dolphins in the Mediterranean with those found in free-ranging, 'healthy' individuals sampled with a biopsy dart and found very different distributions of tissue concentration frequencies between the two sample sets, with more extreme values, both low and high, in the stranded dolphins.

Mass strandings, although devoid of many of these drawbacks, are an occasional and highly unpredictable source of samples.

Direct or incidental catches are good sample sources with all the necessary associated biological data being relatively easy to obtain, but may be limited to a small number of species and geographical regions. In addition, the sex and age composition of the sample may also be biased when compared to the actual population (e.g. see discussions in IWC, 1989).

Biopsy samples collected 'at distance' from free-ranging, apparently healthy cetaceans, are devoid of most of the above shortcomings but have limitations in the associated biological information that can be obtained. Sex, nutritional status and reproductive condition can be assessed from biopsy samples using various molecular and genetic techniques. However, as yet no techniques for determining age, a significant variable in

pollution studies are available (Aguilar and Borrell, 1995). The Workshop **recommends** that research be conducted to determine if it is possible to age individuals from skin or blubber tissue samples. The Workshop noted that IWC/IDCR cruises already undertake biopsy sampling of blue, humpback and right whales on an opportunistic basis. It **recommends** that, where possible, this be extended to biopsy sampling of small cetaceans. This should be considered for all research carried out under IWC auspices.

SC/M95/P14 presented information on a Japanese Government programme 'Project of Global Environment Monitoring with Fishing Vessel Network', scheduled to cover 1992-96. The authors noted that as part of this, Japanese vessels involved in the JARPA programme in the Antarctic (IWC, 1995a, p.28), as well as those contributed for use as part of the IWC/IDCR Southern Hemisphere assessment cruises (IWC, 1995a, p.33), are collecting samples of air and water for pollutant studies. It was commented that this represented a useful addition to studies in a poorly covered area.

5.3.2 Biomarkers

Biomarkers are being developed and used to improve the characterisation of exposure to chemical contaminants in wild populations. This improved characterisation can provide information necessary to link increased exposure to a chemical contaminant to an adverse or deleterious biological effect. However, the use of biomarkers in cetaceans is in its infancy and considerable research is needed to determine dose-response relationships to validate biomarkers, as well as developing appropriate molecular probes (e.g. monoclonal antibodies).

In addition, biomarkers of exposure can be used as an alternative to measurements of concentrations of compounds with the same mechanism of action (e.g. CYP-1A induction for the presence of Ah-responsive compounds).

Initially, at least, priority should be given to biomarkers that have been validated in studies with appropriate surrogate species (e.g. DNA-adducts, MFO induction, measures of immune function). At the second stage the use of other biomarkers should be explored. Samples will need to be collected non-destructively from apparently healthy animals (e.g. biopsy tissue or fluid samples) along relatively well known pollution gradients, and from animals for which as much associated information as possible is available (e.g. sex, age, reproductive status).

The measurement of these biomarkers must be related to the status of the target organ (e.g. MFO induction in skin and the response in the liver). This 'calibration' can be carried out on cetaceans legally taken, incidentally caught or mass stranded. Individuals suspected to be diseased should be avoided to exclude interference of the disease with the biomarker.

As noted above, data for both cetaceans and 'exposure', are needed from non or low-polluted populations to try to establish physiological normality. It is also important that appropriate standardisation and quality assurance controls are established to allow comparison among studies.

5.3.3 Biological variables

The Workshop **recommends** that all available data on each specimen from which material has been taken for pollutant analyses should be properly documented; at least sex, age and nutritional status should be collected whenever possible (SC/M95/P6). Measurement of nutritional status is difficult because reliable condition indices have not been developed for most cetacean species. Blubber thickness is often unreliable and the use of blubber weight and lipid content of the blubber in standardised body locations is recommended. Lipid content is important because, particularly in emaciated individuals, lipid mobilisation may be

coupled with increased blubber water content. Collection of these biological data is important for the interpretation of the toxicological results. Law (1994) provides an example protocol.

5.3.4 Pathology examinations

Necropsies should be carried out by a pathologist preferably with cetacean experience. One suggested protocol for pathological procedures is given in Annex E.

A list of factors to be examined in the context of alterations to reproduction and early development is given under Item 4.1.2.2.

5.3.5 Specimen banking

The Workshop **recommends** that the IWC should encourage and facilitate the standardisation of sampling procedures, storage and collection of associated information among already existing specimen banks and the exchange of relevant information about samples in those banks among institutes carrying out cetacean research.

It also **recommends** that cell types from various organs should be cultured from cetaceans and made available through agencies such as the American Type Culture Collection. This would facilitate studies of biochemical mechanisms and enable dose-response relationships for various endpoints to be established, for comparison with biopsy material.

Tissue samples obtained from stranded animals, biopsies, legal or incidental catches should be archived, frozen or fixed, as possible. The Workshop **recommends** that priority be given to establishing genomic DNA libraries and complementary DNA (cDNA) libraries from different organs and information on archived samples be made available to investigators.

Hindrance to the exchange and transfer of tissue or other samples from marine mammals continues to hamper the progress of investigations that otherwise would be possible. Efforts should be made to facilitate the exchange of materials between investigators around the world.

5.4 Further evaluation of the relationship between toxic burden and impacts

There are about 100,000 synthetic organic compounds currently in use and more are being produced. If each compound is metabolised to an average of three metabolites, then at least 300,000 compounds could be present in the environment. Compounds for which concern has been expressed include: organohalogens, PAHs, metals, endocrine disrupters and organo-tin and organo-mercury. Measurements of levels of these in the environment and in animals are needed for both parent compounds and metabolites of these chemicals (see Item 5.2).

Differences in residue profiles between prey and predators can be used to assess metabolism of compounds. The ICES Marine Chemistry Working Group is developing an approach for analysing PCB patterns in prey and predators and, in this way, identify compounds that are being metabolised by the predator. The general principle of that approach is given by Reijnders and de Ruiter-Dijkman (1995) and Boon *et al.* (1992). In the ICES Working Group approach, log-transformed concentrations of a given congener are plotted against congener 153, a persistent PCB congener that is not readily metabolised. If a congener is metabolised, the slope of the relationship will be < 1. By comparing relative ratios of various individual congeners in marine mammals to the relative ratios in a reference prey organism, one can assess metabolism of congeners without specifically analysing for metabolites.

The hypothesis that cytochrome P450 was responsible for the metabolism of PCBs was confirmed with *in vitro* studies of harbour seal liver (Murk *et al.*, 1994).

Analyses for metabolites should include target organs, storage sites (e.g. blubber and bile) and blood. The relationships among these compartments need to be assessed. There are only a few stable PCB metabolites (Bergman *et al.*, 1994) that are of toxicological concern.

The Workshop noted that in recent years, less research interest appears to have been devoted to metal pollutants in comparison to organochlorines. From the perspective of cetaceans this balance requires redressing somewhat. Some of the relatively small amount of direct evidence with respect to the effect of pollutants is the association of lesions with mercury burden in bottlenose dolphins (Rawson *et al.*, 1993).

The detoxification mechanisms of heavy metals (protein binding and interactions with other elements, such as mercury and selenium) were discussed. The significance of interactions among essential metals and toxic trace elements and, particularly, speciation was noted. The importance of measuring not only total metal levels but also the contribution of the different organic forms as well as concentrations of essential metals was emphasised. It is necessary to determine the physico-chemical form (i.e. speciation) of toxic trace elements in tissues as well as mechanisms of detoxification, with the aim of relating the possible biological effects to the accumulated toxicological active dose. Attention to elements other than cadmium and mercury (those traditionally considered) is warranted. Similarly the potential for differences among subgroups in a population in their ability to detoxify trace elements (e.g. different pilot whale schools at the Faroe Islands - Caurant *et al.*, 1993) requires greater attention. Detoxification mechanisms, including metal binding proteins whose primary function may not be detoxification, should be further elucidated, based on studies of terrestrial mammals and marine invertebrates (e.g. Kagi and Nordberg, 1979; Furness and Rainbow, 1990). Tissues other than the 'traditional' liver and kidney should be examined, because metals may alter the effects of OCs on endocrine functions.

Little information is available on radionuclides and thus the Workshop was unable to evaluate the potential threat posed by this group of pollutants (SC/46/O 8). However, it was agreed that they warranted concern (see Item 5.7) and required further investigation.

In terms of adverse health effects related to pollutants, the most sensitive time period is the embryonic and neonatal development stage (see Item 4.1.2.2). Later stages of life may be useful for monitoring the expression of pathological symptoms and signs, such as cancer in adults. In view of the high sensitivity of the early life-stage and the potential long-term consequences of any effects that are induced, the Workshop **recommends**:

(1) that biological samples from foetal and neonatal cetaceans are collected to obtain measurements of exposure (parent compounds and metabolites) and early biological effects; and
(2) when collecting data through destructive sampling techniques, information from tissues of developing foetuses as well as tissues that are routinely collected through non-destructive techniques should be collected in order to allow the development of models on the potential for foetal exposure when only non-destructive sampling is performed.

5.5 Risk and hazard assessment techniques

Risk assessment generally implies some degree of quantification for an adverse biological effect and in cetaceans only the incidence of cancers in white whales (see Item 4.1.2.4) seems able to meet this requirement. For this reason it was agreed that it was only possible to consider hazard assessment with respect to chemical contaminants in cetaceans and that this might be achieved from the perspective of biomarker studies (see Item 5.3.2).

Biomarkers should be measured, in conjunction with analytical chemical data, along likely gradients of pollution, to try to determine the level of exposure at which an adverse effect

occurs. To achieve this in a reasonable time frame, a small number of appropriate species/populations need to be identified and targeted. It must be recognised that in cetaceans it is difficult to obtain these data and, even when obtained, they must be assessed against a range of non-chemical factors. A suite of biomarkers that have been well validated in other species are needed. Studies focussed on non-destructive sampling should attempt to relate response in biopsy samples to suspected target tissues and determine dose-response relationships. Quality Assurance/Quality Control (QA/QC) procedures should be employed in biomarker studies as well as in analytical determinations.

A major issue that must be addressed is relating biomarker response to detrimental biological effects. It was agreed that studies should focus on species and areas that might be expected to be more likely to enable a linkage to be established between biomarker response and a serious physiological effect as well as the threshold at which this effect occurs. An important caveat is that there is a potential for highly species-specific effects to occur and thus it is not possible to extrapolate among species with a high degree of confidence. It is extremely important that any such studies attempt to elucidate the role of other biological factors (e.g. age, reproductive status) and their influence on biomarker response.

5.6 Trends in global contamination

Information on geographical and temporal trends in contaminants contained in SC/M95/P7, SC/M95/P13, Tanabe *et al.* (1994) and Loganathan and Kannan (1994) were considered. Most information available concerns organochlorines and originates from the Northern Hemisphere. Only limited information is available on heavy metals or from the Southern Hemisphere. There has been a relative increase of PCBs+DDT levels in the tropical belt. Pollutant levels in the water of most rivers and coastal waters in the Northern Hemisphere showed an initial decrease in the late 1970s, but have generally remained constant since then. In open waters such levels are stable or have increased in recent years. Overall, levels in the Southern Hemisphere have increased in recent years, although they are still lower than in the Northern Hemisphere. Information on cetaceans is limited to short periods of time and certain regions, and in most cases follow these general patterns. However, systematic data on temporal trends in contaminants in cetaceans from the Southern Hemisphere are limited to studies on bottlenose and common dolphins in South Africa (De Kock *et al.*, 1994) and minke whales in the Antarctic (SC/M95/P13).

A confounding factor in assessing temporal trends in heavy metals is their natural occurrence, sometimes at high level sources (e.g. Greenland, the Mediterranean) in marine systems.

As noted under Item 5.2, for many 'exotic' compounds, no information on past or current levels is available.

The Workshop recognised that evaluation of temporal and geographical trends requires careful consideration of recent improvements in analytical techniques and capability. Early samples from some regions may have contained significant amounts of unidentified contaminants that were wrongly assigned, such as the insecticide toxaphene eluting with PCB congeners.

The Workshop **recommends** that temporal and spatial trends in the concentrations of pollutants should continue to be examined from different species and geographical regions.

5.7 Identification of additional areas of concern

The Workshop identified a number of subjects of new or future concern: (1) oil pollution; (2) the possible link between chemical pollution and epizootics (see Item 4.1.2.6); (3) marine debris; (4) sewage-related pathogens (Anon., 1993); (5) nutrient related environmental

alterations (e.g. Geraci *et al.*, 1989); and (6) radionuclides. Although outside the scope of this meeting, these issues are matters of concern (see SC/46/O 8 and SC/46/O 14) and should be addressed by the IWC Scientific Committee and in the deliberations of the forthcoming Workshop on the effects of climate change on cetaceans.

The Workshop draws the attention of the Scientific Committee to the report 'Contaminants and Marine Mammals' produced by the Scientific Advisory Committee of UNEP's Marine Mammal Action Plan (UNEP, 1991). The objectives of the recommendations in that report partly overlap with those in the present Workshop Report and include: (a) the evaluation of the health status of marine mammal populations in both polluted and less polluted environments; (b) the development of scientific teams to respond to large-scale mortalities of marine mammals and to evaluate the significant causes and contributing factors underlying such events; (c) the application of scientific knowledge to anticipate, mitigate and prevent further ecosystem deterioration.

The Workshop noted that, as part of the follow up to UNCED, a global intergovernmental meeting was held in November 1995 to discuss a programme of action to address worldwide concern over the impact of anthropogenic contaminants on marine wildlife populations and marine ecosystems; the absence of incontrovertible scientific evidence is not seen as an impediment to the development of an action programme. However, further examination of sources and impacts in marine wildlife populations will be imperative for the continued development of global action and to enable commitments (e.g. Agenda 21) to reduce chemical pollutant emissions 'to levels that are not harmful to man or nature' to be quantified.

6. IMPLICATIONS FOR THE FUTURE WORK OF THE IWC SCIENTIFIC COMMITTEE

This Workshop represents the initial stage of the IWC Scientific Committee's consideration of the broad issue of the effects of environmental change on cetaceans. The Committee has recognised that the normal expertise available to it was insufficient to address this issue; this is reflected in the large proportion of Invited Participants at the Workshop. The Workshop stressed that the impetus generated by its Report and recommendations should not be lost and the Scientific Committee should consider ways to ensure that this does not happen. One way this could be achieved would be for the Committee to invite scientists with the relevant expertise to its regular meetings and for member nations to include such experts on their national delegations. The holding of a future Workshop or Workshops to review progress should also be considered. A mechanism also needs to be developed to synthesise the results of the various topics included in the overall examination of the effects of environmental change on cetaceans. In the shorter term, for example, a number of matters that require further consideration but were outside the scope of this Workshop are noted under Item 5.7 and, in particular, issues that should be examined at the Workshop to be held on climate change are identified under Item 4.2. The importance of cooperating with other international and regional bodies working on related issues (e.g. ICES, IOC, UNEP and the Oslo and Paris Commissions) is stressed.

The Workshop noted that its work and recommendations are of relevance to a wider audience than the IWC's Scientific Committee. It hoped that the research guidelines and recommendations included will be of value to research groups around the world who are beginning to address questions of cetaceans and chemical pollutants, and to stranding networks who are determining the types of analysis that can be carried out for the animals that they encounter.

Since its inception, the primary responsibility of the IWC has been the management of whaling. In the light of the discussion of the value of obtaining samples from 'healthy' animals, the Workshop noted that should animals be taken under IWC regulations, relevant samples should be collected (see Item 5.3). It **recommends** that the Scientific Committee considers making more specific the reference to collection of samples for pollutant analysis in the 'Guidelines for Data Collection and Analysis under the RMP' (IWC, 1995b). Similar samples should be obtained wherever possible from aboriginal subsistence whaling. In addition, the Workshop reiterated the importance of using biopsy techniques to obtain samples from free ranging animals.

While the Workshop believed that all its recommendations are important, it felt that certain specific recommendations should be forwarded by the Scientific Committee to the Commission for more immediate action. In particular those recommendations that will provide results for specific populations and do not need to wait for the completion of the fundamental research for a full, comprehensive analysis of the effect of chemical pollutants on all cetacean species. These are identified under Item 7. The Workshop stresses the need for multidisciplinary focussed programmes of research to be developed and believes that the IWC Scientific Committee should act as a catalyst in this.

7. RECOMMENDATIONS

The Workshop recommendations are included in full in Table 2 but do not follow the order in which they appear in the report. They have been grouped from the general to the specific; Recommendation 1 can be said to summarise the findings of the Workshop.

The Workshop believes that there are sufficient data on the adverse effects of pollutants on the health of other marine mammal and terrestrial species to warrant concern for cetaceans. However, the report and its recommendations show that a considerable amount of fundamental research is needed before it will be possible to adequately address the question of the effects of chemical pollutants on all cetaceans.

Notwithstanding the cautionary note that it is often not appropriate to extrapolate from one species to another, it is clear that if any progress is to be made within a reasonable time frame, a multidisciplinary, multinational focussed programme of research is required that concentrates on those species/areas where there is most chance of success. The Scientific Committee (and the Commission) is strongly urged to consider ways to facilitate the development and execution of such research.

Three species are considered particularly suitable: the bottlenose dolphin; the harbour porpoise; and the white whale.

The bottlenose dolphin

The animals along the coast of central west Florida represent some of the best studied in the world (e.g. Scott *et al.*, 1990). Most of the animals in the Sarasota-Bradenton area have been identified and capture-mark-release techniques have been used since 1970. The animals are thus relatively used to being handled (including the taking of blood samples) and a number of physiological and toxicological studies are underway as noted elsewhere in this report. Associated biological data will therefore often be available for living animals. Local populations are being studied in many parts of the world (e.g. see Würsig and Jefferson, 1990) and thus a 'pollutant concentration gradient' exists; the potential for international cooperation is high. In addition, this species is the most commonly held in captivity (and successfully bred) and thus offers great potential for baseline studies and investigations.

(Text continued on page 26)

Table 2
Summary of recommendations (see text). **especially as part of focused programme on priority species/area.

No.	Recommendation (Agenda Item)	Subject	Methodology	Samples from	Mainly directed at
1	Given the present lack of knowledge on cause-effect relationships between cetacean health and chemical pollutants, the Workshop **recommends** that priority is given to carefully defined and designed studies on the species and in areas where success is more likely, i.e. populations which are in highly polluted areas that are likely to exhibit adverse affects and those which are in pristine or relatively unpolluted areas that may be used to infer baseline biological parameters or physiological 'normality'. [5.3]	General. Cause-effect assessment. Acquisition of baseline data	Multi-disciplinary. (see other Recs below)	One area, wide exposure or 'clean vs contaminated'. For examples see text **	Ecotoxicologists, reproductive physiologists, cetologists
2	The Workshop **recommends** that cetaceans that are currently the object of toxicological analysis be also examined with respect to detailed pathology. [4.1.2.1]	General. Cause-effect	Existing (see Annex E)	All**	Toxicologists, pathologists, cetologists
3	The Workshop **recommends** that monitoring of tissues concentrations is accompanied by monitoring of appropriate biomarkers, pathological examinations and evaluation of incidence of alterations in reproductive biology and early development, as well as collection of appropriate biological data. [5.3]	General. Monitoring	Existing (see other Recs)	All**	See Rec. 1
4	The Workshop **recommends** that temporal and spatial trends in the concentration of pollutants should continue to be sought, from different species and geographical regions. [5.6]	General. Monitoring	Existing	Cetaceans and the environment	IWC and other organisations
5	The Workshop **recommends** that all available data on each specimen from which material has been taken for pollutant analysis should be properly documented; at least sex, age and nutritional status should be collected wherever possible. [5.3.3]	General. Samples	Existing	All (inc. other marine mammals)	Cetologists
6	The Workshop **recommends** that the IWC should encourage and facilitate the standardisation of sampling procedures, storage and collection of associated information among already existing specimen banks and the exchange of relevant information about samples in those banks among institutes carrying out cetacean research. [5.3.5]	General. Samples (banking)	Action by IWC	All	IWC
7	The Workshop noted that IWC/IDCR cruises already undertake biopsy sampling of blue, humpback and right whales on an opportunistic basis. It **recommends** that, where possible, this be extended to biopsy sampling of small cetaceans. This should be considered for all research carried out under IWC auspices. [5.3.2]	General. Samples	Action by IWC	All, especially small cetaceans	IWC/IDCR planners
8	Tissue samples obtained from stranded animals, biopsies, legal or incidental catches should be archived, frozen or fixed, as possible. The Workshop **recommends** that priority be given to establishing genomic DNA libraries and complementary DNA (cDNA) libraries from different organs, and information on archived samples be made available to investigators. [5.3.5]	General. Samples	Existing	All	IWC

continued

No.	Recommendation (Agenda Item)	Subject	Methodology	Samples from	Mainly directed at
9	It also **recommends** that cell types from various organs should be cultured from cetaceans, and made available through agencies such as the American Type Culture Collection. This would facilitate studies of mechanisms, and enable dose-response relationships for various endpoints to be established, for comparison with biopsy material. [5.3.5]	Specific. Samples	Existing	All	Immunologists
10	The Workshop **recommends** that research be conducted to determine if it is possible to age individuals from skin or blubber tissue samples. [5.3.1]	Samples. Age determination	Unknown	Skin samples (biopsy) from animals that can be aged independently	Cetologists, population geneticists
11	The Workshop stressed the need to learn more about the differentiation and development of embryos, foetuses and early postnatal to prepubertal whales and the dynamics of contaminant transfer to the offspring via gestation and milk. The Workshop **recommends** that such studies be developed. They should focus either on the analysis of data from bycaught animals in one area with a great range of exposure, or data from a single species in both relatively clean and contaminated areas, carefully measuring other possible confounding variables (e.g. food supply, density-dependent effects on reproduction). [4.1.2.2]	Reproduction and early development	Largely existing. Multi-disciplinary	Mothers and offspring (incl. foetuses). Bycaught, legally caught or mass stranded. One area, wide exposure or 'clean vs contaminated' populations**	Ecotoxicologists, reproductive physiologists, cetologists, pathologists
12	The Workshop noted that measurement technology has already been established in the field of early development and endocrine disruption. It **recommends** that samples be collected from neonatal and/or juvenile cetaceans (and other marine mammals) as part of a designed study to develop protocols in order to: (i) assay for thyroid hormone, vitamin A and steroid hormone levels in blood plasma; (ii) assay brain and liver samples for thyroid hormones and vitamin A; (iii) assay oestrogen receptor levels in accompanying ovaries, brain and liver; (iv) measure glial fibrillary acidic protein (marker of glial cells) and synaptophysins (marker of neuronal development) in various brain regions; (v) analyse CYP-1A induction in appropriate organs. [4.1.2.2]	Reproduction and early development	Develop for cetaceans	Mothers and offspring (incl. foetuses)**	As Rec. 11
13	In view of the high sensitivity of the early life-stages and the potential long-term consequences of any effects that are induced, the Workshop **recommends**: (1) that biological samples from foetal and neonatal cetaceans are obtained to obtain measurements of exposure (parent compounds and metabolites) and early biological effects; (2) when collecting data through destructive sampling techniques, information from tissues of developing foetuses as well as tissues that are routinely collected through non-destructive techniques should be collected in order to allow the development of models on the potential for foetal exposure when only non-destructive sampling is performed. [5.4]	Reproduction and early development	Existing, see other Recs	Mothers and offspring (incl. foetuses). Bycaught, legally caught or mass stranded**	As Rec. 11

continued

No.	Recommendation (Agenda Item)	Subject	Methodology	Samples from	Mainly directed at
14	The Workshop **recommends** that the high level accumulation of bioactive phenolic PCB metabolites in late gestational foetuses and neonates be further investigated in relation to potential developmental neurotoxic and reproductive effect. [4.1.2.2]	Reproduction and early development	Existing	Foetuses and neonates	As Rec. 11
15	It also **recommends** that detailed pathological analysis of gonads, ano-genital axis and other determinants of sexual development is carried out.	Reproduction and early development	Existing	All, esp. foetuses and neonates	Pathologists, reproductive physiologists
16	The Workshop **recommends** that studies to determine reliable markers of immune function in cetaceans are given high priority. The need for increased numbers of detailed necropsies on cetaceans was also emphasised in order to detect infectious or neoplastic diseases that might result from immunosuppression.[4.1.2.3]	Immunology	General. Develop for cetaceans (and see 1)	All, one area, wide exposure or 'clean vs contaminated' populations**	Immunologists, pathologists
17	The Workshop **recommends** that priority be given to the deveolpment of the following immunological markers: (1) markers for CD 4/CD 8 T-cell surface antigens; (2) monocyte markers; (3) B-cell markers; (4) develop an *in vivo* model of grafting lymphocytes from e.g. cetaceans and mice for studying their functioning towards immunological challenges; (5) further development of *in vitro* immuno-functional tests using white blood cells from cetaceans; (6) more general markers that indicate structural components of immune system, such as blood cell counts; histopathology on thymus, lymph nodes; and immune globulins; (7) markers that indicate susceptibility to infections that are not part of the structural integrity of immune system; (8) vitamin A levels; (9) steroid/thyroid hormone levels. [4.1.2.3]	Immunology	Specific. Develop for cetaceans	All**	Immunologists
18	While a cause-effect relationship cannot be demonstrated (see Item 3.4), the Workshop **recommends** that further investigations are carried out to elucidate the involvement of pollutants in epizootics and, in particular, in cetacean immune competence (see Item 4.1.2.3). [4.1.2.6]	Immunology and epizootics	General. (and see other Recs)	Animals investigated during epizootics (controls needed)	Ecotoxicologists, immunologists, epidemiologists
19	The Workshop **recommend** s that ICES be approached for an evaluation of the effects of pollutants on the abundance and quality of cetacean prey (mainly crustaceans, cephalopods and fish). [4.2]	Effects on cetacean prey	Action by IWC	n/a	ICES
20	It also **recommends** that the IWC Workshop on climate change should give particular consideration to changes in overall productivity and prey species, and how this might be evaluated through predictive modelling. [4.2]	Effects of changes in prey on cetaceans	Action by IWC	n/a	IWC Workshop on climate change
21	The Workshop **recommends** that this proposal be accorded high priority for funding. It also recommends that within this project, tissues are collected for pathological analyses (see Annex E). [5.8]	Heavy metals. Impact assessment	Existing	Pilot whales	IWC for funding, proposers.

The harbour porpoise

The harbour porpoise is one of the most susceptible to incidental capture in fishing gear (e.g. Donovan, 1994). Potentially therefore, a large number of relatively unbiased samples can be obtained (although there appears to be some bias towards young animals) across a 'pollutant concentration gradient', for which all the requisite associated biological data can be obtained (e.g. on both sides of the North Atlantic). Aguilar and Borrell (1995) has summarised the work on pollutants carried out on this species to date. Some harbour porpoises are kept in captivity.

The white whale

As noted throughout the report, the small St Lawrence population is one of the best studied cetacean populations from the perspective of health and pollutants; comparative work with populations from less polluted areas is already underway and could be facilitated by international cooperation (e.g. with Greenland, the Russian Federation and the USA). There are also some animals kept successfully in captivity.

8. PUBLICATIONS

The Report of the Workshop and selected papers will be published in a volume in the IWC special issue series, to be edited by Reijnders, Aguilar and Donovan.

9. ADOPTION OF REPORT

The Report was adopted at 18:30 on 29 March 1995. It was not possible in the time available to complete the editorial work necessary to finalise Item 7. The Workshop delegated this responsibility to Reijnders and Donovan, who were also assigned the task of final editing of the Report for consistency, style and references, and production of the glossary. It was noted that the Report remained confidential until after it was posted to Commissioners. The Workshop especially thanked Helen Sharp who typed the Report throughout the long night of 28 March!

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Annex B

Agenda

1. Introduction (including arrangements for Workshop)
2. Adoption of Agenda
3. Keynote presentations
   - 3.1 Individual variation in contaminant levels
   - 3.2 Xenobiotics and metabolism
   - 3.3 Cancer in cetaceans, a potential biomarker of environmental contamination
   - 3.4 Epidemiology/epizootics and contaminants
   - 3.5 Significance and potential of biomarkers in marine mammal toxicology
4. Effects of chemical pollution on cetaceans
   - 4.1 Direct effects
     - 4.1.1 Lethal effects
     - 4.1.2 Sub-lethal effects
       - 4.1.2.1 Diseases
       - 4.1.2.2 Reproduction and early development
       - 4.1.2.3 Immune suppression
       - 4.1.2.4 Cancer induction and mutagenic effects
       - 4.1.2.5 Behaviour
       - 4.1.2.6 Epizootics
   - 4.2 Indirect effects
5. Research implications
   - 5.1 Consideration of synergistic/cumulative effects
   - 5.2 Exotic compounds
   - 5.3 Adequacy of present monitoring
     - 5.3.1 Sources of samples
     - 5.3.2 Biomarkers
     - 5.3.3 Biological variables
     - 5.3.4 Pathology examinations
     - 5.3.5 Specimen banking
   - 5.4 Further evaluation of the relationship between toxic burden and impacts
   - 5.5 Risk and hazard assessment techniques
   - 5.6 Trends in global contamination
   - 5.7 Identification of additional areas of concern
   - 5.8 Review of submitted proposals
6. Implications for the future work of the IWC Scientific Committee
7. Recommendations
8. Publications
9. Adoption of Report

Annex C

List of Documents

SC/M95

SC/M95/P1. BOON, J.P. Monitoring concentrations of organochlorines, and biochemical and immunological effects in samples of living marine mammals. An introductory note on the possibilities.

SC/M95/P2. MOORE, M.J., MILLER, C.A., WHITE, R.D., SHEA, D., WEISBROD, A.V. and STEGEMAN, J.J. Histological and cytochrome P4501A expression in tissues of pilot whales, *Globicephala melaena*, stranded on Cape Cod, MA USA.

SC/M95/P3. CAURANT, F. Cadmium and mercury in pilot whales: physico-chemical forms of storage and potential hazard to the species.

SC/M95/P4. COLBORN, T. and SMOLEN, M. An epidemiological analysis of persistent organochlorine contaminants in large cetaceans.

SC/M95/P5. CRAIG, A.M., ORPIN, C.G. and BLYTHE, L.L. Biotransformation of marine pollutants, particularly crude oil alkanes, by forestomach bacteria from the bowhead whale. [Research Proposal.]

SC/M95/P6. AGUILAR, A., BORRELL, A. and PASTOR, T. Factors affecting variability of persistent pollutant levels in cetaceans.

SC/M95/P7. BORRELL, A. Summary of temporal trends in pollutant levels observed in marine mammals.

SC/M95/P8. BROUWER, A. Metabolism of xenobiotics in laboratory animals and wildlife species: potential impact on physiology and health.

SC/M95/P9. PEAKALL, D.B. Biomarkers as pollution indicators with special reference to cetaceans.

SC/M95/P10. MARTINEAU, D., LAIR, S., DE GUISE, S. and BELAND, P. Cancer in cetaceans, a potential biomarker for environmental contamination.

SC/M95/P11. SIMMONDS, M.P. Marine mammal mass mortality events: Environmental influences and science.

SC/M95/P12. DONOVAN, G.P. Pollution references on the IWC database.

SC/M95/P13. TANABE, S., AONO, S., FUJISE, Y., KATO, H. and TATSUKAWA, R. Persistent organochlorine residues in the Antarctic minke whale, *Balaenoptera acutorostrata*.

SC/M95/P14. FISHERIES AGENCY GOVERNMENT OF JAPAN. Project of global environment monitoring with fishing vessel network.

SC/M95/P15. KENNEDY, S. Morbillivirus epizootics in aquatic mammals.

SC/46

SC/46/O 8. REIJNDERS, P.J.H. Contaminants and cetaceans: Reasons for concern?

SC/46/O 12. JONES, P.D., HANNAH, D.J., BUCKLAND, S.J., VAN MAANEN, T., LEATHEM, S.V., DAWSON, S., SLOOTEN, E., VAN HELDEN, A. and DONOGHUE, M. Planar chlorinated hydrocarbons in New Zealand marine mammals.

SC/46/O 14. MOSCROP, A. and SIMMONDS, M.P. The significance of pollution for marine cetaceans.

SC/46/O 16. BROWN, K. Requirements for a comprehensive assessment of pollution in cetaceans: quantification, evaluation and absolute threat of pollutants.

SC/46/O 20 (revised). BOWLES, D. An overview of the concentrations and effects of heavy metals in cetacean species.

SC/46/RP4. CAURANT, F., BLOCH, D., MOREAU, A., BALLAN-DUFRANCAIS, C. and ALGOET, M. Histo-pathology of kidney and liver tissues of the pilot whales off the Faroe Islands, related with high levels of cadmium and mercury. [Research Proposal.]

Annex D

Glossary

Aromatic hydrocarbon (Ah) receptor. A protein that binds dioxins, dibenzofurans, non-*ortho* and mono-*ortho* PCBs, and 4 and 5 ring hydrocarbons.

Assays. Procedure for measurement or identification.

Biomarker. A biological response to a chemical or chemicals that give a measure of exposure and sometimes, also of toxic effect.

PCB Congeners. One of the 201 chemical forms of the **P**oly**C**hlorinated **B**iphenyls. These are identified by numbers, e.g. OH-PCB-77, hydroxylated polychlorinated biphenyl, number 77.

Cytochrome P450 1A (CYP-1A). An enzyme found in epithelia and endothelia, that is induced by and metabolises dioxin, dibenzofurans, some PCBs, and 4 and 5 ring hydrocarbons. The protein concentration is commonly measured as a biomarker for the exposure and effect of those compounds.

DNA adducts. Covalent binding of pollutants, especially the polynuclear aromatic hydrocarbons (PAHs), to DNA. This clearly indicates exposure of the organism to the pollutant.

Endocrine disruptor. Any compound that interacts with reproductive physiology to alter normal expression of sexually dimorphic form and/or function.

Ethoxyresorufin-O-deethylase (EROD). A model substrate for metabolism by CYP-1A. The EROD activity is used in conjunction with CYP-1A as a biomarker of exposure to and effect of Ah receptor activating compounds.

Halogenated aromatic hydrocarbons (HAHs). Synthetic compounds containing chlorine, fluorine or bromine atoms, such as PCBs, pesticides and brominated fire retardants.

Hazard assessment. Estimation of potential risk.

Hepa1c1c7. Liver tumour cell line.

Immunosuppression. A measureable alteration in any component(s) of the immune system that is likely to result in increased susceptibility to disease.

Mixed function oxidases (MFOs). A group of enzymes that are capable of metabolising a wide range of natural and unnatural chemicals. The function of these enzymes is to increase excretion of potentially harmful chemicals, but in the case of man-made compounds the metabolites may be more toxic than the original compound.

OH-PCB-77. Hydroxilated polychlorinated biphenyl, number 77.

Persistent induction of biotransformation enzymes. Continous triggering of metabolisation enzymes.

Polychlorinated biphenyls (PCBs). Synthetic compounds used for electrical capacitors in the 1950s to 1970s.

Polycyclic aromatic hydrocarbons (PAHs). Large groups of naturally occurring aromatic compounds containing two or more benzene rings fused together. Also, oil compounds derived directly (petrogenic) or indirectly via combustion (pyrogenic). Some of these compounds, such as benzo(*a*)pyrene, are known carcinogens.

Risk assessment. Estimation of extent of risk.

Residue levels. Concentrations of contaminants in substrate (e.g. soil, fluid, tissue).

T_4-binding competition on TTR. Hepatic thyroxine binding competition on transthyretin.

Xenobiotics. Foreign substances to a living organism (e.g. contaminants).

ABBREVIATIONS OF CHEMICAL COMPOUNDS

3,3',4,4'-TeCB - tetrachlorobiphenyl.
CPs - chlorinated paraffins.
HCB - hexachlorobenzene.
HCHs - hexachlorocyclohexanes.
MSF - methylsulphate.
MSF-PCBs - methylsulphate-PCBs.
OC - organochlorines.
OH - organohalogens.
OH-PCBs - hydroxy-PCBs.
PAH - polycyclic aromatic hydrocarbons.
PBBs - polybrominated biphenyls.
PBDEs - polybrominated diphenylethers.
PCBs - polychlorinated biphenyls.
PCDDs - polychlorinated dibenzo-p-dioxins.
PCDEs - polychlorinated diphenylethers.
PCDFs - polychlorinated dibenzofurans.
PCNs - polychlorinated naphthalenes.
PCTs - polychlorinated terphenyls.
TBT - tributyl tin.
TCP - tris (G-chlorophenyl) methanol.
TCPMe - tris (G-chlorophenyl) methane.
TPT - triphenyl tin.

Annex E

Priorities in Pathology

D. Martineau

INTRODUCTION

The following protocol is intended to further research into the chronic toxicity of organochlorinated compounds (OC) in cetaceans. OC toxicity has been well documented in domestic and laboratory animals. These compounds are known to induce squamous metaplasia of various glands (including mammary glands), to be oestrogenic and to produce mucous metaplasia in glandular stomachs and gastric erosions. A general record of each animal should be kept, including age, sex, reproductive status and, when available, the cause of death. Relevant OC contamination levels in the examined animal should be obtained. The severity and nature of the lesions might be ultimately correlated with OC levels in order to obtain a 'dose-response' relation.

It must be stressed that this protocol is not designed to determine the cause of death. Whenever possible, one should seek ideal conditions for post-mortem examination. These should include a complete, careful examination performed by trained personnel in an appropriate facility. In many field situations, these requirements cannot be fulfilled. Further information is provided in Kuiken and García Hartmann (1993).

GENERAL PROCEDURE

Thin, flat sections (approximately 3mm thick, 1cm wide and 2cm long) should be cut and placed in neutral buffered 10% formalin.

Abnormalities: the size, shape, number and colour of any abnormalities should be described using simple terms, for example: 'a dozen randomly distributed, (1cm-diameter) round, dark red areas surrounded by a pale (2mm thick) halo are present on the lower aspect of the heart'.

SAMPLES REQUIRED FOR DETECTION OF CHRONIC OC TOXICITY

Thyroid glands: cut gland parallel to its long axis, before fixation.

Lungs: total of six sections:

Left lung:	cranial	Right lung:	cranial
	middle		middle
	caudal		caudal

Stomach: a section of the mucosa of each stomach. If ulcers are present, a section of the lesion and a section of normal bordering tissue.

Liver: three sections: middle and both sides.

Kidney: three sections of each: cranial, middle, caudal.

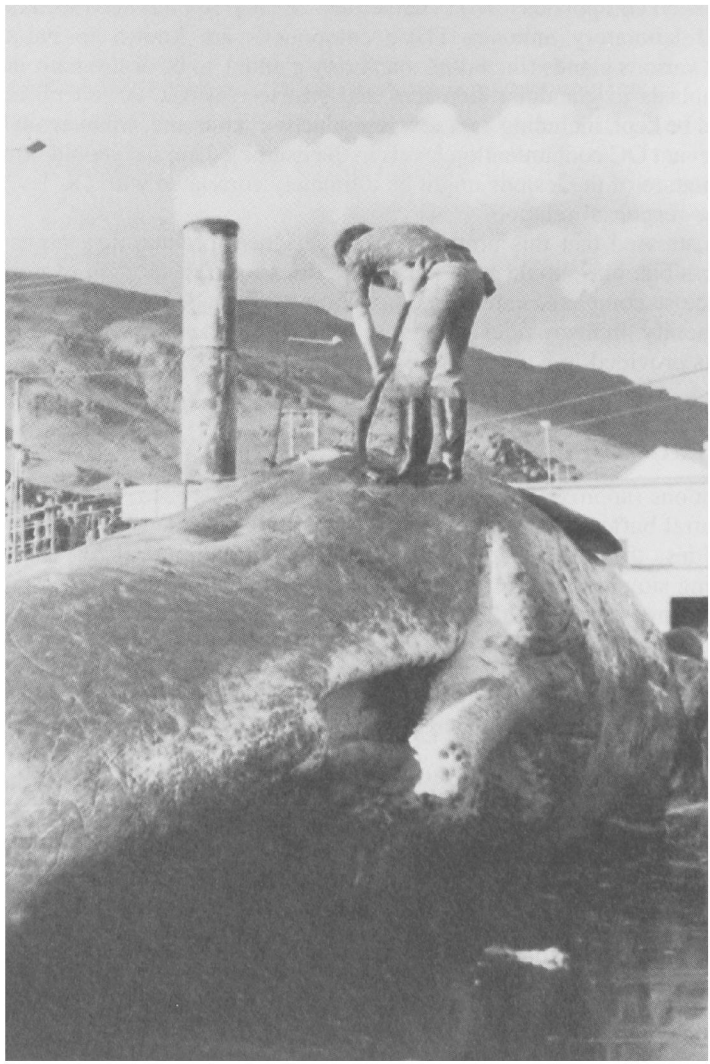
Adrenal glands: cut both glands parallel to their long axis, before fixation.

Mammary glands: three sections of each: cranial, middle, caudal.

Uterus: three transverse sections of each uterine horn at various levels (total of six sections).

REFERENCE

Kuiken, T. and García Hartmann, M. 1993. Proceedings of the First European Cetacean Society Workshop on Cetacean Pathology: dissection techniques and tissue sampling. *ECS Newsletter* 17:1-39.



J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 43–45, 1999

Practical guidelines for postmortem examination and tissue sampling of cetaceans for ecotoxicological purposes

B. CLAUSEN

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INTRODUCTION

Postmortem examinations of marine mammals are undertaken for a number of reasons, *inter alia*: to determine the possible cause of death; to obtain evidence of diseases or pathological changes; and to collect tissue and other samples (e.g. blood and organs) for further research. The latter can provide material for laboratory studies for a variety of subjects including toxicology, immunology, virology, parasitology and histopathology.

The collection of associated biological data such as sex, age, length and nutritional status from each specimen is an essential part of any postmortem examination. Without such data it is very difficult if not impossible to interpret the results of the aforementioned studies.

PATHOLOGICAL EXAMINATIONS

Full scale autopsy guidelines

Necropsies should preferably be carried out in an appropriate facility by a pathologist with experience of cetaceans. Several protocols for postmortem examinations have been developed. A good general field guide on dealing with stranded specimens is provided by Geraci and Lounsbury (1993). In addition, two more detailed sets of guidelines can be recommended. One is the extensive protocol for autopsies of marine mammals produced by Kuiken and Hartmann (1993). The other is a revised and updated version of this for cetaceans given in Law (1994). The latter is particularly relevant in the context of this volume, because as well as guidelines on postmortem examination, it also provides extensive information on the collection and storage of samples, analytical methods and quality control information for both age determination and the determination of trace metals and organochlorines.

Both protocols are comprehensive, detailed and require pathological expertise. However in many cases the conditions required to carry out such optimal, full scale necropsies (including sampling of material) cannot be met. It is for this reason that a more simple protocol is produced here.

Necropsy field guide for laymen

Common problems preventing comprehensive autopsies include the unavailability of a suitable pathologist; a lack of time; the lack of an adequate facility and equipment; the simultaneous stranding of several animals which must be examined in a short period; and the discovery of animals in remote areas from which transport is impossible. It is important to recognise that even in such cases, valuable information can be obtained by a more superficial examination and coarse sampling.

Few tools are essential. These include for example: a knife, a whetstone and rubber gloves; tape measure; camera; notebook/tape recorder; containers for samples.

Serious life threatening infections have occurred among people cutting up marine mammals. Therefore gloves should be worn both for the handling of the animals as well as the necropsy. All wounds or should be carefully washed. Any sign of local infection in the days afterwards must be examined by a medical doctor.

Stepwise postmortem examination and tissue sampling (Items marked with an asterisk are more important in the context of pollution studies)

(1) *Before any cuts are made, the animal should be described, length and sex noted and the state of decomposition indicated (fresh, medium, rotten or falling apart). Although little further information may be obtained from animals in the latter two conditions, it is important because it provides clues for e.g. toxicologists on the suitability of samples for further analyses. Photographs can be extremely useful.

(2) *For odontocetes, at least four teeth should be collected for age determination. (Collection of earplugs for age determination of baleen whales is difficult and probably can only be undertaken by experienced personnel.)

(3) *Examine the animal for wounds, sores and abscesses, and signs of bycatch such as rope scars and flippers or flukes cut off. Even if unfamiliar with pathology the cutter may record large abnormailities like gunwounds, bycatch signs and abscesses etc. Again, photographs can be extremely useful.

(4) *Subsequently, where practical, place the animal on its side, cut through the blubber just over the middle, from the neck to the anus. The skin and the blubber can then be cut and dragged downwards. [Be careful with large whales - the pressure in the body cavity may be so high that the gases and outcoming rotten intestine may overturn the cutter!]. The organs should be taken out one by one, most easily in the following order.

(5) *The genital tract is cut loose from the behind and first loosened from the body when in females, the ovaries can be identified and collected (ovaries are particularly important as they can provide information on the reproductive history of the female). Testes are internal and are attached to the dorsal walls of the visceral cavity.

(6) The digestive tract, including the intestine, stomach, spleen and liver can also be removed from the anus area.. Organs are separated.

(7) Then the kidney and the adrenals in front of the kidneys can be identified up under the back and extracted.

(8) The diaphragm is cut, some ribs are removed and the lungs and heart are taken out and separated.

(9) Afterwards the main organs are examined and, if possible, weighed one by one.

(10) *Record whether the animal is pregnant or lactating, the latter is indicated by yellow-white fluid in the mammary tissue under the blubber on the belly. Special attention should be payed to not overlook small embryos.

(11) The kidneys are examined to see whether they appear similar and for any worms or infections.

(12) The intestine may be opened and the number of parasites estimated.

(13) The stomach is similarly investigated.

(14) The liver is cut and examined for nodules, abscesses and parasites.

(15) The heart is opened and examined for worms.

(16) So are the lungs, via the bronchi.

(17) The parasites are described in terms of e.g. tapeworms, roundworms and the total number estimated. Parasites can be preserved in alcohol (even in gin or whiskey).

(18) *Tissue collected for toxicology should consist of at least a large (e.g. 10 × 10 cm) piece of blubber, muscle (if possible), liver and kidney.
(19) *A piece (*ca* 5 × 5 cm) of liver and of kidney should be separately put in a glass (e.g. a marmalade/jam jar rinsed beforehand with hot water).
(20) *A piece (*ca* 5 × 5 cm) of liver and kidney should be separately packed in plastic (bag or otherwise).
(21) *The labelled material should subsequently be preserved cold and as soon as suitable facilities are available, deep frozen.

The blubber (and sometimes muscle) is used to investigate the levels of the different types of lipophytic pollutants such as organochlorine compounds (PCBs, DDT and other pesticides). The liver and kidney are generally used to analyse levels of heavy metals such as mercury, cadmium and lead.

Without ignoring the toxic impact of heavy metals, it is generally agreed that of the known contaminants, organochlorines are the most likely compounds to affect cetaceans. They are globally widespread and may cause reproductive problems and lower the resistance against disease. Highest priority, therefore should be given to take at least blubber and muscle samples for organochlorine analysis.

Persons who have collected samples but are not in a position to have these analysed should approach the IWC or participants in the workshop, to obtain information on possible options for appropriate analyses to be carried out.

ACKNOWLEDGEMENTS

Thanks are due to the Workshop participants and in particular Alex Aguilar, Greg Donovan and Peter Reijnders.

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Law, R.J. 1994. Collaborative UK Marine Mammal Project: summary of data produced 1988-1992. Fisheries Research Technical Report 97, MAFF, Lowestoft, UK. 42pp.

Towards POLLUTION 2000+

J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 47–53, 1999

SC/49/Rep6

Proposal to the IWC on furthering the recommendations of the Pollution Workshop*

Members: Aguilar, Bjorge, Donovan, Reijnders.

1. INTRODUCTION

The Group met in Texel, the Netherlands, on 3 July 1997 to discuss ways to further the recommendations given in the Bergen Pollution Workshop report as agreed by the Scientific Committee at the 48th Annual Meeting in Aberdeen. Prime consideration was given to Recommendation 1 of that report which in effect summarised the whole meeting. The section in the report leading to that recommendation stated:

> The Workshop believes that there are sufficient data on the adverse effects of pollutants on the health of other marine mammal and terrestrial species to warrant concern for cetaceans. However, the report and its recommendations show that a considerable amount of fundamental research is needed before it will be possible to adequately address the question of the effects of chemical pollutants on all cetaceans.
>
> Notwithstanding the cautionary note that it is often not appropriate to extrapolate from one species to another, it is clear that if any progress is to be made within a reasonable timeframe, a multidisciplinary, multinational focused programme of research is required that concentrates on those species/areas where there is most chance of success. The Scientific Committee (and the Commission) is strongly urged to consider ways to facilitate the development and execution of such research.
>
> Three species are considered particularly suitable: the bottlenose dolphin; the harbour porpoise; and the beluga.

The rationale behind Recommendation 1 is detailed in the Workshop report. It should be noted that by concentrating on this recommendation and its focal species, the group is not implying that research on other species should not be carried out.

Indeed it is hoped that the approach outlined in this proposal may prove useful to researchers working on other species. In particular, with respect to baleen whales, it was noted (O'Shea and Brownell, 1994) that levels of pollutants are low in baleen whales and much below threshold levels presently considered to elicit adverse effects in mammals. Therefore, baleen whales are at this stage not considered suitable priority species to establish the sought cause-effect relationship between cetacean health and chemical pollution. However, the work in progress on North Pacific minke whales (Fujise, pers. comm.) appears promising and further work and presentation of results is encouraged.

2. OBJECTIVES

In an ideal world, the ultimate objective of pollution studies for cetacean management is to determine a predictive model linking tissue pollutant levels with effects at the population level. This is clearly not a realistic short-term goal but it might be achievable in the long-term. Given the wide variety of factors influencing the population dynamics of cetaceans (and indeed any organism), then at best one might eventually be in a position to assign some level of probability of certain effects occurring at the population level, given certain levels of specific pollutants in the body.

* Also printed in *Rep. int. Whal. Commn* 48:425-8 [1998].

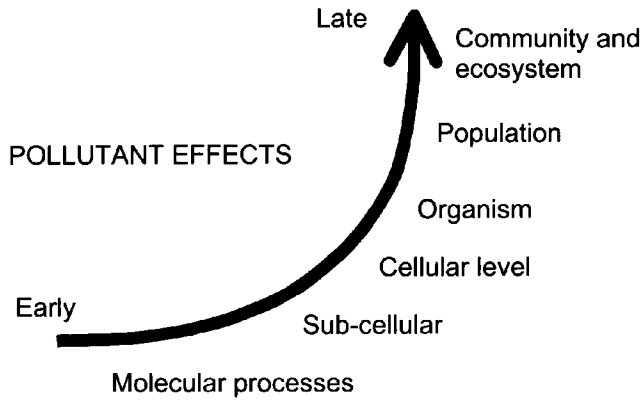

Fig. 1. General action of pollutants.

The general action of pollutants follows the pattern in Fig. 1.

The primary aim of the following proposal is to investigate the first links in the chain, i.e. attempting to determine the relationship between levels in certain tissues and indicators of certain effects. It seemed appropriate to focus on two sets of pollutants: (1) that might provide information of more general applicability, e.g. impact of organochlorines: and (2) that are subject to more local interpretation such as impact of heavy metals. For the latter there are additional complications to their natural high level occurrence in some regions as well as very local high concentrations. It was agreed to focus under (1) on the PCBs for a number of reasons but primarily because more is known about their uptake and metabolisation, as well as the existence of available techniques to indicate exposure and effect.

The process of decision making should follow the path as shown in Fig. 2.

3. SOURCES OF SAMPLES

Suitable samples can be obtained from several sources:

(1) biopsy from free-ranging animals;
(2) captive animals;
(3) temporarily live-captured animals;
(4) by-caught animals;
(5) legally harvested animals.

Not all of the above can provide suitable samples for all of the analyses. Table 1 summarises this applicability.

4. ASSOCIATED BIOLOGICAL INFORMATION

It is clear from the Workshop report that interpretation of the significance of pollutant levels requires knowledge of the sampled animal. Those variables that are particularly important for certain analyses are highlighted in Table 1. In addition, although we are concentrating on the first links of the chain of pollutant-induced effects, it is important to look for certain pathological conditions, especially those that may be associated with pollutants. In cases where sample source allows for detailed pathology, it is considered of relevance that

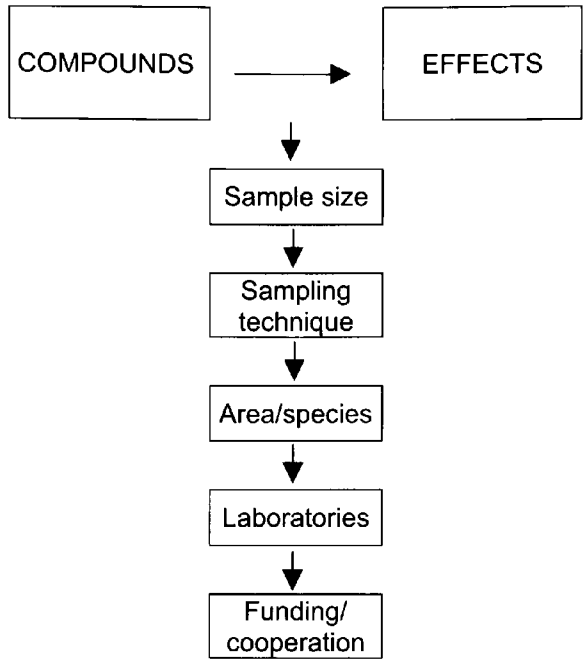

Fig. 2. The decision-making process.

comprehensive pathology is carried out. The Workshop recommendations provide further details but it is clear that changes related to the reproductive system are particularly important.

The following information should be collected for each sampled animal.

(1) Position of capture.
(2) Time of capture/estimated post-mortem time.
(3) Age (teeth), length, sex.
(4) Reproductive condition: collect ovaries, testes samples. Collect whole foetuses and newborn calves where feasible.
(5) Nutritional condition.
(6) Pathology: occlusions and stenosis, collect adrenals, testes, histological liver samples (ribosome density for comparison with enzyme induction).

5. INDICATORS

There are a number of studies in which indicators for pollution exposure and effect are identified. We have focused on eight that we believe hold most promise for cetacean studies (Table 1). The nature of the samples that can be used for each indicator are given. There are some analytical techniques still under development for particular tissues.

In this regard we recommend that an early part of the project should comprise a calibration study to examine: post-mortem times, storage methods and storage times.

With respect to metallothioneins, the Workshop had suggested further investigation of their potential as indicators (Workshop report, item 5.4). This project will allow this to happen.

6. AREA/SPECIES

The Workshop had recommended three focal species which fit well for the PCB studies. We looked at these and tried to identify areas that fulfilled the gradient criterion and where it appeared that reasonable sample sizes could be obtained at least in principle (Table 2).

The Workshop had not commented on focal species/areas for heavy metal analysis. However, we believe that rivers subject to intensive mining operations which determine 'clean' and 'polluted' stream segments would be ideal. For example, in the Amazon river the upper stream is expected to carry only a light heavy metal load whereas parts of the lower river near gold and silver mines are known to be heavily polluted with mercury. The Amazon river dolphin inhabits both the upper and lower parts of the river, and despite being known in some cases to move along considerable distances, appears to occur in essentially local populations. They thus present an excellent subject for studying response variation to differential heavy metal exposure in a relatively homogeneous genetic population. Temporary live-capture of free-ranging individuals is possible.

Table 1

Pollutants and effect indicators to be studies in different cetacean tissues, including feasibility of biopsy sampling and identified potential coordinating/participating laboratories. Key: 1 = Feasible; 2 = Potentially feasible; 3 = Dubious; 4 = Infeasible. A = Age; S = Sex; N = Nutritive condition. GL = Goksøyr lab.; IBN = Institute for Forestry and Nature Research; LUW = Agriculture Univ. of Wageningen (Toxicology Dept.); ML = Martineau lab.; UB = Univ. of Barcelona; US = Univ. of Sienna; UU = Univ. of Utrecht; WH = Woods Hole Oceanographic Institute; WL = Wagemann lab.; YL = Yoshioka lab..

	Potential tissues	Biopsies	Post mortem 24-3hr	Post mortem <3hr	Captive animals	Variables	Laboratories
Pollutants							
PCBs	Blubber	1	1	1	1	S, A, N	UB, IBN
	Blood	4	3	1	1	S, A, N	UB, IBN
Hg, met-Hg	Skin	1	1	1	1	A, S	ICES group
	Liver	4	1	1	4	A, S	ICES group
Cadmium	Skin	1	1	1	1	A, S	ICES group
	Kidney	4	1	1	4	A, S	ICES group
Indicators							
Enzyme induction	Liver	4	1*	1	4	S, A	LUW, US, WH, GL
	Skin	1*	1*	1*	1*	S, A	LUW, US, WH, GL
Sex hormones	Blood	4	2	1	1	S, A	IBN, Hospitals
(oestradiol, testosterone	Muscle	3	2	1	2	S, A	YL
progesterone)	Blubber	1*	2*	1*	1*	S, A	?
Vitamin A	Blood	4	1	1	1	?	LUW, UB
	Liver	4	1	1	4	?	LUW, UB
	Skin	1*	1*	1*	1*	?	LUW, UB
Thyroid hormones	Blood	4	4	1	1		LUW
	Liver	4	4	1	4	S, A	LUW
DNA adducts	Skin	1	1	1	1	A	ML, UU
	Liver	4	1	1	4	A	ML, UU
Porphyrines	Liver	4	2	1	4	?	UB, IBN
	Skin	1*	2*	1*	1*	?	UB, IBN
Luciferase	Blubber	1	1	1	1	?	LUW
	Skin	1	1	1	1	?	LUW
	Blood	4	1	1	1	?	LUW
Metallothioneins	Liver	4	?	1	4	A	LUW,WL
Histopathology	Liver	4	2	1	4	A	LUW,WL

* Analytical technique under development.

Table 2

State of knowledge on cetacean species in specific areas over a pollution gradient, and sampling methods to study pollutant impact.

Sp./pollution level	Study area	Sample source	State of knowledge
Bottlenose dolphins			
High/medium	Florida	Temporary live-capture	V. well-studied already, assoc. biological info. available
	Moray Firth	Biopsy sampling	Small population, some assoc. biological information available
	Mediterranean	Biopsy sampling	Large population, no assoc. biological info.
Light	Mauritania	Biopsy sampling	As above
Harbour porpoises			
High/medium	Gulf of Maine/Bay of Fundy	Bycatch (*ca* 1800)	Studies underway on bycatch
	North Sea	Bycatch (several 000s)	As above
Light	North Norway	Bycatch	
	Greenland	Directed aboriginal hunt	Ease of collecting samples?
White whales			
High	Gulf of St Lawrence	Stranded	Studies underway
Light	Canadian Arctic	Directed aboriginal hunt	Studies underway
	Alaska	Directed aboriginal hunt (400)	Studies underway
Amazon River dolphins	Amazon River	Live capture Biopsy sampling	Studies underway

7. SAMPLE SIZE

It is not possible at this stage to carry out a statistical prediction of the number of samples likely to be necessary to detect significant differences. However, it is clear that sample sizes will probably need to be at least 50 in each 'cell' where the cell will vary by the important variables (e.g. age and sex) as indicated in the table. The total sample needed to collect sufficient animals in each cell will depend on a number of 'sampling selectivity' factors (e.g. are juveniles more likely to be bycaught and do hunters select for larger individuals?) and of course any sex/age segregation in distribution. This needs to be examined in the planning meeting recommended below.

8. LABORATORIES

We recognise that if this project is to succeed it will require the involvement of a number of specialist laboratories. In Table 1 we have given a preliminary list of laboratories/co-ordinators that we know specialise in certain techniques. It is important to remember that while cost is, of course, a factor to be taken into consideration, it is vital that only recognised institutes are involved.

9. FUNDING/CO-OPERATION

It is clear that the cost of this project will be very large although as yet we are not in a position to estimate costs. It is also clear that this will be a multi-year, multi-institution programme. The initial stages will require identifying likely co-operating organisations/institutes/funding agencies. It is unlikely that the IWC alone will be able to fund the whole project!

The example laboratories in Table 1 provide a starting list for collaborative institutions and they can also be approached to see if they are prepared to offer funding 'in kind', e.g. carry out analyses at 'cost' or reduced rates.

We suggest that a number of organisations could be approached for funding/moral support, e.g. ASCOBANS (Eastern North Atlantic harbour porpoises); NAMMCO (Norwegian and Greenlandic harbour porpoises); JCCM (Greenlandic and Canadian white whales); ABWC (Alaskan white whales); ICES; UNEP; IUCN.

10. ORGANISATION

We recommend that the first stage is to organise a planning meeting of interested Institutes and experts. We suggest that the Scientific Committee establishes a working group to further this work. One option is that the group is comprised of the four of us (Reijnders as chief co-ordinator plus Aguilar, Bjørge and Donovan). A first task will be to liaise with the relevant people to clarify the details in Table 1, both from the point of view of sample collection and analyses. A suggested list of tasks and a preliminary list of items to be discussed at the meeting are given below. We **recommend** that the IWC funds this part of the process alone to ensure that progress is made in a timely fashion.

In addition it is clear that the project itself will require an overall co-ordinator/co-ordinating body as it is probable that the project will comprise a number of sub-projects. While the details should probably be finalised at the planing meeting, Reijnders has agreed that he would be prepared to undertake this onerous task.

Finally, we believe that guidelines for timing and publication be agreed early in the process. Given the leading role we would expect the IWC to take we believe it is appropriate to recommend that the resultant papers be published in an IWC Special Issue. It will include an overview of the whole project with an agreed authorship followed by papers for each of the sub-projects.

It is difficult to draw up a realistic time frame for the completion of the project at this stage, as it is dependent on a number of financial and other factors, but we would expect it will take up to five years before publication of the results.

11. ACTION

If this project is to progress beyond the dreaming stage, it is important that a planning meeting is held to develop a fully-fledged proposal. For such a meeting to succeed, a steering group needs to be established as noted under Item 10. We have identified a number of tasks that need to carried out before such a meeting.

(1) Contact institutions that might be interested in collaborating in analyses (i.e. refine the 'Laboratory' column in Table 1) - assigned to Reijnders and Aguilar.
(2) Contact organisations that might be interested in collaborating in data collection (i.e. refine Table 2) - assigned to Donovan and Bjørge.
(3) In the light of (1) and (2) decide if any additional expertise is required - assigned to the Steering Group.
(4) Draw up a list of invited participants - assigned to the Steering Group.
(5) Determine a draft Agenda - assigned to the Steering Group. It will be necessary to request papers on specific topics from certain of the participants.

(6) Determine the logistics of the meeting - Aguilar has indicated that he will be prepared to host the meeting in Barcelona. It might be possible to host the meeting before the next Scientific Committee meeting but this will have to be determined by the Steering Group in the light of points (1)-(4) above. In any event it will be held in the Commission's financial year ending 31 August 1998. The estimated cost of such a meeting is £15,000.

REFERENCE

O'Shea, T.J. and Brownell, R.L. 1994. Organochlorine and metal contaminants in baleen whales - a review and evaluation of conservation implications. *Sci. Total Environ.* 154(2-3):179-200.

J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 55–72, 1999

Planning workshop to develop a programme to investigate pollutant cause-effect relationships in cetaceans:

'POLLUTION 2000+'

INTRODUCTION

The meeting was held at CIDOB (Centre d'Informació i Documentació Internacionals a Barcelona), Barcelona, Spain, from 14-17 March 1999. Local arrangements for the meeting had been made by Aguilar and colleagues at the University of Barcelona and the Fundacio pel Desenvolupament Sostenible. Aguilar welcomed the participants to Barcelona. The list of participants is given as Annex A.

Donovan welcomed the participants on behalf of the IWC. He noted the Commission's increased interest in environmental matters concerning cetaceans, beginning in 1993 (IWC, 1994). The Scientific Committee had agreed to focus its consideration of such matters on two areas: pollution and environmental change. These were the subjects of two Workshops (IWC, 1997; Reijnders *et al.*, 1999). As a result of these, research proposals to address important questions arising were being developed. A meeting had been held on the former (SOWER 2000) in Edinburgh earlier this month (IWC, 1999) and the present Workshop was to further develop an outline proposal (hereafter called POLLUTION 2000+) agreed by the Scientific Committee in 1997 (Aguilar *et al.*, 1998).

2. ARRANGEMENTS FOR THE MEETING

Chairing of the meeting was shared by Aguilar, Donovan and Reijnders. Participants in the Workshop contributed various sections to the draft report. It was agreed that final editing of the report should be undertaken by the co-chairmen. The adopted Agenda is given as Annex B.

3. OBJECTIVES OF THE PROGRAMME

3.1 Review of Aguilar *et al.* (1998)

Donovan introduced Aguilar *et al.* (1998). It had been developed in order to further the main recommendation of the Bergen Workshop (Reijnders *et al.*, 1999, p.22):

> The Workshop believes that there are sufficient data on the adverse effects of pollutants on the health of other marine mammal and terrestrial species to warrant concern for cetaceans. However, the report and its recommendations show that a considerable amount of fundamental research is needed before it will be possible to adequately address the question of the effects of chemical pollutants on all cetaceans.
>
> Notwithstanding the cautionary note that it is often not appropriate to extrapolate from one species to another, it is clear that if any progress is to be made within a reasonable timeframe, a multidisciplinary, multinational focused programme of research is required that concentrates on those species/areas where

there is most chance of success. The Scientific Committee (and the Commission) is strongly urged to consider ways to facilitate the development and execution of such research.

Three species are considered particularly suitable: the bottlenose dolphin; the harbour porpoise; and the white whale.

This outline proposal had been agreed by the Scientific Committee and the Commission in 1997 (IWC, 1998a; b). Subsequently, the proposal was strongly endorsed by ASCOBANS and the ICES Working Group on Marine Mammal Habitat. The latter group is developing a similar research proposal focusing on pinniped species, and has expressed a desire for scientific cooperation with the IWC and suggested exploration of possibilities for joint funding sources.

The present Workshop is a direct result of Aguilar *et al.* (1998) and its terms of reference are to develop and update the outline into a full field and analytical programme. In particular, Aguilar *et al.* (1998) recognised that while in

'an ideal world, the ultimate objective of pollution studies for cetacean management is to determine a predictive model linking tissue pollutant levels with effects at the population level ... this is clearly not a realistic short-term goal but it might be achievable in the long-term'.

Given this, the proposal's primary aim was to attempt to:

'determine the relationship between levels in certain tissues and indicators of certain effects. It seemed appropriate to focus on two sets of pollutants: (1) that might provide information of more general applicability, e.g. impact of organochlorines; and (2) that are subject to more local interpretation such as impact of heavy metals.'

Aguilar *et al.* (1998) then outlined a programme to that aim, covering *inter alia*: sources of samples; associated biological information required; indicators to be examined; potential areas and species to be sampled; sample sizes and potential collaborators.

Aguilar *et al.* (1998) had highlighted potential populations to be studied: harbour porpoises, white whales and bottlenose dolphins (for organochlorines) and Amazon River dolphins (for heavy metals). Subsequent discussion with potential collaborators on the initially proposed project on the Amazon River dolphin has meant that this sub-project is now considered impractical. Thus, it was agreed at the present Workshop that the POLLUTION 2000+ programme should now focus solely on organochlorines (particularly PCBs – see Item 4.1).

During the present Workshop, it was agreed that some clarification of the objectives would be useful. The implication of setting a short-term goal, and the recognition of the Bergen Workshop that 'a considerable amount of fundamental research is needed...', is that the IWC programme is the first stage in an ongoing, and necessarily iterative process. Stages in such a process include:

(1) examining the relationship between tissue levels and biomarkers;
(2) examining the relationship between biomarkers and effects;
(3) examining effects on individuals;
(4) examining how the effects on the individual affect population dynamics.

It was agreed that this first stage of the programme could proceed on two fronts: one, to examine a number of biomarkers (of exposure to and/or effect of PCBs) and try to determine whether a predictive and quantitative relationship with PCB levels in certain tissues exists; the other, to validate/calibrate sampling and analytical techniques to address such questions for cetaceans. Examination of the first requires relatively large sample sizes, and to the extent possible, control for known variables such as age, sex and reproductive condition. It does not require extremely detailed pathology at this stage (see Items 4.4 and 5.4). Examination of the

second may require lower sample sizes but considerably more information. Two important sub-objectives are:

(1) determination of changes in concentrations of variables with post-mortem times;
(2) examination of relationships between concentrations of variables obtained from biopsy sampling with those of concentrations in other tissues that can only be obtained from fresh carcasses.

It is important to stress that the development of POLLUTION 2000+ should not be seen as suggesting that other research on pollutants and cetaceans is not of value. The IWC programme should be seen as a 'core' programme to address some fundamental questions of general applicability (it should be reiterated that the species and areas chosen were chosen because of the likelihood of success and not because of any specific conservation concerns for those species/areas). Its value is immeasurably enhanced by cooperation with existing programmes and as a context for the development of new programmes. The IWC should cooperate with other national and international bodies in a coordinating role (see Item 8). Certain research subjects that are considered of high relevance but that do not fit in the 'core' programme are identified here as 'auxiliary projects'. The IWC should encourage third parties to undertake these projects.

4. IDENTIFICATION OF VARIABLES TO BE MEASURED

4.1 Pollutants

Organochlorine compounds are considered the main focus of the present proposal because their origin is overwhelmingly anthropogenic, they are found at extremely high tissue concentrations in some populations of cetaceans, and they have recognised effects upon wildlife. Moreover, substantial background information exists on their patterns of variation, geographical distribution and tissue kinetics.

4.2 Indicators

4.2.1 Sex hormones

4.2.1.1 REPRODUCTIVE SEASONALITY

Interpretation of sex hormone levels requires knowledge of the natural cycle of hormones in the subject animals. For the bottlenose dolphin, information exists from studies of captive animals and live-recapture studies. This facilitates selection of time periods for sampling of reproductive hormone concentrations relative to contaminant body burdens. Selection of 'quiet periods' when hormonal activity is predictably at a minimum would reduce the kinds of individual variation that might be expected from measuring adults at different points of the reproductive cycle during the general breeding season. Bottlenose dolphin reproductive seasons typically last for several months, occurring during spring, summer and/or autumn (Wells and Scott, In press). However, this may not be a critical period for assessing PCB related hormonal effects. Females may ovulate repeatedly during a given season and males maintain enlarged testes and elevated levels of testosterone over the several month duration of the breeding season. Seasonality varies geographically; for example, dolphins inhabiting different sites in the southeastern USA may exhibit different patterns of seasonality, with the patterns remaining established over multiple generations and even when individuals are transferred to other locations (Urian *et al.*, 1996).

Alternatively, it is possible in some cases to identify the reproductive status of bottlenose dolphins through observational studies. Identifiable dolphins can be monitored closely over time, and the timing of births can be used to identify reproductive status retrospectively. In

some situations, it is possible to conduct hands-on examination and sampling of live dolphins. Such opportunities may allow blood sampling in conjunction with ultrasonic measurement of testis length and diameter, the ultrasonic identification of the presence or absence of a foetus, measurement of a foetus, or ultrasonic identification of a developing follicle to provide information on the reproductive status of individuals.

For the harbour porpoise and white whale, investigation of reproductive tract combined with possible blood analyses from freshly dead animals could facilitate the establishment of hormone profiles throughout the reproductive cycle. Information on basic reproductive biology of harbour porpoises is being obtained from captive animals in Kateminde, Denmark. This includes information on hormone profiles, behaviour, maturity status and different stages in reproductive status using ultrasound and cytology. Investigations of the male cycle are also being planned. The basic reproductive cycle (timing of reproduction, period of reproductive activity, duration of pregnancy) in harbour porpoise has been well documented from individuals taken in commercial fisheries (e.g. Read, 1990 and see Donovan and Bjørge, 1995). Similar data on white whales have been collected on animals taken in subsistence harvests (Braham, 1984; Marine Mammal Commission, 1999).

4.2.2 Enzyme induction

Organochlorines are known to induce metabolic enzymes (the cytochrome P450 isoenzyme system in some cases). Since the same system (hereafter called P450) is involved in the control of endogenous compounds such as steroid hormones, this induction may lead to disturbance in endocrine control. The induction of P450 systems by PCBs has been demonstrated in several marine mammal species. The P450 enzyme system is therefore a useful biomarker for exposure to *inter alia* PCBs.

4.2.3 Thyroid hormones and vitamin A levels

Organochlorines are known to produce alterations of both vitamin A and thyroid hormones, and so are potentially useful biomarkers. Thyroid hormones as well as vitamin A are *inter alia* involved in early development and also in immune function (Marine Mammal Commission, 1999; Reijnders, 1999).

4.2.4 Indicators of immune system status

4.2.4.1 BLOOD CELL PARAMETERS

A number of tests are available to measure immune function in humans and in domestic and laboratory animals. These tests include analysis of immune cells, lymphocyte stimulation tests, cytokine assays, antigen responses and lymphocyte proliferation. Some of these tests have been applied in marine mammals, but others still have to be developed. It is emphasised that a series of blood cell parameters should be used, as the individual tests usually relate to only one specific part of the immune system. Moreover, study of a wide spectrum of blood parameters may enable discrimination of the causes of perturbations, e.g. infection, physical trauma and those produced by toxins.

4.2.4.2 *IN VITRO* TESTS (CELL LINE CULTURES, MICROSOMES) - AUXILIARY PROJECT

From recently harvested, beach cast or euthanised animals, tissues can be collected and maintained in tissue culture media or quickly frozen in tissue culture preservative for establishment of primary cultures and development of immortal cell lines. Establishment of

cultures should allow *in vitro* metabolic and toxicity testing in the species of concern. These techniques are inexpensive alternatives to repeated capture or the maintaining of captive animals. However, one limitation of this approach is the difficulty in extrapolating to whole animal or population level effects. It will, however, provide mechanistic and metabolic information for specific species.

4.2.5 Porphyrins

Porphyrins play a crucial role in the haem biosynthetic pathway. Disruptions of haem biosynthesis (usually referred to as porphyria) by contaminants has been found. Particularly liver porphyrins in wildlife differed markedly after exposure to PAHs. They are therefore good indicators of exposure to *inter alia* PCBs.

4.2.6 DNA adducts (auxiliary project)

The formation of DNA adducts may represent a good measure of the exposure of organisms to PAHs but not PCBs. Since the latter are the main focus of the proposal, DNA adducts are not considered as priority indicators in POLLUTION 2000+.

4.2.7 Luciferase

The proposed luciferase method (see Item 5.2.6) acts specifically via the Ah-receptor route and therefore is a biomarker of exposure to dioxin-type contaminants, as extracted from the tissue(s) of interest. This acts as a measure of dioxin-like exposure for the chemicals present, but not as a measure of the specific chemical basis.

4.3 Biological variables

4.3.1 Body length, sex and age

These are factors significantly affecting both the tissue concentration of organochlorine pollutants in cetaceans and their susceptibility to chemical insult (Aguilar *et al.*, 1999). These variables are also critical for the assessment of reproductive condition, pathology and other variables relevant to the examination of pollutant impact on populations.

4.3.2 Reproductive condition

Reproductive condition affects some of the indicators to be measured (e.g. sex and other hormones). In some species it may also affect the food intake rate or the diet composition and, in this way, it influences nutritive condition. As PCBs are lipophilic, reproductive condition may therefore indirectly affect tissue levels of these compounds. Thus, determination of reproductive status is significant to assess both the observed indicators and the pollutant concentrations.

4.3.3 Nutritive condition

This is significant not only for the assessment of the health status of the individual examined but, because organochlorine compounds are highly lipophilic, changes in blubber layer thickness can have an effect on concentrations of, for example, PCBs in blubber, other organs and blood. Therefore, information on nutritive condition also enables interpretation of pollution concentrations found in tissues.

4.4 Pathology

Detailed necropsies and clinical examinations are required to assess the overall health of an individual and to try to distinguish effects of contaminants from those caused by other known stressors. Pathological effects should help in the interpretation of bioindicator data in relation to contaminant levels.

4.4.1 Developmental stability (auxiliary research)

Also known as developmental homeostasis, this is an indication of the ability of the individual to develop its phenotypic characters. Within a given species, phenotypic heterogeneity among individuals may suggest disturbed development; in some marine mammals (from skull biometric studies), this has been associated with exposure to high levels of PCBs and other pollutants (Zakharov and Yablokov, 1990).

5. ANALYTICAL TECHNIQUES

5.1 Pollutants

5.1.1 Coplanar PCBs

Lipids are extracted from blubber using hexane as a solvent in a Soxhlet apparatus. A portion of this extract is mixed with sulphuric acid for the clean-up. Determination of organochlorine compounds is carried out using standard capillary GC-ECD (Gas Chromatography–Electron Capture Detection) techniques.

PCBs are extracted and cleaned-up from blubber samples by the method of alkaline-alcohol digestion. The extract is run on a 5mm i.d. column packed with 125mg of activated carbon to separate non-ortho coplanar PCB congeners from other PCB isomers. The hexane extract is successively cleaned with 10% fuming sulphuric acid and rinsed in distilled water. The analysis of non-ortho PCB congeners is carried out using a gas chromatograph with mass spectrometer (GC-MS) (Tanabe *et al.*, 1987).

5.2 Indicators

5.2.1 Sex hormone determination

In plasma, serum and urine, sex hormone levels (oestradiol, progesterone, testosterone) can be directly assessed using RIA (Radio-immuno-assay). It may be possible to obtain information on levels from tissue samples by homogenisation in the presence of a solvent, liquid-liquid extraction, GPC (Gel Permeation Chromatography) and RIA with specific antibodies.

5.2.2 Enzyme induction

Concentration and/or enzymatic activity of some proteins are known to change with contaminant exposure. Proteins sensitive to PCB exposure include cytochrome P450 1A for planar PCBs, and P450 2B for some non-planar PCBs. P450 3A is also important for sex steroid metabolism.

P450 1A content can be measured in dermal endothelia, from all cetacean species examined to date, using monoclonal antibody 1-12-3 (Ronis *et al.*, 1989) and other antibodies. A midline slice of epidermis and dermis from the skin/blubber biopsy core is fixed in formalin, and the protein expression measured immunohistochemically. Sections from any routine histology sample may also be stained.

P450 1A, 2B and 3A can be measured for content and activity in liquid nitrogen frozen samples, by immunoblot and catalytic assay respectively. The catalytic assays are often fluorometric, such as for ethoxy-resorufin-O-deethylase (EROD). These are commonly referred to as Phase I enzymes.

5.2.3 Thyroid hormones and vitamin A

Thyroxin (T4) and triiodothyronin (T3) are determined by standard radioimmunochemical methods.

Retinol analysis includes a saponification of the sample with an ethanolic KOH solution and a subsequent extraction with diethyl ether. The extract is analysed by HPLC (High Performance Liquid Chromatography fluoresence analysis) using a reversed phase C18 column, and an ultraviolet detector with a mobile phase of methanol/water.

5.2.4 Indicators of immune system status

5.2.4.1 BLOOD CELL PARAMETERS

5.2.4.1.1 PHENOTYPIC ANALYSES

(1) White blood count – differential smear, total count.
(2) Red blood cell profile.
(3) Lymphocyte sub-populations: analytical flow cytometer and immunohistochemistry.
(4) Leukocyte adhesion proteins - analytical flow cytometry.
(5) Total Ig:

Quantitative: RID – Radial Immunodiffusion; ELISA (Enzyme-linked Immunosorbent Assays); Serum protein electrophoresis.
Qualitative: IEP – Immuno electrophoresis.

(6) Inflammatory cytokines: bioassay, ELISA – in development.
(7) Acute phase proteins: ELISA.
(8) Serum chemistries (complete).

5.2.4.1.2 FUNCTIONAL ASSAYS

(1) Lymphocyte blastogenesis (colorometric; radio-label).
(2) Lymphocyte activation (flow cytometry and Ca^{2+}/IL-2 receptor expression etc.).

5.2.5 Porphyrins

Usually porphyrinogenic effects are measured as the concentration of different porphyrins in liver, urine and blood samples. The measure is the ratio between different porphyrin-derivates.

5.2.6 Luciferase

There are Luciferase induction assays specific for dioxins/PCBs (DR-CALUX) and for (pseudo) oestrogens (ER-CALUX). Lipid extracts from e.g. bloodplasma, liver tissue, are processed over a sulphuric acid-silica column and the residue is added to the culture medium of CALUX cells. Following 24hr exposure, the CALUX cells are lysed and Luciferase activity (light production) is measured in a luminometer. This method directly gives total TEQs of dioxin-like activity/g of lipid (instead of GC-MS congener concentrations multiplied by TEF factors and summed up).

5.3 Biological variables

5.3.1 Age, length and sex

Body length will be measured as the distance (following a straight line but not the contour of the body) from the tip of the upper jaw to the notch between the flukes.

Sex will be determined either by direct examination of the external genitalia in dead or captured individuals or, in dolphins sampled at a distance with biopsy darts, by means of genetic analysis of skin. The skin will be preserved frozen or in DMSO and will be analysed

by means of amplification by PCR of ZFX and ZFY, two specific sex chromosomal DNA regions which present slight differences in their nucleotide sequence (Palsboll *et al.*, 1992).

Age determination will be carried out by counting growth layer groups in the dentine or the cementum of teeth. Teeth must be cleaned but not boiled, and fixed (see field protocol). In the laboratory they will be decalcified in Rapid Bone Decalcifier for the Preparation of Histological Materials (RDO), and longitudinally sectioned in a freezing microtome, stained with Hematoxylin, blued in weak ammonia solution, and mounted onto gelatin coated slides (Hohn and Lockyer, 1995).

5.3.2 Reproductive condition

5.3.2.1 OBSERVATIONS

Long-term monitoring of identified individuals can provide information on reproductive activity/condition. Female reproductive activity can be based on retrospective analysis of the timing of initial appearance of newborn and continued presence of a calf, or oestrus behaviour as indicated by social association patterns. It is possible to generate reproductive histories for identifiable females from some resident bottlenose dolphin populations.

5.3.2.2 LIVE CAPTURE/RELEASE

The following information on reproductive condition can be collected from live-caught animals:

(1) morphometric indications of maturation (length, girth, weight, allometrical differences);
(2) hormone (oestradiol, progesterone and testosterone) levels in blood, faeces, urine, saliva and other matrices as available;
(3) using ultrasound to determine maturity status, seasonal changes or pregnancy by measuring testes length and diameter, foetal presence and corpora lutea, especially valuable in conjunction with hormonal status;
(4) direct measurement or examination of external features (lactation, mammary development, semen in urine samples, ano-urogenital morphometrics).

5.3.2.3 POST-MORTEM (AS ABOVE)

(1) Gross, histologic and cytologic assessment of activity, maturity (spermatogenesis, follicle development, ovarian scars, uterine status) and pathology (see Item 5.4).
(2) Presence and orientation of foetus.
(3) Morphometrics of foetus and reproductive tract.
(4) Mammary development, presence of milk and histology.

5.3.3 Nutritive condition

Nutritive condition will be assessed using a combination of two types of measures: (1) morphometric data (body weight/length ratio, body perimeter at the level of the axillas and at the centre of the anus, thickness of the blubber in the mid body length at the mid point between the dorsal and ventral lines); and (2) total lipid content from at least a blubber sample collected from the dorsal region of the trunk, as determined by Soxhlet extraction of the tissue and subsequent gravimetry, as conducted in PCB analyses. In dead individuals, these measures and the blubber sample will be directly obtained from the corpse. In live-captured individuals, blubber thickness will be determined using ultrasound devices. In remotely biopsied individuals, assessment of nutritive condition will be carried out solely based on the lipid content analysis of the blubber sample (Aguilar and Borrell, 1990; Lockyer, 1995).

5.3.4 Stock identity/individual recognition

Knowledge of stock identity may facilitate the interpretation of contaminant levels relative to exposure history for geographically-based stocks. A variety of tools are available for stock identification; the applicability of any one technique to a given situation varies with the species, and a suite of techniques is most likely to provide suitable information. In recent years, the advent of genetic techniques, particularly analyses of mtDNA, has proved to be a powerful additional tool, especially when used in combination with techniques such as morphometrics, individual identification, examination of parasite loads, contaminant profiles, stomach content analyses and stable isotope analyses.

Individual identification techniques (e.g. photographic identification or genetic fingerprinting) allow the monitoring of individuals over time, including the resampling of individuals throughout their life.

5.4 Pathology

5.4.1 Gross pathology and histopathology of main organs (including dimensions)

Such investigations (e.g. see Politi, 1994) are intended to try to assess general health status.

Gross necropsy starts with history and external examination (skin, orifices, mucosal membranes). At internal examination [organs marked * are to be weighed] liver*, kidney*, heart and lung are of particular toxicological interest. When focusing on the immune system, thymus*, spleen*, lymph nodes and bone marrow deserve attention; when the endocrine system is of concern, attention should be given to ovaries*, uterus (including foetus), testis*, adrenal*, thyroid* and pituitary. For specific investigations, additional tissues (eg. bone, skull) can be sampled (see below).

It is self-evident that sampling and examining additional tissues allows for more 'back-up' material available for future investigative studies.

The above mentioned organs must be sampled for histopathology, usually in buffered formalin.

5.4.2 Health assessment of live animals

In recent years, efforts have been undertaken to proactively assess the health of wild dolphin populations. A Workshop was held in 1993 to develop a means of translating clinical veterinary assessments of individual dolphins to population-level measures of health. A system was devised based on blood chemistry and haematology values obtained from samples collected during capture-release operations. A suite of 19 blood parameters considered to be important indicators of health and physiological processes was identified. These were weighted to take into account variations relative to age, sex, and reproductive condition where appropriate, and given a 'score'. The individual parameter scores are added together to provide a health score for the individual. The distribution of individual scores across the sampled population is examined to evaluate the general health condition of the population at any given time, relative to the Sarasota Bay, USA, reference population (Wells *et al.*, 1995).

An initial testing study with dolphins from Sarasota Bay, FL, Matagorda Bay, TX, and Beaufort, NC, USA, suggested that it provides a relatively accurate representation of the health status of the dolphins, and health score patterns follow seasonal patterns of natural mortality in at least the Sarasota Bay dolphin community. Efforts to refine the system are ongoing. Other parameters under consideration for the model include weight, blubber depth, results of ultrasonic examinations, urine analysis, microbiological analyses and contaminant burdens.

Consideration of a 'health score' as an indicator or biological variable in POLLUTION 2000+ could provide additional insight into the effects of PCBs on these animals. The suite of blood parameters integrated by this model includes the basic indicators commonly used by veterinarians for diagnosing or monitoring a variety of health problems in individual cetaceans.

5.4.3 Secondary sexual characteristics

Routine length measurements should include snout to anus, and anus to posterior and anterior ends of the genital slit. The presence or absence of nipples and/or mammary slits should be recorded for males and females. Photographs should be taken wherever possible.

6. SAMPLING (BY SPECIES, AREA AND VARIABLE)

6.1 Sampling location and target species

An ideal scenario to investigate cause-effect relationships of pollutants in cetaceans would require the following elements:

(1) existence of a sample group inhabiting a pristine (or nearly pristine) environment that permits establishing patterns of variation (e.g. caused by sex, age or nutritive condition) in the proposed indicators in natural conditions;
(2) existence of a strong gradient (i.e. more than one order of magnitude) in exposure to pollutants, as measured by tissue concentrations, either between comparable segments of the same population or between comparable sample groups belonging to different populations of the same species;
(3) the ability to obtain a large enough sample size for each of the groups to be compared, to allow robust statistical analysis (preferably $n > 30$);
(4) field conditions permitting sampling of target tissues in order to allow optimum preservation conditions.

The information contained in Annex C indicates that there is not a single scenario that fulfils all these requirements. The Workshop therefore agreed that separate but complementary sub-projects should be carried out. These will focus on the objectives and groups of indicators for which the respective field conditions are most appropriate. The integration of the results of these sub-projects should provide a comprehensive insight and validate tools and methods that will provide the necessary background for the future phases of the research programme.

Table 1 details the advantages and limitations of each of the sub-projects proposed, and the objectives of the proposed studies.

There are a number of reasons for choosing biopsy-based studies of bottlenose dolphins. One of these is that if it can be shown that biopsy sampling can be used to monitor the 'health-pollutant' status, then this has great potential for future non-invasive monitoring. Another is that it will allow comparison of two 'pristine' areas and the results of this may have implications for interpreting data from different populations. Furthermore it will provide, in collaboration with the bottlenose dolphin live-capture sub-project, baseline information on certain indicators subject to a larger pollutant gradient, including pristine conditions.

The ability to collect large harbour porpoise sample sizes in the Icelandic/Danish and the northwest Atlantic fisheries (see Annex C) render them most useful for attempting to determine if a quantitative and predictive relationship exists between tissue levels and certain indicators.

Table 1

Proposed studies and related details on advantages and limitations, and on objectives, sampling techniques and expected sample sizes.

Species/Areas	Sampling technique	Advantages	Drawbacks	Objectives	*n* over 3 yrs	Priority indicators	Other indicators possible
T. truncatus Sarasota Bay	Live capture	Fresh samples Good biological information on individuals Longitudinal studies and resampling	Limited or no gradient in pollutants No pristine population Inaccessibility to internal organs except for some fresh strandings	Validation of indicators in blood	100	Hormones P450 Porphyrines Immune system Luciferase Vitamin A	DNA adducts Health assess Body condition
T. truncatus Mauritania/Medit./ Bahamas	Remote biopsy	Fresh samples Wide gradient in pollutants Existence of pristine population	Sampling limited to external tissues No biological information on individuals	Validation of indicators obtainable by remote biopsy and comparison between pristine areas	100	Vitamin A P450 Luciferase	DNA adducts
P. phocoena Iceland/Central and Northern North Sea/ Northwest Atlantic	Bycatch	Existence of pristine population Access to internal organs Good biological information on individuals	No fresh samples Moderate gradient in pollutants	Statistical validation between pollutant tissue concentrations and some indicators Establish relationship between 'biopsy' levels and those in other tissues	>200	P450? Porphyrines Sex hormones? Immune system Luciferase? Vitamin A	Gross pathology Early development Bone disorders Reproductive tract Histopathology
P. phocoena e.g. German North Sea/Baltic	Fresh bycaught/ stranded	Reasonable sample size Established programme		Establish post-mortem time relationships Establish relationship between 'biopsy' levels and those in other tissues	*Ca* 60	As many of the suite in Table 2 as possible that are quantifiable	Others

The objectives of relating post-mortem times with pollutant and indicator levels, and of relating levels in biopsy samples with those from other organs and tissues in the body, can be undertaken as part of the same sub-project. These questions can be addressed with smaller sample sizes.

Biopsy-based studies on white whales were also discussed. Due to the limited sample size obtainable for white whales from the St Lawrence Estuary, the inability to establish a significant gradient of tissue concentrations from Arctic whale stocks and the duplication of information acquired in the bottlenose dolphin studies, it was decided not to include the white whales as a study species in the POLLUTION 2000+ programme. However, it should be noted that this does not imply that pollutant studies on white whales are not important or may not be included in future phases of this iterative project.

6.2 Sample quality and preservation methods

The locations selected for the study provide opportunities for sampling using three different means: necropsy of dead animals (either harvested, bycaught in a fishing operation, euthanised or freshly stranded); biopsies obtained from live-captured individuals; and remote biopsies taken from free-ranging individuals. Table 2 details the tissues that can be obtained using each of these techniques.

The condition of the tissues thus obtained is variable and this opportunity allows us to compare 'condition' effects on the variables measured. While remote biopsies and live captures produce fresh tissues, post-mortem times in necropsies vary up to a few days. Some indicators are sensitive to prolonged post-mortem times and this limits their applicability in non-fresh samples. It was agreed that any animal to be sampled for the study should be less than 24 hours post-mortem and the time of sample preservation should also be noted. Table 2 depicts which indicators are likely to provide reliable results in fresh samples (less than 3 hours post-mortem) and in those moderately fresh (between 3 and 24 hours post-mortem).

Table 2 also details optimum conditions for storage, either for short-term periods (few months), long-term storage (few years) or archival purposes (>10 years). Archival of non-used tissues or replicates is important to allow application in the future of more sensible or more reliable techniques that are now under development, or to investigate pollutants or indicators currently not considered.

7. RESPONSIBLE LABORATORIES

7.1 Sample collection

The agreed sub-projects are listed in Table 1. The Workshop is not in a position to finalise details of field collection but nominated the following people who would be responsible for coordinating/obtaining the necessary information required by the Steering Group (see Item 8 below).

(1) Bottlenose dolphin: Sarasota Bay – R. Wells.
(2) Bottlenose dolphin: Mauritania/Mediterranean – A. Aguilar.
(3) Bottlenose dolphin: Bahamas – P. Hammond.
(4) Harbour porpoise: Iceland – G.A. Vikingsson.
(5) Harbour porpoise: Central North Sea – F. Larsen.
(6) Harbour porpoise: Northern North Sea – A. Bjørge.
(7) Harbour porpoise: Northwest Atlantic – T. Rowles.
(8) Harbour porpoise: German North Sea/Baltic – U. Siebert.

Table 2

Indicators to be analysed and sampling and storage methods to be used relative to post-mortem times.
Technique: B: Remote biopsy; D: Necropsy; L: Live capture.

Tissues	Technique	3-24hr post-mortem	< 3hr post-mortem	Short-term storage	Long-term storage	Archival storage
Organochlorines						
Blubber	B, D, L	✔	✔	-20°C	-20°C	-80°C
Whole blood	D, L	✔	✔	-20°C	-80°C	-80°C
Milk	D, L	✔	✔	-20°C	-80°C	-80°C
Sex Hormones						
Blood	D, L	?	✔	Chilled/frozen	-80°C	-80°C
Urine	D, L	?	✔	Chilled/frozen	-80°C	-80°C
Enzyme Induction						
Skin	B, D, L	✔	✔	Neutral formalin/Liquid Nitrogen – see protocol		
Liver	D	No	✔	Neutral formalin/Liquid Nitrogen – see protocol		
Thyroid Hormones (T3 T4)						
Serum	D, L	?	✔	Chilled/frozen	-80°C	-80°C
Vitamin A						
Serum	D, L	?	✔	-20°C	-80°C	-80°C
Liver	D	?	✔	-20°C	-80°C	-80°C
Blubber	B, D, L	?	✔	-20°C	-80°C	-80°C
Blood Cell Parameters						
Whole blood in:						
No anticoagulent (serum)	D, L	No	✔	-80°C	-80°C	-80°C
EDTA (CBC)	D, L	No	✔ (chilled)	No storage		
CPT (Lymphocyte function)	D, L	No	✔ (chilled)	Liquid Nitrogen following preparation		
ECD (Leukocyte sub-pop)	D, L	No	✔ (chilled)	Liquid Nitrogen following preparation		
Lymph node and spleen	D	✔	✔	-80°C/formalin		
Porphyrins						
Blood	D, L	?	✔			
Liver	D	✔	✔			
Urine	D, L	?	✔			
Luciferase						
Blood	D, L	✔	✔			
Blubber	B, D, L	✔	✔			
Skin	B, D, L	✔	✔			

7.2 Analyses

7.2.1 Pollutants and indicators

The Workshop agreed that in order to minimise sources of error, ideally analyses of each variable would be undertaken by a single laboratory. In practice, it was agreed that this should be ensured at least for each species. The Workshop emphasised the need for only experienced (in marine mammals) and quality assured laboratories to be used. Potential laboratories are listed in Table 3. This list is not intended to be exclusive. Final responsibility for the selection of laboratories should lie with the Steering Group (see Item 8). Appropriate cross calibration should occur where necessary.

7.2.2 Biological material

Most standard biological analyses can be carried out by the collecting institution, following a standard protocol to be defined by the Steering Group (see Item 8). However the Workshop made the following observations with respect to certain items.

7.2.2.1 AGEING OF TEETH

The Workshop recommends that at least 20% of teeth from the various areas are cross-read. The final details of this will be decided by the Steering Group.

7.2.2.2 GENETIC DETERMINATION OF SEX/GENETIC PROFILE

A number of institutes carry out such work, including: the University of Barcelona; Portland State University; NMFS; University of Bangor; University of Aarhus; and the University of Kiel.

7.2.3 Routine histopathology (formalin-fixed)

For the bottlenose dolphin studies, this will be applicable to only a few stranded animals. Wells already has an established system for this. However, for the harbour porpoise work, there will be a large number of samples. It was agreed that details for cross-examining slides should be finalised by the Steering Group. This should involve 3-5 workers. Suitable candidates include Wester, Bergmann, Siebert and a number of North American scientists.

8. ORGANISATION/COORDINATION

8.1 Coordinator and steering group

The Workshop identified a number of outstanding and ongoing items at both the scientific and logistical level that can best be addressed by the appointment of a Steering Group. These items are listed in Table 4. Many, if not all, have funding implications. It was agreed that Reijnders should act as the coordinator of the Steering Group and that the Group should comprise a representative from each sub-project (Wells - bottlenose dolphin, live-capture programme; Aguilar - bottlenose dolphin, biopsy programme; Bjørge - harbour porpoise, bycatch programme; Siebert - technique validation/calibration programme); Donovan (IWC); Rowles; plus a statistician/modeller, a biomarker expert and someone with an overview of cetacean biology, pathology, toxicology and veterinary medicine. It was agreed that the latter three should be determined after this Workshop by the Steering Group.

Table 3

Identified potential laboratories involved in variables to be analysed in the different study species.

Items to be studied	Potential laboratories
Pollutants (PCBs)	
Bottlenose dolphin	University of Barcelona; University of Utah; NMFS-NOAA
Harbour porpoise	NMFS Seattle; University of Rostock; University of Barcelona; Free University of Amsterdam
Sex Hormones	
Bottlenose dolphin	University of Miami; Colorado State University; Smithsonian Institution
Harbour porpoise	University of Odense; Heerlen Hospital; competent laboratory
Enzyme Induction	
Bottlenose dolphin	Histological – Woods Hole Oceanographic Institute Laboratories; CSIC (Spain) Enzymatic (frozen) – Woods Hole Oceanographic Institute Laboratories; University of Siena
Harbour porpoise	University of Rostock; Free University of Amsterdam
Thyroid Hormones	
Bottlenose dolphin and harbour porpoise	Free University of Amsterdam
Vitamin A	
Bottlenose dolphin and harbour porpoise	Free University of Amsterdam; University of Barcelona; competent laboratory
Immunology	
Bottlenose dolphin	Blood with no anticoagulent (serum): quantitative/qualitative Ig, inflammatory mediators – University of California, Davis; serum chemistries – competent laboratory Blood in ACD: leukocyte subpopulations – University of California, Davis Blood in CPT: lymphocyte function – University of California, Davis Blood in EDTA: CBC including total and differential cell count – competent laboratory
Harbour porpoise	Blood with no anticoagulent (serum): quantitative/qualitative Ig, inflammatory mediators – University of California, Davis/ University of Kiel; serum chemistries – competent laboratory Blood in ACD: leukocyte subpopulations – University of California, Davis/University of Kiel Blood in CPT: lymphocyte function – University of California, Davis/University of Kiel Necropsy tissue: lymph node and spleen – University of California, Davis/University of Kiel Blood in EDTA: CBC including total and differential cell count – competent laboratory
Porphyrins	
Bottlenose dolphin	Free University of Amsterdam; University of Rochester
Harbour porpoise	Free University of Amsterdam
Luciferase	
Bottlenose dolphin and harbour porpoise	Free University of Amsterdam

Table 4

Ongoing and outstanding items to be addressed by the Pollution 2000+ Steering Group.

Item(s) to be addressed by Steering Group	When
Formulate questions and co-ordinate replies on field logistics	Pre-Grenada
Determine funding requirements, examine implications and priorities, investigate other sources of funding, accounting	Pre- and post- Grenada
Establish field protocols*	Late 1999
Logistics of sample handling e.g. shipping (incl. permits); archiving	Throughout
Co-ordinate analysis of sub-projects, review results, synthesis of sub-project data; review and evaluation and completion of final report; planning for the next phase*	Throughout
Data storage and availability	Throughout

* Organisation of workshops.

8.2 Timetable

The Workshop noted that the IWC would not take a decision on funding until June 1999. It is clear therefore, that fieldwork is unlikely to begin until the year 2000. Although the details will be the responsibility of the Steering Group, it is envisaged that at least three Workshops will be required, probably in December 2001, mid-2004 and early 2005, with the aim of presenting a final report to the IWC at its Annual Meeting in 2005.

8.3 Budget and funding

It is not possible for the Workshop to develop a precise budget, given the need to obtain further details on the field programme and the implications of this for analyses. It therefore agreed that this task should be completed by the Steering Group, based on the requirements given in the Workshop report and attached as an Annex in time for the Scientific Committee meeting in Grenada in May this year [1999] (see Annex C).

Noting the support for this proposal from ASCOBANS, the Steering Group should also contact this and other relevant groups for possible co-sponsorship. NAMMCO has also been examining issues of pollutants and had been invited to attend this workshop but had been unable to attend. They should also be approached as potential collaborators.

When endorsing the IWC proposal for cetaceans, the ICES Working Group on Marine Mammal Habitats had decided to develop a related project for pinnipeds. They met earlier this month [March 1999] in Copenhagen and formulated such a programme. The Workshop agreed that the Steering Group should explore further the possibility of a joint proposal being submitted to the EU.

Even without the ability to develop a detailed budget, it is clear that the total funding required will be extremely high by IWC standards. This had already been pointed out in the original proposal. The Workshop strongly believes that the POLLUTION 2000+ project represents the fundamental research necessary if the effects of pollutants on cetaceans are to be determined. In addition to central IWC funding, therefore, it urges IWC member governments to consider providing support to this project at the national level.

8.4 Report and publications

The Workshop agreed that the final report to the IWC should be completed after a Workshop to review results, probably to be held in early 2005. Given the leading role taken by the IWC in this programme, the Scientific Committee had agreed that it was appropriate that selected

resultant papers should be published as an IWC Special Issue. This does not preclude other papers being published elsewhere. This matter should be discussed further, both within the Steering Group and, if appropriate, with other co-sponsors.

9. ADOPTION OF REPORT

The Report of the Workshop was adopted at the end of the meeting. However, it was agreed that a detailed budget should be drawn up after the meeting by Aguilar, Donovan and Reijnders based on a questionnaire sent out to the institutes/individuals listed in Table 3 and Item 7.1. Details are given in Annex C.

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Annex A

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Annex B

Draft Agenda

1. Introduction
2. Arrangements for the meeting
3. Objectives of the programme
4. Identification of variables to be measured
   - 4.1 Pollutants
   - 4.2 Indicators
   - 4.3 Biological variables
   - 4.4 Pathology
5. Analytical techniques
   - 5.1 Pollutants
   - 5.2 Indicators
   - 5.3 Biological variables
   - 5.4 Pathology
6. Sampling (by species, area and variable)
   - 6.1 Sample size and composition (age, sex, etc.)
   - 6.2 Collection method (including amount of sample required)
   - 6.3 Short-term storage
   - 6.4 Long-term storage and shipment
7. Responsible laboratories
   - 7.1 Sample collection
   - 7.2 Analyses
8. Organisation/coordination
   - 8.1 Coordinator and Steering Group
   - 8.2 Timetable
   - 8.3 Budget and funding
   - 8.4 Report and publications
9. Adoption of report

J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 77–83, 1999

Annex C

POLLUTION 2000+: after Barcelona

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INTRODUCTION

The preceding report of the Workshop addressed the request of the Commission, its Scientific Committee and the SWG on Environmental Concerns (SWGEC) to further develop the research proposal on cetaceans and pollutants, hereafter called Pollution 2000+. The starting point for the Workshop was established by the SWGEC, Scientific Committee and Commission as given in IWC (1998a; b) in which the measured variables - pollutants and biomarkers (indicators of exposure and/or effects), and the target species, had been identified and agreed upon.

PCBs were chosen as model compounds because of their overwhelming anthropogenic origin, very high concentrations in some cetacean populations, recognised effects upon wildlife and the substantial background information already available on patterns in variation, geographical distribution, tissue kinetics and mechanisms of action. By analysing PCBs it was recognised that from the same samples, for no extra cost, information will be obtained on a series of other organochlorines including DDT, DDE, DDD, dieldrin, endrin, eldrin, heptachlor epoxide, lindane, hexachlorobenzene, chlordanes and mirex.

The biomarkers and other indicators previously agreed upon by the SWGEC, Committee and Commission, were discussed and described in more detail in the reports referred to above. These biomarkers are essentially indicators of possible effects on reproduction, early development, the immune system and general health status related largely, but not exclusively, to PCB-exposure.

With respect to target species, it was agreed in Barcelona that of the four identified species (bottlenose dolphin, harbour porpoise, white whale and Amazon river dolphin), only two species will be examined in this proposal. The possibilities to obtain adequate matching samples (size, type covering a sufficient gradient of pollution), precluded the inclusion of the white whale and the Amazon river dolphin in Pollution 2000+. However, it was also noted that pollutant studies on these species (and indeed others) are important and may be included in future phases of this iterative project. Interested groups are encouraged to undertake such studies. The collection and at least archival of samples from these populations should be encouraged by IWC.

Last year, the Scientific Committee stressed, and this was again clearly stated in Barcelona, that the programme was intended to specifically address the main recommendation of the IWC Pollution Workshop. Researchers were encouraged to address the other recommendations of that Workshop and consider other species and sources of samples. The priorities of the research programme were not meant to imply that other approaches were untenable, but rather, that it was important for the IWC to focus its effort on particularly important questions that would have wide ranging benefits to studies of cause-effect relationships in cetaceans.

As clearly stated in Barcelona:

> 'It is important to stress that the development of the IWC programme should not be seen as suggesting that other research on pollutants and cetaceans is not important. The IWC programme should be seen as a 'core' programme to address some fundamental questions. Its value is immeasurably enhanced by cooperation with existing programmes and as a context for the development of new programmes. The IWC should cooperate with other bodies in a coordinating role.'

Focusing on PCBs and these two species increases the power of the experimental design (i.e. increased sample size within a species) to better determine if the proposed biomarkers will be useful in discriminating the populations at greatest risk to organochlorine effects. It is also intended to produce a model for studies of other contaminants in other species and areas, by bringing together biologists, (toxico) pathologists and others in a multidisciplinary collaborative programme.

It is also worthwhile reiterating that samples will be archived for further analyses outside the core programme following the guidelines listed in table 2 of the Barcelona Workshop report. The Workshop had also encouraged auxiliary projects to be taken up by national groups and other institutions. For example, the assessment of new or recently found compounds in cetaceans, such as organotins and polybrominated biphenyls, is, of course, of relevance to cetaceans.

DEVELOPING A BUDGET

In Barcelona it was noted that it

> 'was not possible for the Workshop to develop a precise budget, given the need to obtain further details on the field programme and the implications of this for analyses. It therefore agreed that this task should be completed by the Steering Group, based on the requirements given in the Workshop report and attached as an Annex in time for the Scientific Committee meeting in Grenada in May this year.'

In practice, this required sending out questionnaires to the identified field research contacts and the potential laboratories identified in the Barcelona Workshop report. Final replies to these questionnaires were not received until the start of the meeting. It has therefore not been possible to circulate and receive comments back from the full Steering Group identified in the Barcelona Workshop report - thus only the above-named authors are responsible for this document.

ESTIMATED COSTS

The questionnaire asked all potential institutes to provide estimates of the costs involved in their participation. An overview of the responses is given in Appendix 2. The Workshop had recommended that it should be the decision of the Steering Group to decide on the final institutions, with the primary consideration being given to quality assurance followed by cost. The total budget for all of the analyses identified in the Barcelona Workshop report is *ca* £1,300,000. Thus the prophecy from the Bergen Workshop and IWC (1998) has been fulfilled - this type of research programme is very expensive.

IWC (1998, p.427) had noted that the project would be a very large, co-operative programme - one that the Commission alone would be unable to fund. The level of support already expressed for this proposal is extremely encouraging. The programme as outlined in IWC (1998) was strongly endorsed by ASCOBANS at its Meeting of Parties. The recent Advisory Committee meeting of ASCOBANS also endorsed the Barcelona Workshop report on the basis of the summary prepared by Reijnders (the Committee's rules meant that the written report could not be submitted as a document to that meeting). IWC (1998) was also endorsed by ICES and used by them to develop a similar programme for pinnipeds.

Although it has not been possible to calculate the exact value of the 'in-kind' funding offered by the cooperating institutions, even a crude estimate reveals that over £200,000 is being offered and probably considerably more. Further potential funding sources include: the European Commission, the joint USA-EU programme; the Nordic Council of Ministers; and certain Fishermen's Associations. It is to be hoped that IWC member nations may also offer direct or indirect funding in addition to any core IWC funding. Similarly, one might hope that various non-governmental organisations might be prepared to contribute.

It was foreseen that one of the tasks of the coordinator of POLLUTION 2000+ would be to follow up on these and other sources of funding. This is an important part of the initial segment of the programme.

REFINING THE PROPOSAL

Based on the Barcelona Workshop report the following two short-term objectives were identified for POLLUTION 2000+:

(a) to select and examine a number of biomarkers of exposure to and/or effect of PCBs and try to determine whether a predictive and quantitative relationship with PCB levels in certain tissues exists;
(b) to validate/calibrate sampling and analytical techniques to address such questions for cetaceans, specifically:
    (i) determination of changes in concentrations of variables with post-mortem times;
    (ii) examination of relationships between concentrations of variables obtained from biopsy sampling with those of concentrations in other tissues that can only be obtained from fresh carcasses.

Given these objectives and the levels of resources and effort necessary to examine them, we propose that the work be divided into two phases; information from Phase 1 is important in providing the calibration/validation tools necessary to better focus and design Phase 2. The importance of calibration studies for the interpretation of pollutant-related data has been stressed both in Bergen and subsequently. Data from Phase 1 will provide information not only essential for completing Phase 2 of POLLUTION 2000+ but also of fundamental importance to many research programmes examining issues of chemical pollutants and cetaceans. Thus, Phase 1 concentrates largely on objective (b) above and comprises two sub-projects: (1) effect of post-mortem time; and (2) relationship between information obtained from biopsy samples with that obtained from live-captured animals or carcasses (either from bycaught or freshly stranded animals).

Phase 1 data are to be analysed before embarking on Phase 2. The need for a Workshop or Workshops to evaluate data from the project is clearly stated in the Barcelona Workshop report. This will inevitably result in a revised programme to be presented to the Committee and the Commission. However, it would be misleading if the likely funding levels for Phase 2 of the programme were not also presented to the Commission.

Effect of post-mortem time

Changes in levels of contaminants and indicators of exposure are known to occur after death due to the inevitable physiological changes and breakdown of tissue (e.g. see Barcelona Workshop report). It is essential that these changes are quantified to determine the effect of post-mortem time on levels in the various tissues if the implications of measured levels of these in animals whose time to death is uncertain are to be correctly interpreted with respect to concentrations in the living animal.

Information from biopsy samples

In order to look at potential effects of pollutants at the population level (the ultimate aim of the research programme; Aguilar *et al.*, 1998), it is necessary to try and develop techniques that will allow collection of data from large numbers of free-living animals. One such method is obtaining biopsy-samples. However, to interpret the results from such samples it is essential to know how those levels obtained via such techniques (skin/blubber) relate to other tissues which are in practice obtained via live-capture (blood) and necropsies (e.g. liver, kidney and muscle). In this way we will acquire more insight into how far it might be possible to relate some indicators of exposure found through biopsy techniques to indicators and associated pathological findings obtained via necropsies.

It is therefore extremely important that such 'calibration' studies are undertaken before embarking on Phase 2 involving bycaught or freshly stranded animals. It should be possible to address both questions via the same sampling regime. The post-mortem experiment could be carried out on a selected subset of the biopsy calibration experiment animals. The absence of a suitable source of fresh carcasses of bottlenose dolphins means that the calibration experiments will be carried out on harbour porpoises. The choice of sampling area or areas needs to be decided by the Steering Group.

It can be seen that Phase 1 includes the field research component as well as analyses of the bottlenose dolphin sub-project in the Sarasota Bay area, and the field research component of the bottlenose dolphin sub-project in Mauritania, Bahamas and the Mediterranean, but, that only the PCB analyses are being undertaken as part of Phase 1. The rationale for the latter is that (a) it takes advantage of existing field work; and (b) it will enable selection of a single 'unpolluted' area on which to focus the Phase 2 segment. The remaining indicator analyses from the samples collected in Phase 1 will be undertaken as part of Phase 2, depending upon the findings of Phase 1.

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International Whaling Commission. 1998a. Chairman's Report of the Forty-Ninth Annual Meeting. *Rep. int. Whal. Commn* 48:17-52.

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Appendix 1

BRIEF OUTLINE OF THE EXPERIMENTAL DESIGN OF THE CALIBRATION SUB-PROJECT

The importance of age and sex in interpreting tissue levels of contaminants is well known. Therefore any such study must consider at least the following categories if practical: immature males; adult males; immature females; adult females - pregnant; adult females - resting; adult females - lactating.

For the post-mortem sub-project, the calibration exercise must cover the likely range of time to death for bycaught animals under usual fishing operations. It is suggested that for each individual, samples are taken for the following times to death: 0, 3, 6, 12, 18 and 24hrs. Attention should be given to storing the carcass in a manner likely to most closely resemble the conditions of a bycaught animal left in the nets. It is important that animals that are known to have very recently (<15mins) died are used. The 0hr measurements are the equivalent of a biopsy sample. For Phase 1, five animals in each category should be

examined. Whether further analyses are required for Phase 2 will be determined after the results from Phase 1 become available.

A full autopsy must be undertaken after the last samples have been taken and where possible, the variables outlined in Table 1 must be measured.

Table 1

Variables to be measured.

Variable	Tissue
PCBs	Blubber
	Blood
	Milk
Porphyrines	Blood
	Liver
	Urine
Immune system	Whole blood (see Table 2)
Thyroid hormones	Serum
P450	Skin
	Liver
Luciferase	Blood
	Blubber
	Skin
Sex hormones	Blood
	Urine
Vitamin A	Blubber
	Blood
	Liver

Appendix 2

BUDGET FOR PHASE 1 OF POLLUTION 2000+

1. Administration

This is an extremely important item if the project is to succeed. £20,000 is required.

2. Post-mortem calibration study - harbour porpoises - five animals in each class

(1) Due to the ability to sample blood, it is assumed that analyses will only be carried out for 0, 3, 6 and 24hrs (i.e during necropsy);

(2) In order to obtain sufficient 0hr specimens for the various age-sex classes, it is necessary to sample more widely. For those animals sampled that are not freshly dead, tissue will be archived for analysis under Phase 2 of the programme. The number of sampled animals necessary to obtain sufficient animals in each class is difficult to determine, but for purposes of the budget it is assumed that about 120 animals will be sufficient. Sampling may occur in Iceland, Norway, Denmark, USA and Germany.

Samples	Variable	Tissue	Cost per sample (£)	Total (£)
180	PCBs	Blubber	105	18,900
120		Blood	105	12,600
180	Vitamin A	Blubber	85	15,300
180		Liver	85	15,300
180		Blood	85	10,200
120	Porphyrines	Blood	65	7,800
180		Liver	65	11,700
180		Urine	65	11,700
120	Immune system	Whole blood	256	30,762
180	Thyroid hormones	Serum	8	1,435
180	P450	Skin	0	0
180		Liver	66	11,932
120	Luciferase	Blood	216	25,888
180		Blubber	232	41,819
180		Skin	332	59,741
120	Sex hormones	Blood	10	1,200
Sub-total				276,278
Field costs				25,000
Sub-project cost				**301,278**

3. Bottlenose dolphin sub-project:

Sample size	Field costs (£)	Analysis	Tissue	Total (£)
Mauritania				
15	12,900	PCBs	Blubber	1,575
Bahamas				
15	5,000	PCBs	Blubber	1,575
Mediterranean				
15	10,600	PCBs	Blubber	1,575
Sub-total				33,225
Sarasota Bay				
30	6,000	PCBs	Blubber	3,150
30			Blood	3,150
10			Milk	1,050
30		Hormones	Blood	300
30		Enzyme induction	Skin	1,071
30		Porphyrines	Blood	1,950
30			Urine	1,950
30		Immune system	Whole blood	7,691
30		Thyroid hormones	Serum	239
30		Luciferase	Blood	6,472
30			Blubber	6,970
30			Skin	9,957
30		Vitamin A	Serum	2,550
30			Blubber	2,550
Sub-total				55,050
Sub-project cost				**88,275**

Papers

J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 83–116, 1999

Biological factors affecting variability of persistent pollutant levels in cetaceans[1]

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ABSTRACT

The main biological factors responsible for the variability of pollutant concentrations in cetaceans are reviewed. Diet is significant because many pollutants are concentrated through food webs. This explains most interspecific differences in pollutant levels and it may also contribute to variation among populations of the same species or even among different components of the same population when diet is subject to age-related or sex-related variations. The effect of body size is complex. Excretion rate and activity of detoxifying enzymes decrease as body weight increases, processes which would lead to higher pollutant concentrations in large animals. In contrast, a high metabolic rate, which is inversely correlated to body size, is associated with high pollutant concentrations. These opposing effects usually result in higher residue levels in smaller individuals. Body composition affects the contribution of each body compartment to the overall pollutant load. Therefore, the body load of lipophilic pollutants will strongly depend on the relative mass of blubber, a variable that shows a threefold variation among cetacean species or, in seasonal feeders, among individuals. Nutritive condition also affects the dynamics of lipophilic pollutants. Lipid mobilisation results in an increase in residue levels, but this variation is not as large as a purely concentrative model would suggest because of enhancement of detoxification processes following a rise in tissue pollutant concentrations. Disease affects pollutant levels in different ways: impoverishing nutritive condition; altering normal physiological functions; and depressing reproduction therefore reducing reproductive transfer in females. The combined result of these processes is usually an increase in pollutant levels in diseased individuals. The concentration of lipophilic pollutants normally increases with age in males because input exceeds the ability of the organism to excrete pollutants. Variable proportions of the pollutant load are transferred to offspring during gestation and lactation, for which reason tissue concentrations in females decrease or stabilise, thus producing lower residue levels than in males. However, because not all compounds are transferred at the same rate, their relative abundance varies with age and sex. Intensity of reproductive transfer is also associated with the reproductive traits of the species, particularly the length of lactation. With the exception of zinc, concentrations of heavy metals increase with age in both sexes but, by contrast with lipophilic pollutants, concentrations in females are similar or higher than in males. The significance of these factors of variation should be taken into account when designing sampling methodology, comparing sample groups, or evaluating toxicological impact.

KEYWORDS: POLLUTION; HEAVY METALS; ORGANOCHLORINES; REPRODUCTION; BIOACCUMULATION; BIOMAGNIFICATION; CETACEANS-GENERAL; BOTTLENOSE DOLPHINS; HARBOUR PORPOISE; SPOTTED DOLPHIN; MINKE WHALE; FIN WHALE; SPERM WHALE; RIGHT WHALE; BOWHEAD WHALE

INTRODUCTION

Exposure of a given organism to a given pollutant is commonly monitored through the concentration of the targeted pollutant in selected tissues of the organism. This is clearly easier and more straightforward than measuring direct intake through food or other sources

[1] A version of this paper was submitted to the IWC Scientific Committee as SC/M95/P6.

of exposure and has been used extensively to monitor population exposure and to identify components of the ecosystem that are susceptible to pollutants.

However, when a sufficiently large number of individuals belonging to the same population have been studied, a substantial variation in tissue residue levels has been observed. This suggests that components of the same population, although sharing the same ecosystem, are not identically exposed to xenobiotics and that their capacity to excrete these pollutants is also different. Proper knowledge of the patterns of variation of pollutant levels within populations is necessary in order to assess the impact of xenobiotics.

During the 1970s, the discovery that some cetacean species carried extremely high levels of pollutants in their tissues, particularly heavy metals and organochlorines, raised concerns about the effects of these compounds on their survival, particularly in association with other threats including direct exploitation, incidental mortality in fishing gear and destruction of habitat. This led to attempts to improve the monitoring of xenobiotic exposure. There is now a considerable body of literature on the tissue levels of isolated individuals or larger samples from cetacean populations. However, results are often difficult to compare and the extent of exposure is difficult to assess because of substantial variation in tissue levels among individuals of different sex, age, reproductive status or nutritive condition. Cetaceans are long-lived and their growth period is protracted. Many are seasonal feeders and their body compositions undergo drastic changes throughout the year, with reproduction involving considerable energy expenditure and transfer of organic constituents to offspring. These factors combine to create large individual variation in pollutant levels.

The present paper reviews information available on sources of individual variation of pollutant levels in cetaceans. This is relevant not only to the design of surveys in this field, but also to the reliable assessment of population exposure to a given compound.

MAIN FACTORS AFFECTING INDIVIDUAL VARIATION

Diet

Most persistent contaminants are incorporated into the body of mammals via food, and thus in pollution studies of cetaceans it has been accepted as a general axiom that diet determines the xenobiotic load of a species. While this may not be always true[2], in absolute terms, intake via food represents the bulk of pollutant intake.

The effect of diet is particularly significant because many persistent pollutants increase their concentrations through the food web, and therefore tissue concentrations in top predators are much higher than those in organisms feeding at low trophic levels. This increase in concentration of a substance in an organism compared to that in its food is commonly known as biomagnification and it depends on a variety of factors. In small animals with gills, equilibrium partitioning of chemicals between body lipids and the environment appears to be the main factor regulating pollutant accumulation. However, in air-breathing predators such as marine mammals, biomagnification is thought to occur because the mass of the pollutant is largely conserved along the food chain, while the food through which it is transferred is partly transformed into energy or excreted (Janssen *et al.*, 1993).

Biomagnification is usually defined as the ratio of concentrations between predator and prey. However, this is rather simplistic because other factors, such as the physical and chemical properties of the compound, the existence of other routes of exposure and/or the physiological and biochemical make-up of the animal also play a significant role in the

[2] Rawson *et al.* (1995) have suggested that inhalation is a significant source of HgSe in bottlenose dolphins.

process. This explains the increasing criticism that models based on simplistic assumptions of food chain structure have attracted in recent years (Janssen *et al.*, 1993; James and Kleinow, 1994).

The potential for biomagnification varies greatly among pollutants. It is generally accepted that it is high for most organochlorine compounds, particularly those with high molecular weight and abundant chlorine substitutions, e.g. DDT, many PCBs, chlordane, toxaphene (especially nonachlorobornanes) and polychlorinated terphenyls (PCTs). Among the PCBs, biomagnification potential varies with structure and it has been shown that congeners with vicinal unsubstituted positions (especially *meta* and *para*) are selectively metabolised by marine mammals (Boon *et al.*, 1992). Polybrominated biphenyls (PBBs) behave like PCBs. Dibenzodioxins and dibenzofurans are less lipophilic and easier to degrade, so their biomagnification potential appears lower (Ballschmiter *et al.*, 1989; Rappe and Buser, 1989). Heavy metals constitute a heterogeneous group. Mercury is usually accepted as being bioaccumulative, whereas cadmium is not; data on lead and zinc are inconclusive (Laws, 1981; Kay, 1985; Bowles, 1999). Taking into account the limited information available, it appears that the potential for biomagnification of radionuclides and polyaromatic hydrocarbons (PAHs) by marine mammals is low (Anderson *et al.*, 1990; Calmet *et al.*, 1992). In general, fish are considered to be better metabolisers of PAHs than molluscs, for which reason it is likely that biomagnification of these compounds will be lower in fish-eating cetaceans than in those feeding on cephalopods (Law and Whinnett, 1992).

However, direct evidence for biomagnification occurring in cetaceans is limited. Table 1 shows available information on tissue levels of some pollutants and concentrations in their food, together with the biomagnification factor. These data should be viewed with some caution because the comparison of the whole prey is usually made against a single tissue of the cetacean. Moreover, the prey analysed, although selected in every case to account for a representative sample of the cetacean diet, clearly will not contain the identical pollutant loads that a cetacean would encounter in diverse combination of prey species. However, the results from the different surveys and species are reasonably consistent and some general patterns may be found. The biomagnification factor of all organochlorine compounds and mercury appears extremely high (on several occasions exceeding over 100-fold), whereas elements such as chromium, nickel, copper, zinc, cadmium and lead approached unity (and indeed were quite often lower than one), suggesting that biomagnification does not occurr for these elements.

For those pollutants in which biomagnification is significant, diet is undoubtedly a key factor determining resultant tissue concentrations. Indeed, it is expected to explain most of the interspecific variation occurring in cetacean species inhabiting the same waters. However, irrespective of the overall biomagnification factors, some organisms may display a specificity for the accumulation of a given compound and this may lead to increased levels of such a compound in subsequent levels of the food chain. For example, nickel levels in baleen whales are comparatively higher than those in toothed whales because of the ability of krill to concentrate this metal.

It is important, however, to remember that diet may vary substantially at the intraspecific or even intrapopulation levels. In particular, variation in diet associated with age and sex, especially in sexually dimorphic species, has been reported for a number of species, including sperm whales (Clarke *et al.*, 1993), white whales (Seaman *et al.*, 1982), spotted dolphins (Bernard and Hohn, 1989) and harbour porpoises (Recchia and Read, 1989). This variation may be caused by the lower diving capacity of the smaller individuals and the resultant differences in prey size by younger individuals, or differential requirements in composition of diet during different growth or reproductive states. Such shifts in diet may,

Table 1

Biomagnification factors (available in the literature) of cetaceans in relation to their food. Factors are calculated from concentrations expressed on an extractable lipid basis in the case of organochlorines and on a fresh weight basis in the case of trace elements. m: muscle; l: liver; k: kidney; Magn: magnification factor; N: number.

Compound	Species	N	Tissue	N	Food	Magn.	Reference
PCB	*D. leucas*	-	blubber	-	fish	8.0	Muir *et al.* (1992)
	T. truncatus	1	blubber	2	fish	510.6	Morris *et al.* (1989)
	S. coeruleoalba	1	blubber	2	fish	21.6	Morris *et al.* (1989)
		1	blubber	-	squid + fish	11.0	Tanabe *et al.* (1981b)
	P. phocoena	4	blubber	2	fish	38.9	Morris *et al.* (1989)
tDDT	*D. leucas*	-	blubber	-	fish	10.0	Muir *et al.* (1992)
	T. truncatus	1	blubber	2	fish	569.3	Morris *et al.* (1989)
	S. coeruleoalba	1	blubber	2	fish	70.5	Morris *et al.* (1989)
		1	blubber	-	squid + fish	12.0	Tanabe *et al.* (1981b)
	P. phocoena	4	blubber	2	fish	19.8	Morris *et al.* (1989)
HCB	*D. leucas*	-	blubber	-	fish	3.0	Muir *et al.* (1992)
	T. truncatus	1	blubber	2	fish	89.9	Morris *et al.* (1989)
	S. coeruleoalba	1	blubber	2	fish	56.1	Morris *et al.* (1989)
		1	blubber	-	squid + fish	23.0	Tanabe *et al.* (1981b)
	P. phocoena	4	blubber	2	fish	27.4	Morris *et al.* (1989)
tHCH	*D. leucas*	-	blubber	-	fish	1.4	Muir *et al.* (1992)
	S. coeruleoalba	1	blubber	-	squid + fish	6.4	Tanabe *et al.* (1981b)
Dieldrin	*T. truncatus*	1	blubber	2	fish	1,723.9	Morris *et al.* (1989)
	S. coeruleoalba	1	blubber	2	fish	64.3	Morris *et al.* (1989)
	P. phocoena	4	blubber	2	fish	65.2	Morris *et al.* (1989)
Total-Hg	*M. monoceros*	-	liver	-	fish	163.0	Muir *et al.* (1992)
	S. coeruleoalba	6	m+l+k	5	squid	125.0	Itano *et al.* (1984b)
		6	m+l+k	10	fish	175.0	Itano *et al.* (1984b)
		1	muscle	2	fish	8.0	Morris *et al.* (1989)
	T. truncatus	1	muscle	2	fish	10.0	Morris *et al.* (1989)
		1	muscle	-	fish	24.0	Moreno *et al.* (1984)
	P. phocoena	2	muscle	2	fish	9.0	Morris *et al.* (1989)
Methyl-Hg	*S. coeruleoalba*	4	m+l+k	5	squid	57.0	Itano *et al.* (1984a)
		4	m+l+k	10	fish	69.0	Itano *et al.* (1984a)
Cd	*M. monoceros*	-	liver	-	fish	80.0	Muir *et al.* (1992)
	S. coeruleoalba	25	muscle	3	squid	0.0	Honda and Tatsukawa (1981)
		25	liver	3	squid	0.4	Honda and Tatsukawa (1981)
		25	muscle	2	fish	2.5	Honda and Tatsukawa (1981)
		25	liver	2	fish	263.0	Honda and Tatsukawa (1981)
Cu	*S. coeruleoalba*	25	muscle	3	squid	0.1	Honda and Tatsukawa (1981)
		25	liver	3	squid	0.2	Honda and Tatsukawa (1981)
		25	muscle	2	fish	0.9	Honda and Tatsukawa (1981)
		25	liver	2	fish	3.0	Honda and Tatsukawa (1981)
		1	muscle	2	fish	0.6	Morris *et al.* (1989)
	T. truncatus	1	muscle	2	fish	0.7	Morris *et al.* (1989)
	P. phocoena	1	muscle	2	fish	0.6	Morris *et al.* (1989)
Fe	*S. coeruleoalba*	25	muscle	3	squid	2.1	Honda and Tatsukawa (1981)
		25	liver	3	squid	2.9	Honda and Tatsukawa (1981)
		25	muscle	2	fish	15.0	Honda and Tatsukawa (1981)
		25	liver	2	fish	20.1	Honda and Tatsukawa (1981)
Mn	*S. coeruleoalba*	25	muscle	3	squid	0.6	Honda and Tatsukawa (1981)
		25	liver	3	squid	6.7	Honda and Tatsukawa (1981)
		25	muscle	2	fish	0.2	Honda and Tatsukawa (1981)
		25	liver	2	fish	2.1	Honda and Tatsukawa (1981)

continued

Table 1 continued

Compound	Species	N	Tissue	N	Food	Magn.	Reference
Ni	*S. coeruleoalba*	25	muscle	3	squid	0.6	Honda and Tatsukawa (1981)
		25	liver	3	squid	1.5	Honda and Tatsukawa (1981)
		25	muscle	2	fish	0.8	Honda and Tatsukawa (1981)
		25	liver	2	fish	2.0	Honda and Tatsukawa (1981)
		1	muscle	2	fish	1.0	Morris *et al.* (1989)
	T. truncatus	1	muscle	2	fish	1.0	Morris *et al.* (1989)
	P. phocoena	2	muscle	2	fish	0.7	Morris *et al.* (1989)
Pb	*M. monoceros*	-	liver	-	fish	0.1	Muir *et al.* (1992)
	S. coeruleoalba	25	muscle	3	squid	0.7	Honda and Tatsukawa (1981)
		25	liver	3	squid	2.0	Honda and Tatsukawa (1981)
		25	muscle	2	fish	0.4	Honda and Tatsukawa (1981)
		25	liver	2	fish	1.3	Honda and Tatsukawa (1981)
		1	muscle	2	fish	0.9	Morris *et al.* (1989)
	T. truncatus	1	muscle	2	fish	0.9	Morris *et al.* (1989)
	P. phocoena	2	muscle	2	fish	0.7	Morris *et al.* (1989)
Zn	*S. coeruleoalba*	25	muscle	3	squid	0.3	Honda and Tatsukawa (1981)
		25	liver	3	squid	1.2	Honda and Tatsukawa (1981)
		25	muscle	2	fish	1.2	Honda and Tatsukawa (1981)
		25	liver	2	fish	4.0	Honda and Tatsukawa (1981)
		1	muscle	2	fish	0.4	Morris *et al.* (1989)
	T. truncatus	1	muscle	2	fish	0.4	Morris *et al.* (1989)
	P. phocoena	2	muscle	2	fish	0.8	Morris *et al.* (1989)
Se	*S. coeruleoalba*	6	m+l+k	5	squid	9.0	Itano *et al.* (1984a)
		6	m+l+k	10	fish	7.0	Itano *et al.* (1984a)

on some occasions, involve substantial variation in the type of organism consumed or even in the trophic level exploited. For example, after being weaned, juvenile harbour porpoises base their diet on euphausiids while their mothers are feeding on euphausiid predators (Smith and Read, 1992). In addition, lactating spotted dolphins consume mainly fish, whereas pregnant females feed almost exclusively on squid in order to cope with different nutritional requirements at each reproductive stage (Bernard and Hohn, 1989). This may have consequences not only for the absolute amount of pollutants ingested, but also on their relative abundance. For example, cadmium tissue concentrations in species consuming squid are higher because this prey carries high levels of this metal (Szefer *et al.*, 1994).

These age- or sex-related variations in diet undoubtedly influence intrapopulation variation in pollutant levels, although the recognition of this effect may be complex unless shifts in diet are longstanding. For example, Tanabe *et al.* (1984) found that younger Southern Hemisphere minke whales carried higher concentrations of DDTs and PCBs in their tissues than mature ones. This contrasts with what would be expected according to typical age-related trends in males of other species, and this author associated this apparent anomaly with a shift in diet with age. Thus, immature minke whales remain at lower latitudes during the summer and feed not only on euphausiids but also on copepods and fish, whereas adult individuals migrate to higher latitudes and base their diet solely on (less polluted) euphausiids.

Finally, it should be taken into account that diet is also likely to affect the activity of enzymes responsible for detoxification. For example, the MFO system is a multi-enzymatic substrate-inducible complex and has been found to be more active in species with a wide dietary spectrum than in those that feed on a limited number of species. MFO induction is also dependent on the type of food consumed. The ability to detoxify foreign compounds is higher in herbivores than in carnivores because the former are more often exposed to natural

toxic chemicals than the latter. This link between an ability for detoxification and diet has been put forward to explain differences in adaptation to pollution in a number of avian and mammalian species (e.g. Walker, 1980; Focardi *et al.*, 1988; Fossi *et al.*, 1988) and is also likely to play a role in the dynamics of pollutants in cetaceans.

Body size

Body size plays a complex role in interspecific variation in the accumulation pattern of pollutants. On the one hand, the elimination rate of foreign compounds per unit body weight in mammals decreases as body weight increases (Parke, 1980). This is also true for the activity of detoxifying enzymes, particularly microsomal mono-oxygenase systems which contain cytochrome P450 forms (Walker, 1980). These two factors would, in principle, combine to favour the accumulation of higher pollutant levels in species of large size. On the other hand, however, there is an inverse relationship between metabolic rate and body size and, because metabolic rate is also usually correlated with an ability to accumulate pollutants in vertebrates (Moriarty, 1984), higher pollutant accumulation rates are in principle to be expected in smaller species.

Although these two effects are opposed, in most species the influence of the metabolic rate outstrips that of the other two factors combined. Thus, in models of pollutant accumulation, the concentration factor is largely dependent on the predator's daily rate of food consumption as a proportion of the predator's body weight (inversely correlated to body size) and, of course, on the mean concentration of pollutant within the prey (Moriarty, 1984). Therefore, smaller animals overall tend to carry larger body loads of pollutants relative to their body weight in spite of high enzymatic activity and elimination rates.

Variation in body size among cetaceans is dramatic. Some representatives of the families Delphinidae, Phocoenidae and Pontoporidae weigh, when adult, only about 30-40kg, while the larger Balaenopterids can reach a body mass exceeding 150 metric tonnes (Evans, 1987). This represents about a 4×10^3 increase, by far the largest variation range in any mammal taxon. This of course has consequences for observed interspecific variation in pollutant levels. For example, Henry and Best (1983) found in southern Africa that minke whales (*ca* 10 tonnes) carried about 50% higher DDT concentrations in their blubber than fin whales (*ca* 80 tonnes), despite both being krill-eating species. Moreover, dieldrin was detected in measurable quantities in minke whales but not in fin whales. In the North Atlantic, Borrell (1993b) found that blubber organochlorine concentrations in male sperm whales (*ca* 50 tonnes) were about 20% of those found in male long-finned pilot whales (*ca* 1.5 tonnes) from the same waters, again despite the fact that both species are teutophagous and therefore feed on similar food resources.

The effect of body mass is not usually taken into account when studying interspecific variation in pollutant levels, and much of the observed variation is usually attributed to dissimilarities in diet. This lack of information makes it difficult to predict the actual influence of body mass on pollutant residue concentrations. A simple model can be proposed if it is accepted that the body load of a given pollutant is directly proportional to the amount of that pollutant absorbed by the intestine. In turn, assuming a given concentration of pollutant in the food, the quantity of pollutant absorbed is directly proportional to the amount of food ingested. Efficiency of food assimilation in cetaceans has been suggested to be about 80% (Lockyer, 1981), but a similar figure is not available for most pollutants. Although the amount of biomass of food ingested will of course depend on a number of factors such as the availability and quality (mainly calorific content) of that food, it is directly proportional to the metabolic rate of the individual and therefore its body mass. Thus, the biomass of food ingested (I) relative to body mass (M) has been calculated (Innes *et al.*, 1986) as:

$$I = 0.42M^{0.67}$$

where I is expressed in kg per day and M in kg.

The mean tissue concentration of a given persistent pollutant in the body of a mammal can be calculated as:

Mean tissue concentration = pollutant body load ÷ body weight

Taking this into account, the relationship between tissue concentration of a given pollutant, the body load of this pollutant and the body mass of the species concerned, can be assumed to vary following the pattern in Fig. 1. This shows that once body size reaches over 10,000kg, mass is of little importance in determining tissue concentrations. In smaller species, however, the effect of body size on the relationship is dramatic. As a consequence of a rapid increase in metabolic rates at lower body mass, both body loads and, particularly, tissue concentrations, increase remarkably. Indeed, this effect is probably more significant in explaining variations in tissue concentrations of different species found in the same waters than small differences in diet or in other biological traits.

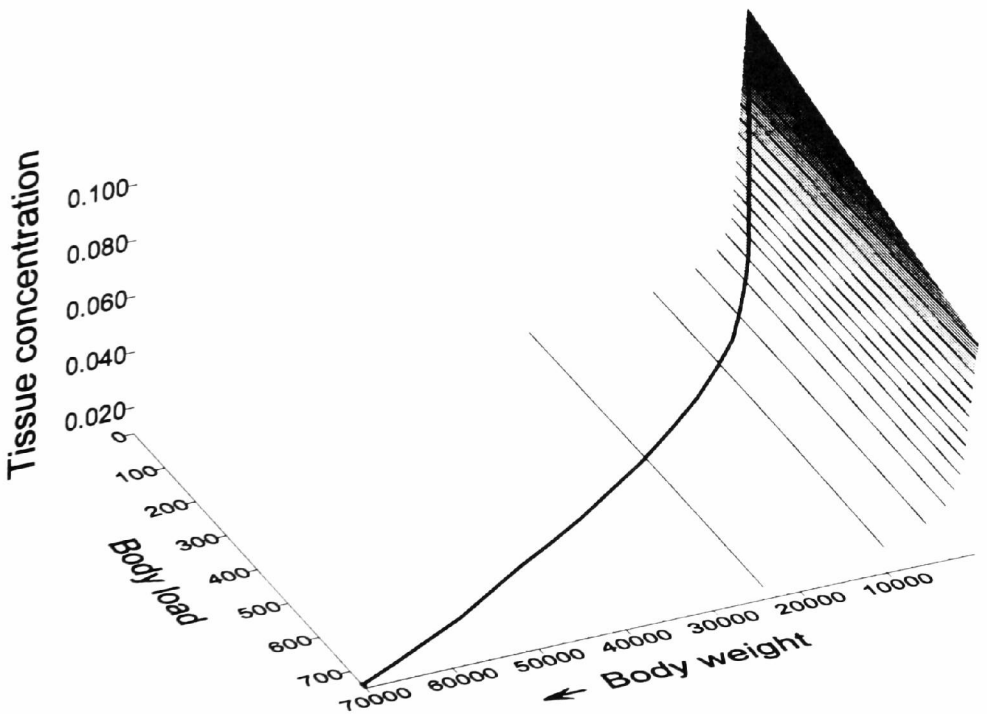

Fig. 1. Theoretical relationship between body mass, body load of a given pollutant, and tissue concentration of that pollutant in the body of a cetacean.

Body composition

The distribution pattern of pollutants in the body of an organism is complex, but largely depends upon the physical and chemical properties of the compounds involved. Some pollutants, such as organochlorines, organobromines and polyaromatic hydrocarbons, are

lipophilic and therefore accumulate in fat-rich tissues. This property means that an organism with a large fat compartment will have a large capacity for retaining these chemicals (Aguilar, 1985). Thus, about 70-95% of the total body load of lipophilic xenobiotics in cetaceans is located in the blubber (Table 2). Non-lipophilic pollutants depend on more complex rules of accumulation, although their distribution patterns still follow chemical affinities. Mercury, cadmium, zinc and other heavy metals accumulate mainly in the liver, muscle and kidneys (i. e. Honda *et al.*, 1982; André *et al.*, 1990a). However, others behave differently. For example, lead is mostly retained in bone because its biochemical behaviour is similar to that of calcium, and in man 90% of the lead present in the body is contained in bone. Furthermore, the biological half-life of lead in bone is about five years, while that in soft tissues is only 3-4 weeks (Fridberg, 1985). Radionuclides accumulate more readily in liver than in muscle (Calmet *et al.*, 1992).

Table 2

Contribution of main body compartments to the total load of pollutants present in the bodies of cetaceans. n.a.: not analysed.

Number	Compound	% Blubber	% Muscle	% Liver	% Kidney	% Bone	% Intestine	% Lung	Reference
S. coeruleoalba									
1	tDDT	95.10	4.30	0.20	0.20	n.a.	n.a.	n.a.	Tanabe *et al.* (1981a)
	PCB	95.00	4.60	0.10	0.10	n.a.	n.a.	n.a.	Tanabe *et al.* (1981a)
	BHC	90.00	8.40	0.40	0.30	n.a.	n.a.	n.a.	Tanabe *et al.* (1981a)
	tHCH	91.30	6.50	0.80	0.40	n.a.	n.a.	n.a.	Tanabe *et al.* (1981a)
S. coeruleoalba									
1	tDDT	93.50	4.60	0.23	0.73	n.a.	n.a.	n.a.	Fukushima and Kawai (1981)
	PCB	92.10	6.20	0.53	0.61	n.a.	n.a.	n.a.	Fukushima and Kawai (1981)
	BHC	92.00	5.60	0.67	0.45	n.a.	n.a.	n.a.	Fukushima and Kawai (1981)
S. coeruleoalba									
25	tDDT	96.94	1.81	1.21	0.05	n.a.	n.a.	n.a.	Borrell (1993b)
	PCB	95.58	2.91	1.54	0.09	n.a.	n.a.	n.a.	Borrell (1993b)
M. stejnegeri									
1	tDDT	98.40	1.40	0.08	0.01	n.a.	n.a.	n.a.	Miyazaki *et al.* (1987)
	PCB	97.80	2.00	0.20	0.02	n.a.	n.a.	n.a.	Miyazaki *et al.* (1987)
G. melas									
20	tDDT	99.50	0.40	0.09	0.04	n.a.	n.a.	n.a.	Borrell (1993b)
	PCB	99.00	0.85	0.12	0.03	n.a.	n.a.	n.a.	Borrell (1993b)
B. physalus									
26	tDDT	78.30	12.30	0.10	0.10	9.20	n.a.	n.a.	Aguilar and Borrell (1994a)
	PCB	76.50	13.90	0.10	0.10	9.40	n.a.	n.a.	Aguilar and Borrell (1994a)
S. coeruleoalba									
14	Hg	4.49	57.79	27.93	n.a.	n.a.	n.a.	n.a.	Itano *et al.* (1984a)
14	Se	14.91	37.28	25.35	n.a.	n.a.	n.a.	n.a.	Itano *et al.* (1984a)
11	Methyl-Hg	2.20	88.40	3.37	n.a.	n.a.	n.a.	n.a.	Itano *et al.* (1984a)
S. attenuata									
44	Hg	38.05	26.52	29.72	n.a.	n.a.	1.27	2.04	André *et al.* (1990a)
S. attenuata									
27	Cd	n.a.	18.62	23.63	2.63	n.a.	18.25	9.22	André *et al.* (1990b)

This heterogeneous affinity of pollutants for different parts of the body and the relative importance of these different parts in relation to body mass are significant factors determining the amount of pollutants retained by an organism. In cetaceans, the relative contribution of most tissues and organs to the composition of the body is relatively constant, the only significant difference being blubber. In general, large species tend to have less

blubber relative to body mass (Ryg *et al.*, 1993). For example, the percentage of blubber mass in relation to body mass in northern Atlantic waters may increase from 15-19% in large baleen whales (Lockyer, 1976; Lockyer and Waters, 1986) to 25% in medium-sized odontocetes such as pilot whales (Lockyer, 1993) and 45% in harbour porpoises (Slijper, 1958), the smallest of the odontocetes inhabiting the region.

Substantial variation may also be found both among different taxonomic groups and among individuals or species subject to different climates. In right and bowhead whales, both members of the family Balaenidae, blubber constitutes about 40-45% of body mass (Lockyer, 1976; George *et al.*, 1988), while in fin or sei whales of similar mass belonging to the Balaenopteridae, it only contributes about 15-19% (Lockyer, 1976; Lockyer and Waters, 1986). In beaked whales inhabiting cold waters (e.g. northern bottlenose whales), the contribution of blubber to body mass is 40-45% (Benjaminsen and Christensen, 1979), but it is as low as 20-22% in temperate water beaked whales such as Cuvier's or Blainville's (Ross, 1984).

The implications of such variation for the accumulation rates of lipophilic xenobiotics in cetaceans have not been investigated thus far, but it is likely that the fatter the individual, the higher its pollutant load, as has been observed in fish, birds and terrestrial mammals (Samiullah, 1990).

In addition, body composition affects the relative contribution of each body part to the overall pollutant body load. Table 2 details the available information on the percentage contribution of five main body parts to pollutant load in cetaceans. As mentioned above, blubber contributes to the bulk of organochlorine contaminant load, both because of its lipid richness and its large contribution to body mass. Although the lipid content of muscle is low, it is also an important reservoir of organochlorines because of its large contribution to body mass. In some cetaceans (mainly the baleen whales and large toothed whales) bone is very porous and contains abundant lipid reserves; in these cases, bone also contains a significant portion of the total organochlorine load. The contribution from liver, kidneys and other viscera is negligible. Although the data are limited, it appears clear that the contribution of blubber load of organochlorines to total body load is much higher in the small or medium-sized odontocetes (90-99%) than in the larger fin whale (76-78%), as expected from the blubber mass/body mass relationships mentioned above. Data on trace elements are unfortunately not available to allow comparison among species, although muscle appears to be the compartment containing the largest heavy metal loads.

Nutritive condition

As seen above, fat is one of the main constituents of the cetacean body. One of its major functions is to serve as an energy store, for which reason its contribution to body mass strongly depends on the condition of the individual. In species subject to strict seasonal migratory and feeding regimes (e.g. most baleen whales), body fat may vary dramatically through the year. It has been calculated that some baleen whales increase their body weight by 50-100% by the end of the feeding period, mainly because of fat accumulation (Lockyer and Brown, 1981). Indeed, the massive size of baleen whales has been associated with the need to accumulate substantial amounts of lipid reserves to cope with migratory and reproductive requirements during periods of low or no feeding (Brodie, 1975).

Variation in nutritive condition affects not only the volume of the fat compartment but also its composition. Thus, in baleen whales, blubber lipid richness may vary from over 88% in a fat, pregnant female, to as low as 34% in a resting, post lactating whale (Aguilar and Borrell, 1990). Similar, although less marked, variations may be observed in the lipid content of other tissues. Muscle of the posterior part of the trunk in pregnant whales accumulates

17-19% of lipid, whereas in lactating females it only averages 11.5% (Lockyer, 1987). Changes in the lipid content of bone, kidneys and other organs are also significant and have been described in a number of species.

Seasonal fluctuations in the nutritive condition of odontocetes do not appear to be as large as in mysticetes. Variation in blubber thickness is usually moderate and not strictly related to reproductive status, indicating that adequate food supply is generally available to provide the energetic requirements. Changes in lipid content and mass of internal organs are also moderate when compared to baleen whales (Gambell, 1972; Read, 1990; Lockyer, 1993).

It is obvious that variation in lipid richness has implications for the dynamics of lipophilic contaminants, although the actual effects on tissue concentrations induced by this variation are not so clear. When lipids are mobilised, two extreme processes are possible: either pollutants leave the tissues together with the lipids to which they are bound, or they remain in the tissue. In the first case, tissue concentrations will remain constant; in the second, they will increase proportionally to the amount of lipids mobilised. Studies suggest that, despite substantial variability between species or even within individuals, the actual process lies somewhere between the two extremes. In other words, lipid mobilisation results in an increase in the levels of residues, but the variation is not as high as a purely concentrative model would suggest. The reasons for this intermediate accumulative process are unclear, but it appears that partial mobilisation of the more polar fraction of the xenobiotic load and enhancement of metabolising and excretory capabilities when tissue pollutant concentrations rise, attenuates the increase produced by lipid mobilisation (Aguilar, 1987).

Calculation of tissue xenobiotic concentrations in relation to the lipid content of the tissue instead of its fresh weight partially account for such variations, but do not totally solve the problem. The relationship between PCB concentrations in the blubber (expressed on a lipid basis) and the lipid content of this tissue, in a sample of Mediterranean striped dolphins (Aguilar and Borrell, 1994a) shows that even taking into consideration blubber lipid richness, PCB concentrations are negatively correlated with fat content (Fig. 2). This indicates that some build-up of pollutants occurred in dolphins in poor nutritive condition. This increase is due to the fact that lipids are more readily mobilised from the blubber than lipophilic pollutants are and, therefore, the reduction in lipid mass is not coupled with a parallel reduction in xenobiotic mass (Aguilar, 1987). This effect has long been recognised in the dynamics of lipophilic contaminants in marine mammals (e.g. Addison and Smith, 1974; Drescher *et al.*, 1977), and has critically complicated surveys in which substantial variation of nutritive condition of individuals occurred (e.g. Hall *et al.*, 1992; Kuiken *et al.*, 1994).

It is unclear whether changes in nutritive condition may affect tissue concentrations of non-lipophilic pollutants. The most extensive mass changes when a cetacean grows thin or fat occur in the blubber, and therefore these changes mostly affect lipophilic pollutants. However, reduction of protein mass and liver enlargement in individuals in poor nutritive condition are also recognised in mammals (Spinage, 1985; Ortiz, 1987; Watkins *et al.*, 1991). Such changes are likely to influence the dynamics of accumulation of certain non-lipophilic pollutants that concentrate in these tissues, such as most heavy metals (see Table 2). Further research should be carried out to ascertain the effect of mass change in tissues other than blubber on the compartmentation and mobilisation of non-lipophilic pollutants.

Incidence of disease

Most pollutant surveys in cetaceans undertaken to date have been carried out on stranded specimens. Although in most cases the cause of death of these animals is unknown, except in areas where fishing interactions are frequent, disease is certainly the most likely source of mortality (except perhaps, in cases of mass strandings). There are several reasons why a

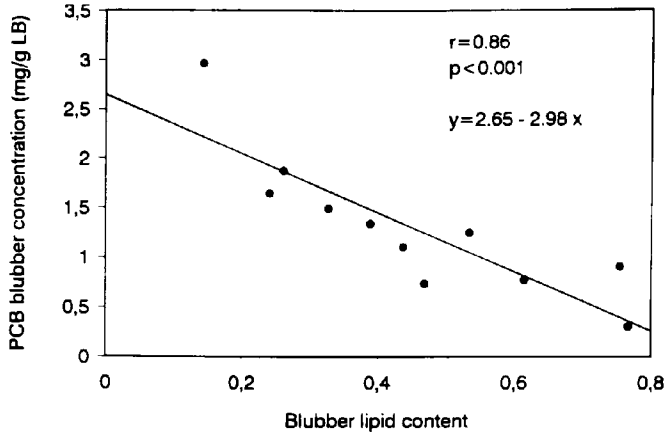

Fig. 2. Relationship between PCB concentration in the blubber of 15-19 year old male Mediterranean striped dolphins, expressed on a lipid basis, and the lipid content of the tissue.

diseased cetacean may be likely to carry abnormal pollutant loads in its body. For example, an animal that has been sick for a long period is likely to be undernourished or to have fed on food resources different from those that constitute its usual diet. In addition, many diseases affect metabolic centres and thus the capacity to metabolise or excrete pollutants may be affected. Therefore, it is questionable whether the pollutant concentrations in the tissues of these cetaceans are representative of normal conditions. The effects of a shift in diet or of fat mobilisation on tissue xenobiotic levels, particularly of those that are lipophilic, have been discussed above. In general terms it is to be expected that a rise in concentrations occur when the individual grows thin. However, the effects of physiological or metabolic alterations remain mostly unclear.

In females subject to severe long-term disease, it is likely that reproduction is altered or discontinued. In these situations, age-related accumulation trends will shift from the usual decreasing pattern in females to one of progressive accumulation, similar to that typical of males. For example, Martineau *et al.* (1987) found that DDT and PCB concentrations increased with age in stranded female white whales from the St Lawrence Estuary. An effect of this type probably explains the abnormally high levels of organochlorines observed in stranded female cetaceans, which in some cases even exceeded those of males (e.g. common dolphins in O'Shea *et al.*, 1980).

The results of studies performed on marine mammals affected by viral epizootics in recent years have repeatedly indicated increased concentrations of pollutants in individuals killed by the disease than in those that survived it (Kuehl *et al.*, 1991; 1994; Hall *et al.*, 1992; Aguilar and Borrell, 1994a). The existence of a cause-effect relationship between susceptibility to the disease and abnormally high pollutant levels has been the subject of substantial controversy and remains unclarified (Kennedy, 1999). Possible explanations include depressed immunocompetence caused by pollutants, mobilisation of pollutants stored in reserve tissues in individuals thinned by the disease, or alterations in physiological functions leading to increased concentrations (Aguilar and Borrell, 1994a).

Lipophilic xenobiotics, both because of the immunodepressive capacity of some of them and their association with lipid dynamics (and therefore with nutritive condition) have centred most discussions on the effect of disease on pollutant levels or vice versa. It is likely,

however, that many other pollutants may be affected when the normal physiological functions are altered. Further research is needed to clarify this subject, particularly if stranded cetaceans are to be used for monitoring population exposure.

Age and sex

Most cetaceans inhabit locations far from point sources of pollution and are therefore only affected by xenobiotics that are persistent, i.e. those that are not readily decomposed by the environment (once released). In many cases, particularly for highly lipophilic chemicals, persistence is associated with being accumulative, which means that the pollutant is retained by body tissues and its half-life in the organism is long.

By definition, for a pollutant to be accumulative, its input should in principle exceed the ability of the organism to excrete it. In other words, its intake rate should be initially greater than the combination of its metabolisation and excretion rates. In this situation, concentrations in tissues are expected to increase progressively with age throughout the life of the individual, the slope of the increase being proportional to the difference between the intake rate and the excretion plus metabolisation rates. This increase is further enhanced by the limited ability of foetuses and calves to biotransform xenobiotics. In humans, for example, cytochrome P450 activities are 20-50% of adult activities during the foetal stage (Sipes and Gandolfi, 1991). No similar calculations have been performed for cetaceans, but the activity of degradative enzymes in foetal minke whales (*Balaenoptera acutorostrata*) has been found to be extremely low (Goksøyr *et al.*, 1988). This handicapped detoxification ability is related to the biochemical differentiation of both the rough and smooth endoplasmic reticulum of hepatocytes, which is not complete during the early stages of life.

However, almost invariably, homeostatic responses elicit physiological mechanisms to counteract or destroy unwanted chemicals. When these mechanisms are successful, the initial increase in tissue concentrations levels off and the organism reaches an equilibrium in which enhanced degradation capabilities balance new pollutant intake. In this situation, the slope of the relationship between age and tissue pollutant concentrations tends to level off in older individuals.

Therefore, the pattern of variation of a given pollutant depends on its difficulty of excretion, its capacity to activate metabolisation processes and its resistance to these processes. While the physical and chemical properties of the different xenobiotics are very variable, the physiology of the detoxification processes is quite homogeneous among taxonomically related species. For this reason, age-related patterns observed for a given compound are similar among different cetacean species. Furthermore, transfer during gestation and lactation to offspring plays a key role in determining age-related trends in the tissue concentration of certain pollutants in females. Moreover, a marked difference between males and females in the toxicologic response to a number of xenobiotics has been noted. Capacity for detoxification in female mammals is usually lower than in males. Apparently, the balance between male and female sex hormones is important in determining the activity of cytochrome P450 and other enzymes responsible for pollutant degradation (Sipes and Gandolfi, 1991). Therefore, both sexes should be examined separately.

Fig. 3 shows a hypothetical age-relationship of tissue concentrations for organochlorine and other persistent lipophilic pollutants. This has been extracted from commonly observed patterns in different cetacean species available in the literature (Table 3). In males, concentrations tend to increase rapidly during the juvenile stage, but the slope slowly levels off in older individuals until a plateau is reached. This levelling-off is probably a combination of reduced daily feeding rate in adults and enhanced metabolisation and excretion rates when pollutant levels build-up. Table 3 details case-studies available in the literature in which age-related patterns were investigated (only surveys where $n > 20$).

Table 3

Age-related variation in pollutant levels observed in different cetacean species.
*: sample including males and females, n.s.: non significant trend.

Compound	Species	Tissue	Area	No.	Trend	Reference
MALES						
PCB	*B. physalus*	blubber	E.North Atlantic	69	increase	Aguilar and Borrell (1988)
	B. acutorostrata	blubber	Antarctic	20	increase	Tanabe *et al.* (1986)
	B. acutorostrata	blubber	Antarctic	59	increase	Tanabe *et al.* (1995)
	D. leucas	blubber	West Greenland	71	n.s.	Stern *et al.* (1994)
	G. melas	blubber	Faroe Islands.	30	n.s.	Borrell *et al.* (1995a)
	T. truncatus	blubber	E.South Africa	52	increase	Cockcroft *et al.* (1989)
	S. coeruleoalba	blubber	N.W. Mediterranean	38	increase	Borrell (1993b)
	P. phocoena	blubber	Bay of Fundy	61	increase	Gaskin *et al.* (1983)
	P. phocoena	blubber	Scandinavia	34	increase	Kleivane *et al.* (1995)
	P. dalli	blubber	N.W.North Pacific	40	increase	Subramanian *et al.* (1987)
	P. blainvillei	blubber	Northern Argentina	43	n.s.	Borrell *et al.* (1995)
tDDT	*B. physalus*	blubber	E.North Atlantic	69	increase	Aguilar and Borrell (1988)
	B. acutorostrata	blubber	Antarctic	20	increase	Tanabe *et al.* (1986)
	B. acutorostrata	blubber	Antarctic	59	increase	Tanabe *et al.* (1995)
	D. leucas	blubber	West Greenland	71	n.s.	Stern *et al.* (1994)
	G. melas	blubber	Faroe Islands.	30	n.s.	Borrell *et al.* (1995)
	T. truncatus	blubber	E.South Africa	52	increase	Cockcroft *et al.* (1989)
	S. coeruleoalba	blubber	N.W. Mediterranean	38	increase	Borrell (1993b)
	P. phocoena	blubber	Bay of Fundy	60	increase	Gaskin *et al.* (1982)
	P. phocoena	blubber	Scandinavia	34	increase	Kleivane *et al.* (1995)
	P. dalli	blubber	N.W.North Pacific	40	increase	Subramanian *et al.* (1987)
	P. blainvillei	blubber	Northern Argentina	43	increase	Borrell *et al.* (1995)
tHg	*B. acutorostrata*	liver	Antarctic	96	increase	Honda *et al.* (1987)
	P. macrocephalus	muscle	South Australia	313	decrease	Cannella and Kitchener (1992)
	M. monoceros	blubber	Baffin Island	49 *	increase	Wagemann *et al.* (1983)
		muscle	"	58 *	increase	"
		liver	"	38 *	increase	"
		kidney	"	55 *	increase	"
	M. monoceros	muscle	West Greenland	28	increase	Hansen *et al.* (1990)
		liver	"	26	increase	"
		kidney	"	28	increase	"
	P. phocoena	liver	Norway	56	increase	Teigen *et al.* (1993)
		kidney	"	56	n.s.	"
		muscle	Bay of Fundy	22	increase	Gaskin *et al.* (1972)
		muscle	Bay of Fundy	68	increase	Gaskin *et al.* (1979)
		liver	"	44	increase	"
		kidney	"	26	increase	"
Cd	*B. physalus*	liver	E.North Atlantic	35	increase	Sanpera *et al.* (1995)
		kidney	"	36	n.s.	"
	B. acutorostrata	liver	Antarctic	96	inc-dec	Honda *et al.* (1987)
	M. monoceros	blubber	Baffin Island	45 *	decrease	Wagemann *et al.* (1983)
		muscle	"	58 *	increase	"
		liver	"	38 *	decrease	"
		kidney	"	55 *	increase	"
	M. monoceros	muscle	West Greenland	25	n.s.	Hansen *et al.* (1990)
		liver	"	27	increase	"
		kidney	"	28	n.s.	"

continued

Table 3 continued

Compound	Species	Tissue	Area	No.	Trend	Reference
Co	*B. acutorostrata*	liver	Antarctic	96	n.s.	Honda *et al.* (1987)
Cu	*B. physalus*	muscle	E.North Atlantic	29	n.s.	Sanpera *et al.* (1995)
		liver	"	35	n.s.	"
		kidney	"	35	decrease	"
	B. acutorostrata	liver	Antarctic	96	n.s.	Honda *et al.* (1987)
	M. monoceros	blubber	Baffin Island	45 *	n.s.	Wagemann *et al.* (1983)
		muscle	"	58 *	n.s.	"
		liver	"	38 *	n.s.	"
		kidney	"	55 *	n.s.	"
Ni	*B. acutorostrata*	liver	Antarctic	96	n.s.	Honda *et al.* (1987)
Pb	*B. acutorostrata*	liver	Antarctic	96	n.s.	Honda *et al.* (1987)
	M. monoceros	blubber	Baffin Island	45 *	n.s.	Wagemann *et al.* (1983)
		muscle	"	58 *	n.s.	"
		liver	"	38 *	decrease	"
		kidney	"	55 *	n.s.	"
Zn	*B. physalus*	muscle	E.North Atlantic	33	n.s.	Sanpera *et al.* (1995)
		liver	"	35	n.s.	"
		kidney	"	32	increase	"
	B. acutorostrata	liver	Antarctic	96	n.s.	Honda *et al.* (1987)
	M. monoceros	blubber	Baffin Island	45 *	decrease	Wagemann *et al.* (1983)
		muscle	"	58 *	n.s.	"
		liver	"	38 *	n.s.	"
		kidney	"	55 *	n.s.	"
	M. monoceros	kidney	West Greenland	27	n.s	Hansen *et al.* (1990)
Se	*M. monoceros*	blubber	Baffin Island	47 *	n.s.	Wagemann *et al.*(1983)
		muscle	"	58 *	n.s.	"
		liver	"	38 *	decrease	"
		kidney	"	55 *	n.s.	"
	M. monoceros	kidney	West Greenland	27	increase	Hansen *et al.* (1990)
	P. phocoena	liver	Norway	56	increase	Teigen *et al.* (1993)
		kidney	"	56	n.s.	"
FEMALES						
PCB	*B. physalus*	blubber	E.North Atlantic	97	decrease	Aguilar and Borrell (1988)
	D. leucas	blubber	West Greenland	67	decrease	Stern *et al.* (1994)
	G. melas	blubber	Faroe Islands	69	decrease	Borrell *et al.* (1995)
	G. macrorhynchus	blubber	Japan	24	n.s.	Tanabe *et al.* (1987)
	T. truncatus	blubber	E.South Africa	52	decrease	Cockcroft *et al.* (1989)
	S. coeruleoalba	blubber	N.W. Mediterranean	33	decrease	Borrell (1993b)
	S. coeruleoalba	blubber	Japan	40	n.s.	Fukushima and Kawai (1981)
	P. phocoena	blubber	Bay of Fundy	39	n.s.	Gaskin *et al.* (1983)
	P. dalli	blubber	N.W.North Pacific	26	n.s.	Subramanian *et al.* (1987)
	P. blainvillei	blubber	North Argentina	31	n.s.	Borrell *et al.* (1995)
tDDT	*B. physalus*	blubber	E.North Atlantic	97	decrease	Aguilar and Borrell (1988)
	D. leucas	blubber	West Greenland	67	decrease	Stern *et al.* (1994)
	G. melas	blubber	Faroe Islands	69	decrease	Borrell *et al.* (1995)
	G. macrorhynchus	blubber	Japan	24	n.s.	Tanabe *et al.* (1987)
	T. truncatus	blubber	E.South Africa	52	decrease	Cockcroft *et al.* (1989)
	S. coeruleoalba	blubber	N.W. Mediterranean	33	decrease	Borrell (1993b)
	P. phocoena	blubber	Bay of Fundy	55	decrease	Gaskin *et al.* (1982)
	P. dalli	blubber	N.W.North Pacific	26	n.s.	Subramanian *et al.* (1987)

continued

Table 3 continued

Compound	Species	Tissue	Area	No.	Trend	Reference
tHg	*B. acutorostrata*	liver	Antarctic	39	inc-dec	Honda *et al.* (1987)
	P. macrocephalus	muscle	South Australia	100	n.s.	Cannella and Kitchener (1992)
	M. monoceros	blubber	Baffin Island	49 *	increase	Wagemann *et al.* (1983)
		muscle	"	58 *	increase	"
		liver	"	38 *	decrease	"
		kidney	"	55 *	increase	"
	M. monoceros	muscle	West Greenland	31	increase	Hansen *et al.* (1990)
		liver	"	30	increase	"
		kidney	"	32	increase	"
	D. leucas		Canadian Arctic			Wagemann *et al.* (1990)
	P. phocoena	liver	Norway	36	increase	Teigen *et al.* (1993)
		kidney	"	36	n.s.	"
		muscle	Bay of Fundy	45	increase	Gaskin *et al.* (1979)
		liver	"	24	increase	"
Cd	*B. physalus*	liver	E.North Atlantic	35	increase	Sanpera *et al.* (1995)
		kidney	"	35	increase	"
	B. acutorostrata	liver	Antarctic	39	increase	Honda *et al.* (1987)
	M. monoceros	blubber	Baffin Island	45 *	decrease	Wagemann *et al.* (1983)
		muscle	"	58 *	increase	"
		liver	"	38 *	increase	"
		kidney	"	55 *	increase	"
	M. monoceros	muscle	West Greenland	27	n.s.	Hansen *et al.* (1990)
		liver	"	31	n.s.	"
		kidney	"	32	increase	"
Co	*B. acutorostrata*	liver	Antarctic	39	n.s.	Honda *et al.* (1987)
Cu	*B. physalus*	muscle	E.North Atlantic	37	n.s.	Sanpera *et al.* (1995)
		liver	"	37	decrease	"
		kidney	"	36	decrease	"
	B. acutorostrata	liver	Antarctic	39	n.s.	Honda *et al.* (1987)
	M. monoceros	blubber	Baffin Island	45 *	n.s.	Wagemann *et al.* (1983)
		muscle	"	58 *	n.s.	"
		liver	"	38 *	decrease	"
		kidney	"	55 *	n.s.	"
Fe	*B. acutorostrata*	liver	Antarctic	39	increase	Honda *et al.* (1987)
Ni	*B. acutorostrata*	liver	Antarctic	39	n.s.	Honda *et al.* (1987)
Pb	*B. acutorostrata*	liver	Antarctic	39	n.s.	Honda *et al.* (1987)
	M. monoceros	blubber	Baffin Island	45 *	n.s.	Wagemann *et al.* (1983)
		muscle	"	58 *	n.s.	"
		liver	"	38 *	increase	"
		kidney	"	55 *	n.s.	"
Zn	*B. physalus*	muscle	E.North Atlantic	38	decrease	Sanpera *et al.* (1995)
		liver	"	37	n.s.	"
		kidney	"	35	increase	"
	B. acutorostrata	liver	Antarctic	39	n.s.	Honda *et al.* (1987)
	M. monoceros	blubber	Baffin Island	45 *	decrease	Wagemann *et al.* (1983)
		muscle	"	58 *	n.s.	"
		liver	"	38 *	n.s.	"
		kidney	"	55 *	increase	"
	M. monoceros	kidney	"	31	increase	Hansen *et al.* (1990)

continued

Table 3 continued

Compound	Species	Tissue	Area	No.	Trend	Reference
Se	*M. monoceros*	blubber	Baffin Island	47 *	n.s.	Wagemann *et al.* (1983)
		muscle	"	58 *	n.s.	"
		liver	"	38 *	increase	"
		kidney	"	55 *	decrease	"
	M. monoceros	kidney	West Greenland	28	increase	Hansen *et al.* (1990)
	P. phocoena	liver	Norway	36	increase	Teigen *et al.* (1993)
		kidney	"	36	n.s.	"
MALES AND FEMALES TOGETHER						
tHg	*D. leucas*	muscle	West Greenland	24	n.s.	Hansen *et al.* (1990)
		liver	"	23	increase	
		kidney	"	20	increase	"
	D. leucas	muscle	Canadian Arctic and	107	n.s.	Wagemann *et al.* (1990)
		liver	St Lawrence estuary	139	increase	"
		kidney	"	137	increase	"
	G. melas	liver	Faroe Islands	92	increase	Caurant *et al.* (1994)
		kidney	Faroe Islands	54	increase	"
	S. coeruleoalba	blubber	Japan	36	increase	Itano and Kawai (1981)
		muscle	"	38	increase	"
		liver	"	34	increase	"
	S. coeruleoalba	muscle	Japan	51	increase	Honda *et al.* (1983)
		liver	"	45	increase	
		kidney	"	20	increase	"
	S. coeruleoalba	liver	W. Mediterranean	27	increase	André *et al.* (1991)
	S. attenuata	muscle	E. tropical Pacific	31	increase	André *et al.* (1990a)
		liver	"	33	increase	"
		kidney	"	31	n.s.	"
	P. phocoena	muscle	W. Greenland	78	increase	Paludan-Muller *et al.* (1993)
		liver	"	78	increase	"
		kidney	"	78	increase	"
		skin	"	78	increase	"
Methyl-Hg	*S. coeruleoalba*	muscle	Japan	31	n.s.	Itano and Kawai (1981)
As	*G. melas*	liver	Faroe Islands	92	n.s.	Caurant *et al.* (1994)
		kidney	Faroe Islands	97	n.s.	"
Cd	*B. physalus*	liver	Iceland	39	n.s.	Sanpera *et al.* (1995)
		kidney	"	49	n.s.	"
	B. acutorostrata	liver	Antarctic	135	increase	Honda *et al.* (1987)
	D. leucas	muscle	West Greenland	24	n.s.	Hansen *et al.* (1990)
		liver	"	23	increase	"
	D. leucas	muscle	Canadian Arctic and	108	n.s.	Wagemann *et al.* (1990)
		liver	St Lawrence estuary	139	increase	"
		kidney	"	137	increase	"
	G. melas	liver	Faroe Islands	120	increase	Caurant *et al.* (1994)
		kidney	Faroe Islands	97	increase	"
	S. coeruleoalba	muscle	Japan	59	increase	Honda *et al.* (1983)
		liver	"	57	increase	"
		kidney	"	30	n.s.	"
	S. attenuata	muscle	E. tropical Pacific	21	increase	André *et al.* (1990b)
	P. phocoena	muscle	W. Greenland	78	increase	Paludan-Muller *et al.* (1993)
		liver	"	78	increase	"
		kidney	"	78	increase	"
		skin	"	78	increase	"

continued

Table 3 continued

Compound	Species	Tissue	Area	No.	Trend	Reference
Co	*B. acutorostrata*	liver	Antarctic	135	n.s.	Honda *et al.* (1987)
Cu	*B. physalus*	muscle	Iceland	36	decrease	Sanpera *et al.* (1995)
		liver	"	38	n.s.	"
		kidney	"	37	decrease	"
	B. acutorostrata	liver	Antarctic	135	n.s.	Honda *et al.* (1987)
	D. leucas	muscle	Canadian Arctic and	107	decrease	Wagemann *et al.* (1990)
		liver	St Lawrence estuary	139	decrease	"
		kidney	"	137	decrease	"
	G. melas	liver	Faroe Islands	122	increase	Caurant *et al.* (1994)
		kidney	"	97	increase	"
	S. coeruleoalba	muscle	Japan	59	n.s.	Honda *et al.* (1983)
		liver	"	57	decrease	"
		kidney	"	30	decrease	"
Fe	*B. acutorostrata*	liver	Antarctic	135	increase	Honda *et al.* (1987)
	S. coeruleoalba	muscle	Japan	59	increase	Honda *et al.* (1983)
		liver	"	57	n.s.	"
		kidney	"	30	n.s.	"
Ni	*B. acutorostrata*	liver	Antarctic	135	n.s.	Honda *et al.* (1987)
	S. coeruleoalba	muscle	Japan	59	increase	Honda *et al.* (1983)
		liver	"	57	increase	"
		kidney	"	30	n.s.	"
Pb	*B. acutorostrata*	liver	Antarctic	135	n.s.	Honda *et al.* (1987)
	S. coeruleoalba	muscle	Japan	59	increase	Honda *et al.* (1983)
		liver	"	57	increase	"
		kidney	"	30	n.s.	"
Zn	*B. physalus*	muscle	Iceland	33	n.s.	Sanpera *et al.* (1995)
		liver	"	38	decrease	"
		kidney	"	33	n.s.	"
	B. acutorostrata	liver	Antarctic	135	n.s.	Honda *et al.* (1987)
	D. leucas	muscle	West Greenland	24	n.s.	Hansen *et al.* (1990)
		liver	"	23	n.s.	"
		kidney	"	20	n.s.	"
	D .leucas	muscle	Canadian Arctic and	108	n.s.	Wagemann *et al.* (1990)
		liver	St Lawrence estuary	139	n.s.	"
		kidney	"	137	n.s.	"
	G. melas	liver	Faroe Islands	122	n.s.	Caurant *et al.* (1994)
		kidney	"	97	increase	"
	S. coeruleoalba	muscle	Japan	59	decrease	Honda *et al.* (1983)
		liver	"	57	decrease	"
		kidney	"	30	n.s.	"
Se	*D. leucas*	muscle	West Greenland	24	n.s.	Hansen *et al.* (1990)
		liver	"	23	increase	"
		kidney	"	20	n.s.	"
	D. leucas	muscle	Canadian Arctic and	105	n.s.	Wagemann *et al.* (1990)
		liver	St Lawrence estuary	111	n.s.	"
		kidney	"	115	n.s.	"
	G. melas	liver	Faroe Islands	94	increase	Caurant *et al.* (1994)
		kidney	"	54	increase	"
	S. coeruleoalba	blubber	Japan	36	n.s.	Itano and Kawai (1981)
		muscle	"	38	increase	"
		liver	"	34	increase	"

In the great majority of cases a positive correlation was found between age and pollutant concentrations and, when this was not the case, trends were unclear but never indicated a decrease in concentrations.

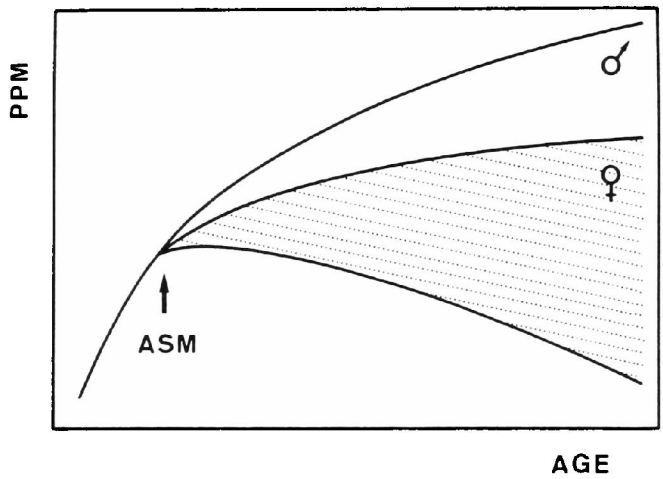

Fig. 3. Typical age-related variation of concentration of organochlorine and other persistent lipophilic pollutants in the tissues of cetaceans.

In females, observed patterns were quite different. Lipophilic chemicals easily traverse placental membranes and are therefore transferred to the foetus. This passage is easier for chemicals of low molecular weight, which in organochlorines is associated with low chlorination of the molecule, than for those with high weight, usually associated with a high number of chlorine substitutions (Juchau, 1983). Moreover, lipophilic compounds are also readily transferred to milk (Ridgway and Reddy, 1995). Again, there are some differences depending on the physical and chemical properties of the compound; the highly chlorinated organochlorines are transferred less efficiently from the body lipid deposits to the circulatory system, and from there to milk, than those lowly chlorinated (Aguilar and Borrell, 1994c).

The discharge occurring during reproduction produces a change in the age-related pattern in females since the onset of reproduction. The initial increase during the juvenile stage slows down and concentrations either increase at a lower rate than in males, stabilise, or decrease (Fig. 3). Logically, the magnitude of this change depends on the intensity of the reproductive transfer and this will, in turn, depend on the physical and chemical properties of the compound and the biological traits of the species involved.

Table 4 details available information on the percentage of organochlorine body load of the female transferred to the offspring during a single pregnancy or lactation. As can be seen, transfer is much larger during lactation (range: 7-98% depending on species and compound) than during pregnancy (range: 0.5-9.4%). This is explained by the large amount of lipids transferred during lactation to the calf, which is much larger than that deposited in the foetus. The total amount of organochlorines transferred during a complete reproductive cycle is estimated to range from 7-100%, depending on species and compound.

However, irrespective of the intensity of this transfer, this discharge will produce lower concentrations of lipophilic pollutants in the tissues of adult females than in those of adult males. Table 5 details differences observed between males and females (only surveys with $n > 25$ were considered). In the totality of the cases, males presented higher concentrations

Table 4

Reproductive transfer of pollutants, calculated as percent transfer in relation to maternal body load, for different pollutants and cetacean species. n: number of individuals used to calculate the pollutant transfer; M: males; F: females; F(l): lactating females; F(p) pregnant females.

Species	n						Transfer			Reference
	F	M	F(l)	milk	F(p)	foetus	Gest.	Lact.	Total	
tDDT										
B. physalus	82	59	-	-	-	-	-	-	11.8	Aguilar and Borrell (1994b)
G. melas	-	-	21	21	11	11	7.8	80.0	87.8	Borrell *et al.* (1995)
T. truncatus	-	-	-	-	-	-	-	-	80.0	Cockcroft *et al.* (1989)
S. coeruleoalba	-	6	7	-	1	1	4.2	91.0	95.2	Fukushima and Kawai (1981)
S. coeruleoalba	1	-	-	1	1	1	4.7	94.6	99.3	Tanabe *et al.* (1981a,1982)
PCB										
B. physalus	82	59	-	-	-	-	-	-	5.3	Aguilar and Borrell (1994)
G. melas	-	-	21	21	11	11	6.1	95.5	101.6	Borrell *et al.* (1995a)
T. truncatus	-	-	-	-	-	-	-	-	80.0	Cockcroft *et al.* (1989)
S. coeruleoalba	-	6	7	-	1	1	3.8	88.0	91.8	Fukushima and Kawai (1981)
S. coeruleoalba	1	-	-	1	1	1	4.0	91.1	95.1	Tanabe *et al.* (1981a,1982)
HCB										
S. coeruleoalba	-	6	7	-	1	1	6.3	72.0	78.3	Fukushima and Kawai (1981)
S. coeruleoalba	1	-	-	1	1	1	9.4	98.0	107.4	Tanabe *et al.* (1981a,1982)
tHCH										
S. coeruleoalba	1	-	-	1	1	1	8.9	93.5	102.4	Tanabe *et al.* (1981,1982)
tOCs										
P. phocoena	-	-	-	-	-	1	15.0	-	-	Duinker and Hillebrand (1979)
Hg										
S. coeruleoalba	-	-	-	-	1	1	0.4	-	-	Itano *et al.* (1984b)
Se										
S. coeruleoalba	-	-	-	-	1	1	0.9	-	-	Itano *et al.* (1984b)
Methyl-Hg										
S. coeruleoalba	-	-	-	-	1	1	1.0	-	-	Itano *et al.* (1984b)

than females. The difference between sexes ranged from about a two-fold variation (Baird's beaked whales from Japan - Subramanian *et al.*, 1988; minke whales from the Antarctic - Tanabe *et al.*, 1986), to over a six-fold variation (bottlenose dolphins from South Africa - Cockcroft *et al.*, 1989; white whales from the St Lawrence - Martineau *et al.*, 1987).

The above mechanisms of accumulation, degradation or excretion obviously affect the various organochlorine compounds in a different manner depending on their chemical structure and physico-chemical properties. As a consequence, their relative abundance in tissues will not only depend on that in the environment, but also on the age, sex and reproductive history of the individual involved. Thus, in marine mammals the ratios tDDT/PCB and DDE/tDDT have usually been found to increase in males and to decrease in females. This obviously results in both ratios being typically higher in adult males than in adult females (Subramanian *et al.*, 1987; Aguilar and Borrell, 1988; Borrell, 1993a; Stern *et al.*, 1994; Borrell *et al.*, 1995; 1996).

In trace elements, age-related variation patterns are not so homogeneous and predictable (Fig. 4). Cadmium and mercury concentrations are low at birth and increase progressively with age in both sexes. Levels of selenium are highly correlated with those of mercury (Koeman *et al.*, 1973) and, therefore, also increase with age. However, for all three elements, the slope of the trend is frequently steeper in females, for which reason adult females often carry higher tissue levels of these three compounds than males (Table 5).

Table 5

Male-female difference in pollutant concentrations determined for various pollutants and cetacean species. n.s.: non significant trend.

Compound/Species	Tissue	Area	No.	Difference	Reference
PCB					
B. physalus	blubber	E.North Atlantic	101	higher in males	Aguilar and Borrell (1988)
B. borealis	blubber	Iceland	40	higher in males	Borrell (1993a)
	blubber	Canadian Arctic	75	higher in males	Muir *et al.* (1990)
	blubber	West Greenland	89	higher in males	Stern *et al.* (1994)
G. melas	blubber	Faroe Islands	99	higher in males	Borrell *et al.* (1995)
T. truncatus	blubber	E.South Africa	31	higher in males	Cockcroft *et al.* (1989)
	blubber	Gulf of Mexico	26	higher in males	Kuehl and Habler (1995)
S. coeruleoalba	blubber	N.W. Mediterranean	58	higher in males	Borrell (1993b)
P. phocoena	blubber	Bay of Fundy	40	higher in males	Gaskin *et al.* (1983)
	blubber	British waters	28	higher in males	Kuiken *et al.* (1994)
	blubber	Denmark	37	higher in males	Clausen and Andersen (1988)
P. dalli	blubber	N.W.North Pacific	27	higher in males	Subramanian *et al.* (1987)
tDDT					
B. physalus	blubber	E.North Atlantic	101	higher in males	Aguilar and Borrell (1988)
B. borealis	blubber	Iceland	40	higher in males	Borrell (1993a)
D. leucas	blubber	Canadian Arctic	75	higher in males	Muir *et al.* (1990)
	blubber	West Greenland	89	higher in males	Stern *et al.* (1994)
G. melas	blubber	Faroe Islands	99	higher in males	Borrell *et al.* (1995)
T. truncatus	blubber	E.South Africa	29	higher in males	Cockcroft *et al.* (1989)
	blubber	Gulf of Mexico	26	higher in males	Kuehl and Habler (1995)
S. coeruleoalba	blubber	N.W. Mediterranean	58	higher in males	Borrell (1993b)
P. phocoena	blubber	Bay of Fundy	47	higher in males	Gaskin *et al.* (1982)
	blubber	Denmark	37	higher in males	Clausen and Andersen (1988)
P. dalli	blubber	N.W.North Pacific	27	higher in males	Subramanian *et al.* (1987)
Dieldrin					
D. leucas	blubber	Canadian Arctic	75	higher in males	Muir *et al.* (1990)
T. truncatus	blubber	Gulf of Mexico	26	higher in males	Kuehl and Habler (1995)
HCB					
D. leucas	blubber	Canadian Arctic	75	higher in males	Muir *et al.* (1990)
P. phocoena	blubber	British waters	28	higher in males	Kuiken *et al.* (1994)
T. truncatus	blubber	Gulf of Mexico	26	n.s.	Kuehl and Habler (1995)
alfa HCH					
D. leucas	blubber	Canadian Arctic	75	n.s.	Muir *et al.* (1990)
P. phocoena	blubber	British waters	28	higher in males	Kuiken *et al.* (1994)
Methyl-Hg					
B. physalus	muscle	N-E Spain	30	n.s.	Sanpera *et al.* (1993)
tHg					
B. physalus	muscle	N-E Spain	30	n.s.	Sanpera *et al.* (1993)
	liver	"	30	n.s.	"
P. macrocephalus	muscle	South Australia	414	higher in females	Cannella and Kitchener (1992)
M. monoceros	muscle	West Greenland	59	higher in females	Hansen *et al.* (1990)
	liver	"	56	higher in females	"
	kidney	"	60	higher in females	"
G. melas	liver	Faroe Islands	92	higher in females	Caurant *et al.* (1994)
	kidney	"	54	n.s.	"
T. truncatus	liver	Gulf of Mexico	27	higher in males	Kuehl and Habler (1995)
S. coeruleoalba	muscle	Japan	51	n.s.	Honda *et al.* (1983)
	liver	"	45	n.s.	"
S. attenuata	muscle	E. tropical Pacific	31	higher in females	André *et al.* (1990a)
	liver	"	33	higher in females	"
	kidney	"	31	higher in females	"

continued

Table 5 continued

Compound/Species	Tissue	Area	No.	Difference	Reference
tHG cont.					
P. phocoena	liver	Norway	92	n.s.	Teigen *et al.*(1993)
	kidney	"	92	n.s.	"
	muscle	Bay of Fundy	113	higher in females	Gaskin *et al.* (1979)
	liver	"	68	higher in males	"
	kidney	"	42	higher in females	"
As					
G. melas	liver	Faroe Islands	92	n.s.	Caurant *et al.* (1994)
	kidney	"	54	n.s.	"
Cd					
B. physalus	liver	E.North Atlantic	70	n.s.	Sanpera *et al.* (1995)
	kidney	"	71	n.s.	"
B. physalus	liver	Iceland	39	n.s.	Sanpera *et al.* (1995)
	kidney	"	49	n.s.	"
G. melas	liver	Faroe Islands	120	higher in females	Caurant *et al.* (1994)
	kidney	"	54	n.s.	"
S. coeruleoalba	muscle	Japan	59	n.s.	Honda *et al.* (1983)
	liver	"	57	n.s.	"
	kidney	"	30	n.s.	"
S. attenuata	muscle	E. tropical Pacific	27	n.s.	André *et al.* (1990b)
	liver	"	27	n.s.	"
	kidney	"	27	n.s.	"
Cu					
B. physalus	muscle	E.North Atlantic	66	higher in males	Sanpera *et al.* (1995)
	liver	"	72	n.s.	"
	kidney	"	71	n.s.	"
B. physalus	muscle	Iceland	36	n.s.	Sanpera *et al.* (1995)
	liver	"	38	higher in males	"
	kidney	"	37	n.s.	"
G. melas	liver	Faroe Islands	120	higher in females	Caurant *et al.* (1994)
	kidney	"	97	n.s	"
S. coeruleoalba	muscle	Japan	59	n.s.	Honda *et al.* (1983)
	liver	"	57	higher in males	"
	kidney	"	30	n.s.	"
Fe					
B. acutorostrata	liver	Antarctic ocean	135	higher in males	Honda *et al.* (1987)
S. coeruleoalba	muscle	Japan	59	n.s.	Honda *et al.* (1983)
	liver	"	57	higher in males	"
	kidney	"	30	n.s.	"
Mn					
S. coeruleoalba	muscle	Japan	59	n.s.	Honda *et al.* (1983)
	liver	"	57	higher in females	"
	kidney	"	30	n.s.	"
Ni					
S. coeruleoalba	muscle	Japan	59	higher in males	Honda *et al.* (1983)
	liver	"	57	higher in males	"
	kidney	"	30	n.s.	"
Pb					
S. coeruleoalba	muscle	Japan	59	higher in males	Honda *et al.* (1983)
	liver	"	57	higher in males	"
	kidney	"	30	n.s.	"

continued

Table 5 continued

Compound/Species	Tissue	Area	No.	Difference	Reference
Zn					
B. physalus	muscle	E.North Atlantic	71	n.s.	Sanpera *et al.* (1995)
	liver	"	69	n.s.	"
	kidney	"	67	n.s.	"
	muscle	Iceland	35	n.s.	Sanpera *et al.* (1995)
	liver	"	35	n.s.	"
	kidney	"	33	n.s.	"
G. melas	liver	Faroe Islands	120	n.s.	Caurant *et al.* (1994)
	kidney		97	n.s.	"
S. coeruleoalba	muscle	Japan	59	n.s.	Honda *et al.* (1983)
	liver	"	57	higher in males	"
	kidney	"	30	n.s.	"
Se					
G. melas	liver	Faroe Islands	92	higher in females	Caurant *et al.* (1994)
	kidney	"	54	n.s.	"
P. phocoena	liver	Norway	92	n.s.	Teigen *et al.* (1993)
	kidney	"	92	n.s.	"

This difference exists despite the fact that there is apparently no impediment to the transplacental transport of these elements and that transfer to milk occurs, although it is reduced. The only information on percentage of body load transfer through reproduction available for cetaceans is the study by Itano *et al.* (1984b) on striped dolphins, which indicated a gestational transfer of only 0.4-1% of maternal load of mercury and selenium to the foetus. The ratio foetal concentration/maternal concentration for a given tissue is also indicative of gestational transfer. Honda and Tatsukawa (1981) and Honda *et al.* (1986) calculated these ratios for a number of heavy metals in the striped dolphin and found values usually below unity, which indicates that some restriction exists for the placental passage of these elements.

Data on lactational transfer are even more sparse. No calculations on percentage of body load of trace elements through milk are available for cetaceans. In humans, maternal milk usually contains about 5% of the mercury concentration of maternal blood (Goyer, 1991). In cetaceans, information on heavy metal content in milk is restricted to a single study on mercury and selenium in the striped dolphin (Itano *et al.*, 1984b) but extremely low levels of lactational transfer are suggested.

It appears that it can be generally accepted that the reproductive transfer of mercury, selenium or cadmium is negligible and unlikely to affect the elemental load of the mother. Although this would explain a similarity in levels of these chemicals in both sexes, it does not justify the higher tissue concentrations usually detected in females. The cause for this dissimilarity remains unclear. It has been suggested that in sexually dimorphic species, it is due to the dilution of elements in the body of the males, which are larger (Caurant *et al.*, 1994). However, this does not explain maintenance of the difference in the long-term, neither does it explain the fact that the same difference has been observed in species which are not sexually dimorphic (Table 5), such as the spotted dolphin and harbour porpoise. Indeed, it is more likely that this age-related variation is associated with a difference in metabolic pathways linked to hormone cycles, obviously different in both sexes, as suggested by Caurant *et al.* (1994).

A significant portion of the mercury present in the tissues of marine mammals is found speciated, mainly as methylmercury (CH_3Hg), a much more toxic form than inorganic mercury. Information about sex or age-related trends in concentrations of speciated forms is

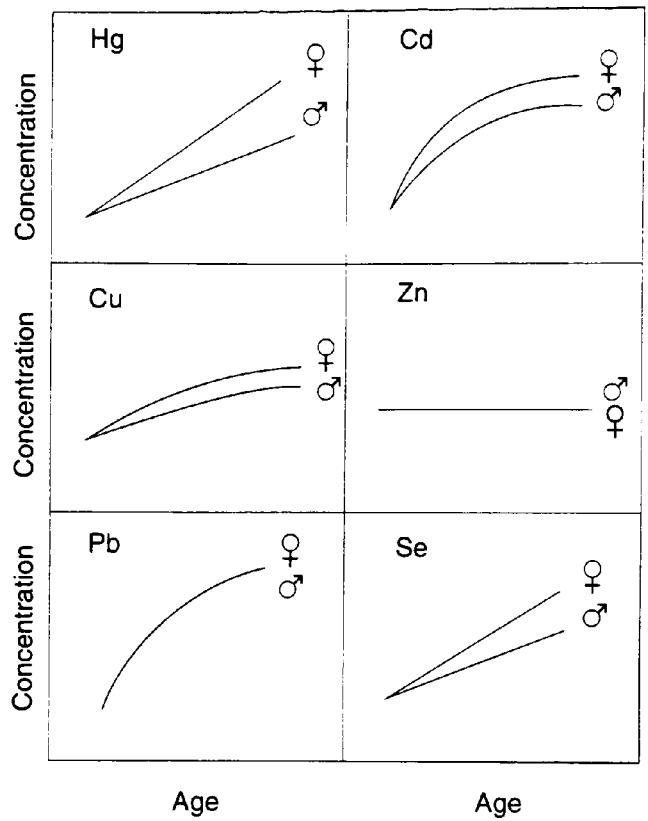

Fig. 4. Typical age-related variation of concentration of selected trace elements in the tissues of cetaceans.

scarce and limited to CH_3Hg, which also appears to increase with age in both sexes (Reijnders, 1980; Sanpera *et al.*, 1993). However, marine mammals have a well known ability to demethylate CH_3Hg and, because this process is progressive throughout the life of the individual, its relative abundance, as measured by the index $\%CH_3Hg/tHg$, has been found to decrease with age (Reijnders, 1980).

Lead behaves differently however. In mammals its concentration in different tissues follows variable age-related trends, but it accumulates markedly in bone and kidneys (Goyer, 1991). Therefore, body loads tend to progressively accumulate throughout life, although a levelling-off of this increasing trend may occur at advanced ages (Fig. 4). Lead is able to cross the placenta and it may be found in the milk in low quantities (Honda *et al.*, 1983; 1987; André *et al.*, 1990a). Usually, levels in males are somewhat higher than in females (Tables 3 and 5) although the information available is insufficient to allow reliable quantification of reproductive transfer.

Data on other heavy metals is more sparse. Copper often shows modest increases with age although in some cases it may be stable throughout the life of the individual or even decrease (Table 3 and Fig. 4). It readily passes the placental membranes and levels in the foetus are usually higher than in the mother (Fujise *et al.*, 1988; Law *et al.*, 1992). However, in terms of body loads, this transfer is probably negligible and tissue concentrations are generally slightly higher in males than in females (Table 5). This difference is apparently due to

sex-related differences in hormone metabolism (Caurant *et al.*, 1994) as suggested for mercury and cadmium. Zinc did not show any trend associated with age and no differences were found between the sexes. Placental transfer is apparently low (Law *et al.*, 1992). Data on cobalt, arsenic and nickel are insufficient to draw conclusions about age-related or sex-related variations in cetaceans, although data from other mammals suggest that, if existing, these should be moderate (Goyer, 1991).

No information is available on age- or sex-related variation in either sex or reproductive transfer of radionuclides, polyaromatic hydrocarbons or hydrocarbons in general.

Reproductive biology

As seen above, some pollutants are transferred from the reproducing female to the offspring, both during gestation and lactation. There is considerable uniformity in the basic traits associated with pregnancy in cetaceans. Thus, the duration of gestation and the size of neonates relative to that of their mothers, and therefore the relative amount of biomass transferred, are generally constant (Perrin and Reilly, 1984). In contrast, there is large variability among species in the duration of lactation and, consequently, in the amount of biomass transferred. In baleen whales, lactation is short and typically extends over a period of about 5-10 months (Lockyer, 1984), apparently because transition to independent feeding does not require complex learning. In toothed whales, in contrast, behaviour associated with capture of prey is complex, requires considerable training and lactation is therefore more protracted (Brodie, 1969). Its duration ranges from slightly over one year in small delphinidae to about 7-13 years in sperm whales (Best *et al.*, 1984; Perrin and Reilly, 1984).

This large variation in the length of lactation entails substantial interspecific variation in the amount of pollutants transferred. Indeed, in those chemicals that are excreted with the milk, like most lipophilic compounds, reproductive transfer is directly related to duration of lactation. Fig. 5 shows the relationship between the extent of the adult male-female difference in the levels of PCBs and lactation length in nine cetacean species. Pollutant data for producing this relationship have been extracted from Table 5, and lactation length of the various species from Braham (1984), Gaskin *et al.* (1984), Lockyer (1984) and Perrin and Reilly (1984). There is a significant ($p < 0.05$) correlation between the two variables, the sex-related difference being low in baleen whales and other cetaceans with short lactation periods, but high in small delphinidae with protracted lactation lengths.

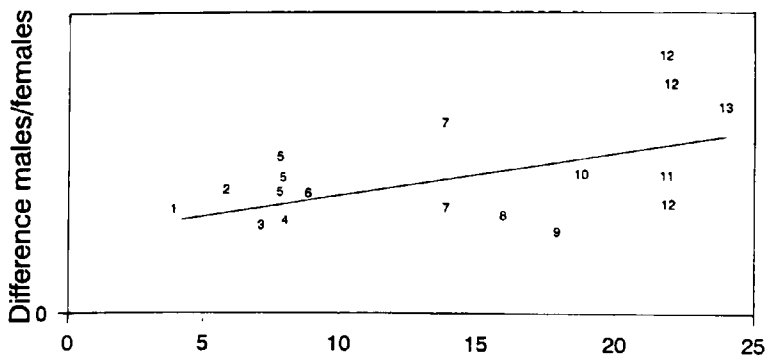

Fig. 5. Relationship between length of lactation and adult male-female differences in PCB blubber concentrations in different species of cetaceans. $y = 1.76 + 0.081x$; $r = 0.53$; $p < 0.05$.
Key: 1 = *B. acutorostrata*; 2 = *B. borealis*; 3 = *E. robustus*; 4 = *B. physalus*; 5 = *P. phocoena*; 6 = *P. blainvillei*; 7 = *S. coeruleoalba*; 8 = *D. delphis*; 9 = *L. acutus*; 10 = *G. macrorhynchus*; 11 = *G. melas*; 12 = *D. leucas*; 13 = *P. dalli*.

In addition, the relative proportion of the organochlorine load transferred to offspring was estimated to be much lower in fin whales (range 3-27%) than in bottlenose dolphins (*ca* 80%) or striped dolphins (72-91%), again reflecting differences in the length of lactation period (Aguilar and Borrell, 1994c). Furthermore, the age-related patterns of variation of pollutant levels in females may reflect changes in reproductive activity with age. For example, Tanabe *et al.* (1987) found that, after the typical decrease in organochlorine concentrations that follow the beginning of reproduction in adult female short-finned pilot whales, a secondary increase appeared in individuals above the age of 25, which corresponds to a slowing-down of reproductive activity in the species.

IMPLICATIONS OF THE EXISTENCE OF INDIVIDUAL VARIABILITY

Sampling techniques and source

As mentioned above, many surveys attempt to monitor pollutant levels in cetacean populations using stranded individuals. This approach has a number of drawbacks, some of which can be readily solved if factors inducing individual variation are taken into account, while others are more difficult or impossible to overcome. The significance of disease and nutritive condition on the tissue levels has already been discussed and their effect may (or may not) be accounted for if corrections for lipid content of the tissues or proper identification of cause of death is possible. However, strandings also suffer from a number of other shortcomings, as discussed below.

The age composition of stranded cetaceans reflects the pattern of the age-specific mortality rate rather than the actual age-structure of the population. Neonates, weaners and senescent individuals are usually more common among strandings than juveniles and young mature animals, which comprise most of the actual population. In some conditions, even the sex ratio may be biased. Calzada *et al.* (1994) examined the age and sex composition of the striped dolphins killed by the 1990-1992 Mediterranean morbillivirus epizootic and found an abnormally high relative abundance of calves and old individuals in the sample. Similar results were obtained by Härkönen and Heide-Jørgensen (1990) in a similar study on the 1988 harbour seal epizootic in the Kattegat-Skagerrak area.

Another problem is that samples from stranded cetaceans are almost invariably collected at unknown post-mortem times and this is likely to have an effect on tissue pollutant concentrations. Some organic compounds are very volatile and they may abandon the cellular structure if the carcass is subject to direct sun or wind exposure, conditions likely to occur in a stranded cetacean. Borrell and Aguilar (1990) examined variation in organochlorine levels in tissues from a dolphin corpse left outdoors and found that the lipid content and concentrations of DDTs and PCBs significantly decreased in muscle and blubber after a few days of exposure to temperate weather conditions. Although similar studies have not been carried out on other organic pollutants or on heavy metals, it is likely that changes of this nature may also occur for these compounds.

The combined effect of these factors is difficult to predict. Impoverished nutritive condition is expected to increase tissue pollutant levels. Exposure to outdoor conditions will work in the opposite direction. Incidence of disease may handicap the ability of an individual to excrete pollutants. The effect of a biased age-composition of the sample or sex ratio may operate in any direction. It is clear that this makes samples from strandings difficult to work with, and ones from which spurious conclusions can be easily drawn.

Fig. 6 shows the frequency distributions of PCB concentrations in Mediterranean striped dolphins found washed ashore and in free-ranging individuals sampled using biopsy techniques, and therefore considered to be more representative of the actual population. Both samples were collected during the same time period (1987-1994) and region (northeastern

coast of Spain). Stranded individuals affected by the Mediterranean morbillivirus epizootic were not included in this sample, nor were biopsies from free-ranging dolphins collected in 1990, the year when the event affected the sampling area. As can be seen, concentrations found in dolphins sampled with the biopsy dart (with the exception of a single individual that carried abnormally high concentrations), follow quite closely a normal distribution. The concentrations found in stranded dolphins, in contrast, exhibit a more irregular distribution, with more extreme values, both above and below the majority of the live, free-ranging population. The reasons for this are unclear, although it is likely that undernourished individuals comprise the most highly polluted component of the stranding sample, while individuals with long post-mortem times comprise the less polluted one. In conclusion, a stranded sample is considered to be a poor representation of the true population, particularly if the sample size was small and is therefore likely to produce biased results.

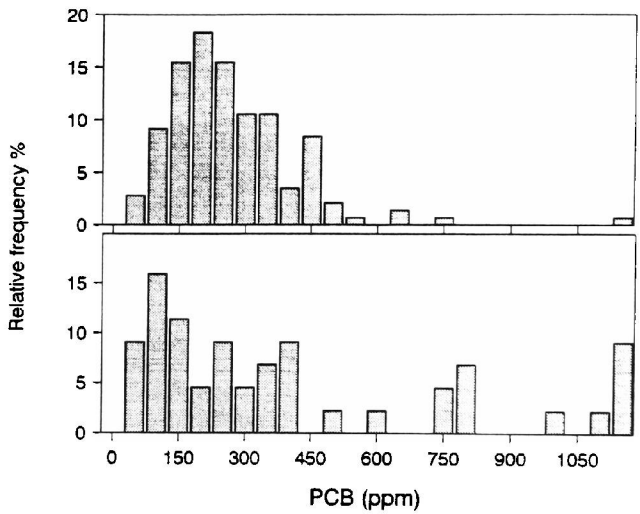

Fig. 6. Frequency distributions of PCB concentrations in the blubber of free-ranging (above) and stranded (below) Mediterranean striped dolphins.

Samples obtained from whaling operations or fisheries' bycatch do not suffer from most of these drawbacks (except the potential bias in age and sex structure due to differential catchability or selection by fishermen) and are likely to be more representative of the actual population. However, relying on these operations for pollutant sampling has obvious logistical difficulties and also limits the availability of samples to a small number of species and geographical regions.

Collection of biopsies from free-ranging cetaceans appears to be a practical alternative and many researchers have shifted to this technique in recent years (Aguilar and Borrell, 1994b). Biopsies can be collected from a reasonable number of individuals, the tissues obtained are fresh, samples can be considered to be a reasonable representation of the population and the collection technique is essentially unharmful to the sampled individual. However, the technique does have a number of limitations. Currently-used biopsy darts are only capable of extracting skin and the superficial layers of the blubber. In large cetaceans these

superficial layers are mostly devoted to thermoregulation, so the sample collected may not be fully representative of the body load of certain lipophilic pollutants (Aguilar and Borrell, 1991). This limitation may be solved using a dart capable of penetrating the whole blubber thickness in order to collect a sample containing a full representation of the blubber strata (Lambertsen *et al.*, 1994), but this type of dart is obviously more invasive than those acting only at the superficial layers.

An important limitation is that, because samples are collected from free-ranging individuals, no information is usually available on their main biological characters, many of which are relevant to a proper evaluation of the levels of pollutants present in the tissues. However, recent developments have solved some of these limitations. Body size can be measured by photogrammetric techniques, e.g. gender determined using a number of techniques based on DNA analysis (e.g. Baker *et al.*, 1991; Palsbøll *et al.*, 1992) and nutritive condition assessed through the richness in triglycerides of the blubber layer (Aguilar and Borrell, 1990). It is likely that in the near future it will be possible to determine reproductive condition of individuals by analysing the hormone content of blubber, but we lack methods to determine other key factors affecting pollutant tissue levels, such as age.

Interpretation of tissue levels and loads

The patterns described above are reasonably consistent across many species and populations. However, deviations are not exceptional and researchers should be aware that substantial variation occurs among species, populations or even population components.

A paradigmatic example is the sperm whale, as the sexes distribute differently. Females of all ages and juveniles inhabit tropical and temperate waters throughout the year and comprise the so-called 'nursery' schools (Best *et al.*, 1984). Some large males move into these schools for undetermined periods of time, but for much of the time adult males move to cooler waters. This segregation implies a substantial difference in the diet, on the pollutant profile and the content of the food resources on which the different population components feed. In addition, adult males are capable of undertaking much longer and deeper dives, and consume prey located at different depths than those typical of females and juveniles. This difference in diet occurs even when both sexes share geographically identical feeding grounds. The size of the consumed prey is also highly variable, being much larger in adult males than in females or juveniles of either sex (see a review of sperm whale feeding in Clarke, 1980). Further, males show a strong tendency to take other prey than squid, whereas females seem to be much more dependent on cephalopoda. Besides these differences in feeding regime, the species is highly dimorphic and the body mass of adult males is about 3-4 times that of adult females. Since adult males inhabit colder waters and are subject to strict and more energy-demanding migratory regimes, the relative contribution of blubber to body mass is about 25%, as compared to 18-20% in females (Mizue, 1951; Best *et al.*, 1984; Evans, 1987).

These differences among sexes or age-classes obviously have an impact on the pollutant concentrations that sperm whales carry in their tissues. In effect, they follow none of the patterns of variation described above. Levels in females are higher and different in profile than males, and age-related variation in males is non-existent; a decrease in levels has even been suggested for at least the first quarter of the lifespan (Aguilar, 1983; Henry and Best, 1983).

Sperm whales are perhaps a remarkable example of complexity with regards to population biology among cetaceans, but sex- or age-related variation in diet, daily food intake rate, body size, body composition, or behaviour, are frequent among cetaceans and should be carefully considered when interpreting pollutant loads.

Toxicological implications of individual variability in pollutant levels

The toxicological implications of the age-related patterns observed for most pollutants are not obvious. While it is true that pollutant levels are usually low in young animals and high in adults, their actual impact on the individual is not necessary proportionate. Neonates and calves exhibit greater sensitivity than adults to certain toxicants, particularly carcinogens. For example, polycyclic aromatic hydrocarbons do not usually induce liver cancer in adult laboratory animals, but do so when administered to newborns because of the rapid growth of their liver. Furthermore, at advanced ages, the biotransformation capacity of hepatic microsomes weakens and many biochemical and physiological functions such as renal and hepatic blood flows or the efficiency of the urinary and biliary excretory systems decrease; thus older animals may have an increased tissue sensitivity to some toxins (Sipes and Gandolfi, 1991; Williams and Weisburger, 1991).

These changes associated with ageing point to a relatively higher sensitivity to xenobiotics in both the young and the old. However, the ability to biodegrade compounds is not necessarily a recipe for minimising their effect (Reijnders, 1994; Reijnders and de Ruiter-Dijkman, 1995). Some pollutants, such as certain PCB congeners, DDT or lead, generate degraded or biotransformed forms that are more toxic than their respective parent compounds. Moreover, certain chemicals, (e.g. carbon tetrachloride) are inactive in their original form and require biotransformation to exert their toxic effect. In these cases, sensitivity to the agent will follow a reverse trend and exposure to the chemical is expected to be less hazardous in both younger and older animals.

Gender also plays a key role in the detoxification process. As seen above, the enzyme system of males is better equipped to cope with xenobiotics than that of females, so the capacity to degrade foreign chemicals is higher in males. This difference appears to be linked to the balance of sex hormones, and artificial alteration of this balance in laboratory animals results in biotransformation rates of females approaching those observed in males, and vice versa. However, this potential response in males should not be taken as direct evidence that they are less susceptible to the toxic impact of chemicals than females. Indeed, if the toxic effect is produced by a metabolite or reactive intermediate instead of the parent compound, males will show a greater susceptibility to the agent. For example, male rats are more likely to suffer hepatic injury by carbon tetrachloride than females because, in the latter, degradation and subsequent formation of toxic forms is a lower process (Sipes and Gandolfi, 1991).

In summary, it is important to remember that, as when interpreting tissue pollutant levels, a toxicological evaluation should only be attempted when taking into account the available knowledge on the biology of the species, and of the population, population component or individual subject to study.

ACKNOWLEDGEMENTS

We thank J.U. Skaare (State Veterinary Laboratories, Oslo, Norway) and R. Law (Fisheries Laboratory, Essex, UK) for their revision of an early version of the manuscript. This research was funded by the *Comisión Interministerial de Ciencia y Tecnología* (CICYT), project AMB-399/94, and the *Dirección General para la Conservación de la Naturaleza* of Spain. A. Borrell was supported by a postdoctoral contract from the *Comissió per a Universitats i Recerca* (RED) of Catalonia.

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J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 117–124, 1999

Biomarkers as pollution indicators with special reference to cetaceans[1]

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ABSTRACT

The limited information available on biomarkers in cetaceans and pinnipeds is reviewed. The main problems with their application to cetaceans are the difficulties in obtaining suitable tissue material and in carrying out experimental work to relate pollutant levels and effects. A white whale population from the Gulf of St Lawrence has been found to have a high incidence of tumours and the presence of adducts was related to exposure to benzo(a)pyrene (PAH), a well known carcinogen. Some research has been carried out on induction of Mixed Function Oxidases (MFOs) (mainly cytochrome P450 system) in cetaceans, but the results are not yet conclusive. Studies on other groups of animals suggest that MFOs may be valuable biomarkers, particularly if techniques to measure them in biopsy skin samples are further developed. The goal should be that wild cetaceans are physiologically normal, biomarkers can be used to identify populations whose physiological functions are outside normal limits because of excessive exposure to pollutants. Since no pristine environments currently exist, measurements of biomarkers along gradients of pollutant exposure are needed to establish physiological normality in cetaceans.

KEYWORDS: BIOMARKERS; DNA ADDUCTS; HAZARD ASSESSMENT; IMMUNOSUPPRESSION; PHYSIOLOGY; POLLUTION-HAHs; POLLUTION- ORGANOCHLORINES; POLLUTION-PESTICIDES; WHITE WHALE; STRIPED DOLPHIN; FIN WHALE; MINKE WHALE; SHORT-FINNED PILOT WHALE; DALL'S PORPOISE; PINNIPEDS

INTRODUCTION

At a recent symposium organised by the European Science Foundation (Peakall and Walker, 1994) biomarkers were defined as:

> 'A biological response to a chemical or chemicals that gives a measure of exposure and, sometimes, also of toxic effect'.

The term 'biological response' is usually taken to be any response (biochemical, physiological, pathological or behavioural) up to the organisational level of the intact animal. Biological responses at higher organisational levels, such as population or community structure, are usually termed 'bio-indicators'.

Biomarkers are often divided into 'biomarkers of exposure' and 'biomarkers of effect', but this is misleading. All biomarkers indicate exposure and demonstrate an effect of some sort or another; if an additional term is thought necessary it should be 'biomarkers of toxic or adverse effect'.

[1] This paper was originally submitted to the IWC Scientific Committee as SC/M95/P8.

The main reason for using biomarkers is to overcome the difficulty in conventional toxicology of making a hazard assessment under field conditions. The basic approach of conventional toxicology is to measure the amount of the chemical present and then relate that, via animal experiments (or correlation to observed adverse effects), to adverse effects caused by the chemical. While we have a large amount of data on the levels of pollutants in marine mammals, we have comparatively little information on the effects that these pollutants have on them (Reijnders *et al.*, 1999).

BIOMARKERS: DIFFICULTIES WITH MARINE MAMMALS

Although biomarkers are becoming widely used in environmental assessment there are two specific difficulties in the use of them to study marine mammals and, more specifically, cetaceans.

(1) Collection of samples

There have been strenuous efforts to conserve cetaceans and thus, more than for almost any other group of animals, the taking of animals for scientific study is controversial (e.g. Donovan, 1992). Therefore, samples must either be obtained non-destructively or obtained from animals that have already died.

(2) Practical difficulties with experimental studies

No experimental studies on the effects of pollutants on cetaceans have been carried out to date and thus we have to depend on either correlations between effects seen and pollutant levels without being able to confirm a cause-and-effect relationship, or on extrapolation from other groups of animals. In the context of the latter approach, the experimental data available on pinnipeds will be briefly reviewed below. Another potential approach, already used on a number of species, but as yet rarely on cetaceans (see Busbee *et al.*, 1999), is the use of tissue culture for experiments.

BIOMARKER DATA AVAILABLE IN CETACEANS

DNA adducts

Although the covalent binding of environmental pollutants to DNA (adduct formation) is a clear demonstration of exposure to these agents, it is merely an indication of possible adverse effects. Any alteration to genetic material must be taken seriously but it should be remembered that a range of repair mechanisms exist.

The group of compounds that have been studied in the most detail for adduct formation are the polynuclear aromatic hydrocarbons (PAHs); some, such as benzo(a)pyrene (BaP), are known carcinogens. There are a considerable number of techniques available for analysing their adduct formation (see Shugart, 1993) that can be broadly allocated to two categories, those that measure the total number of adducts and those that identify specific adducts.

BaP-DNA adducts were found in the brain tissue of three white whales (*Delphinapterus leucas*) found dead in the St Lawrence (Martineau *et al.*, 1988). In a later study, ten out of eleven white whales examined had these adducts in the brain and they were also present in the six livers examined (Martineau *et al.*, 1994). By contrast, no such adducts were found in the brains of four white whales killed by native hunters in the Mackenzie Delta region of the

Arctic (Martineau *et al.*, 1994). The finding of BaP-adducts in the brains of the St Lawrence white whales can be correlated to the high incidence (19%) of tumours found in whales that underwent post-mortems (Martineau *et al.*, 1999).

In contrast, studies by Ray *et al.* (1991) found no differences in the total number of adducts in the livers of eight white whales from Hudson Bay, six from the Mackenzie Delta or four from the St Lawrence.

More extensive studies, preferably measuring both total PAH adducts and BaP-adducts, are needed. Since DNA adducts can be measured in skin samples (L. Shugart, pers. comm.) it should be possible to obtain these samples non-destructively from free-ranging cetaceans using biopsy techniques (Aguilar and Borrell, 1994). However, it will be necessary to establish relationships between the levels of adducts in the skin and in target organs using material that is available for autopsy.

Mixed Function Oxidase induction

The Mixed Function Oxidases (MFOs) or mono-oxygenases are a group of enzymes that are capable of being induced by a wide range of substances, both natural and man-made. They form an important defence mechanism, which initially evolved to enable animals to deal with plant or animal toxins in their diet by increasing the rate of metabolism of these toxins. In the modern world, the same system is often called into play by man-made chemicals, such as organochlorines.

A study based on skin biopsy samples from seven striped dolphins (*Stenella coeruleoalba*) and nine fin whales (*Balaenoptera physalus*) from the Mediterranean Sea has been reported by Fossi *et al.* (1992). The MFO activity in the striped dolphins was four times higher than in the fin whales and this was ascribed to the higher levels of PCBs and total DDT in the dolphins; however, this variation is well within the inter-species variation that has been reported, even between fairly closely related species (Walker, 1980). The important point of this rather preliminary study is that MFO activity in the skin, although much lower than the activity in the liver (Watanabe *et al.*, 1989), can be readily measured.

The characteristics of the cytochrome P450 system of the minke whale (*Balaenoptera acutorostrata*) have been studied (Goksøyr *et al.*, 1985; 1986). Studies on three additional species of cetaceans have been reported by Watanabe *et al.* (1989). While this is valuable baseline information it does not provide pollutant related information. The capacity of the MFO systems of cetaceans to be induced and thus aid in the metabolism and excretion of organochlorine compounds has been reviewed by Tanabe and Tatsukawa (1992). They concluded that 'these animals have a low capacity for degradation of these contaminants resulting from a specific mode of cytochrome P450 drug-metabolising enzyme systems'.

One of the clearest indications that induction of the MFO system is occurring comes from the correlation of the activity of one of the MFO enzyme (7-ethoxyresoresufin O-deethylase) with the total PCB concentration in the livers of short-finned pilot whales, *Globicephala macrorhynchus* (Watanabe, cited in Tanabe and Tatsukawa, 1992). In addition, strong correlations of cytochrome CYP1A with the level of toxic PCB congeners have been reported by White *et al.* (1994) for the white whale.

Plasma hormone levels

There have been few published studies relating hormone levels to pollutant levels in blood samples. Subramanian *et al.* (1987) examined the correlations of hormone levels with the levels of PCBs and DDE in the blood of the Dall's porpoise (*Phocoenoides dalli*). A modest negative correlation between the level of testosterone and DDE was determined but no relationship was found between testosterone and total PCBs, or aldosterone levels with either pollutant.

Immunological studies

Despite the importance of the immune system in the link between pollutants and disease, no detailed pollutant-related immunological studies on cetaceans have been reported. However, a number of immunological studies on cetacean plasma are underway that will provide useful reference material and which could be adapted to such work (e.g. Abbott, 1979; Bossart, 1984; Brown *et al.*, 1988; St Aubin *et al.*, 1990; Kennedy *et al.*, 1991; Domingo *et al.*, 1992; Kumar and Cowan, 1994; De Guise *et al.*, 1996; Erickson *et al.*, 1995; Lahvis *et al.*, 1995).

EXPERIMENTAL STUDIES WITH PINNIPEDS

In the absence of significant experimental studies on the effects of pollutants on cetaceans it is worthwhile briefly reviewing the data that are available on seals. Although both pinnipeds and cetaceans are marine mammals it should be pointed out that these two groups are not phylogenetically closely related and furthermore are known to differ in at least some responses to contaminant exposure, e.g. MFO-induction (Tanabe and Tatsukawa, 1992; Reijnders, 1994).

Studies have been carried out by Reijnders and co-workers on harbour seals (*Phoca vitulina*) which were fed fish from highly polluted areas such as the Baltic or Wadden Seas, the control group being fed fish from the relatively clean North Atlantic. In the first study (Reijnders, 1986) a significant decrease in reproductive success was found in the group fed with the fish from polluted areas. However, no differences were found in the levels of circulating hormones. Studies were also carried out on the levels of vitamin A and thyroid hormone levels (Brouwer *et al.*, 1989). Significantly lower levels of plasma retinol, total and free thyroxine and triiodothyronin were found in those seals on the contaminated diet. In a subsequent experiment a variety of immunological tests were carried out and it was found that there was functional impairment of both the innate and adaptive immune systems in the seals fed on herring from the Baltic (De Swart *et al.*, 1994; Ross *et al.*, 1995). Vitamin A levels were also significantly lower in this group, confirming earlier studies.

HAZARD ASSESSMENT USING BIOMARKER DATA

In hazard assessment using biomarker data, it is necessary to define what 'no hazard' is. Peakall (1992) argued that a reasonable position is that 'the physiological functions of organisms, outside the exclusion zone, should be within normal limits'. The rationale for having an 'exclusion zone' in hazard assessment is that one cannot expect physiological normality in the immediate area of, for example, an industrial port, although in general it would not be considered acceptable to pollute an entire estuary. Fortunately, for marine mammals, the question of 'exclusion zones' can be ignored. For cetaceans, therefore, it can be argued that a reasonable standard could be that human activities do not cause their physiological function to be outside the normal limits.

Such an approach can be compared to a thorough human medical check-up where a range of tests are carried out and action is taken only if the measurements (cholesterol level, blood pressure, etc.) are outside normal limits. The advantages of the approach are twofold.

(1) It is, initially, independent of the pollutants involved and thus avoids the problem of mixtures and unknown substances. Only if abnormal function is found is it necessary to make detailed investigations.
(2) It is philosophically defensible. It recognises that pollutants will be present (analytical chemistry is too good for zero to be a practical objective) and rather stresses that the

function of the animals living in the area should be normal. Thus it provides a realistic target for remedial action.

The major limitations of the approach are as follows.

(1) The definition of physiological normality presents two difficulties: no completely pristine environment now exists; and there are the previously mentioned practical difficulties of working with cetaceans, particularly the large whales and beaked whales. Areas of low pollution and, more specifically, measurements along gradients are needed to establish, as far as possible, physiological normality.
(2) It requires that a sufficient range of tests are available to be able to determine with confidence that physiology is indeed normal and that all major classes of pollutants have been covered.
(3) It does not address the question as to whether harm is caused by the altered physiological state. This needs further investigation in terms of costs to the individual/population involved.

The first and second limitations are, at the moment, the most serious with respect to cetacean studies. More data are needed to establish normality and more tests (e.g. non-destructive tests for immunological studies) need to be developed. The third point may be controversial but physiological normality does not seem an unreasonable goal for free-living open ocean animals.

CONCLUSIONS

At present there are few studies on cetaceans that can be directly related to the issue of biomarkers. Clearly much more work is needed. One example of the type of study that could be useful is that of the population of white whales that live in the polluted St Lawrence Estuary, Canada. The main findings of a number of years of study were a high prevalence of tumours (19% of the 73 whales autopsied - Martineau *et al.*, 1999) and a high incidence of lesions to the digestive system (53% of examined animals) and mammary glands (45% of adult females examined) (Béland *et al.*, 1993). A *prima facia* case can be made for the involvement of chemicals based on the presence of BaP-DNA adducts in the brain at levels associated with carcinogenesis in laboratory animals. A healthy Arctic population of the same species had neither a high prevalence of tumours nor detectable levels of BaP-DNA adducts in the brain.

High levels of PCBs (and other organochlorines) have been recorded in these white whales and other cetaceans. But although there is evidence that the capacity of the MFO system in cetaceans is relatively low compared to other orders of mammals and that some induction of MFOs has occurred, no definite proof of harm has been generated.

Nevertheless, concerns remain in relation to this group of compounds. Firstly, there is good evidence that present levels are causing problems for pinnipeds in the Baltic and Wadden Seas (Helle *et al.*, 1976; Reijnders, 1980). Secondly, although there are bans and restrictions on PCBs in many countries, it is likely that the release of these persistent compounds into the environment will continue for many years to come (IWC, 1997).

RECOMMENDATIONS

(1) Biomarkers (certainly DNA-adducts and MFO induction and, if possible, others) must be measured in skin samples collected along known pollution gradients and be related to the levels of pollutants measured in the same samples. Blood samples can also be used to examine biomarkers such as adduct formation with haemoglobin and enzyme activity;

although this requires the capture of animals. It is important to measure biomarkers in healthy living animals rather than stranded specimens. Examples of gradients that could be studied include the white whale in the St Lawrence compared to those in the Arctic, and harbour porpoises in the Baltic, North Sea and open Atlantic (e.g. IWC, 1999).

(2) Measurements of biomarkers in the skin must be related to those in other organs (brain for adducts, liver for MFO induction) which are normally used. This should be carried out with animals legally taken or killed by accident rather than on stranded animals, again to ensure that physiological normality is more probable.

(3) If such information is to be accepted and used in the conservation of cetaceans these biomarker studies must be coordinated. This is needed to ensure that both measurements of biomarkers and residue levels are comparable, and that the framework of the studies is organised so that they can be used as part of the regulatory process.

Finally, it is vital to have much more information on the effects of pollutants on cetaceans. Additional collection of residue data alone is not helpful and indeed, it can be argued that analytical chemical studies should only be undertaken in support of detailed biological studies.

ACKNOWLEDGEMENTS

The author wishes to thank J. Stein, C. Walker and two anonymous reviewers for their help with this manuscript.

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J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 125–148, 1999

An overview of the concentrations and effects of metals in cetacean species[1]

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ABSTRACT

Data are presented on the biomagnification rates, accumulation and concentrations of metals in cetacean species. Concentrations of metals predominantly occur in the soft tissues, although zinc and lead concentrate in the skin and bones. Rates of uptake are dependent upon metal availability, the species' dietary preference and chemical reactions between contaminants. Differences in concentrations occur according to the sex and age of the animal, with certain metals displaying age-related trends. Mercury is the only metal which shows both biomagnification at all levels of the food chain and a positive correlation with age at all stages during a cetacean's life. Differences in concentrations occur between baleen species and toothed cetaceans. Levels tend to be lower in baleen whales, primarily due to a shorter food chain (resulting in lower bioconcentration factors) and as the principal prey species are taken from lower parts of the food chain. A number of storage and detoxifying mechanisms have been recorded in many species that may alter the effects of high metal concentrations. Data on the effects of metal toxicity in cetacean species are sparse, but tolerance limits have been proposed for mercury and cadmium. These are compared with high concentrations recorded in certain species and possible effects extrapolated. Effects of toxicity may alter depending on the species, age and sex of the animal, but indications of toxic effects have been reported. Finally, the possibility of determining regional hot-spots, where background pollution levels are high, from concentrations of mercury reported in cetacean species, are examined.

KEYWORDS: POLLUTION; BIOACCUMULATION/MAGNIFICATION; HEAVY METALS; TOXICITY; REVIEW

INTRODUCTION

This paper is a review of the literature on the incidence of metals in cetacean species, and is intended to provide a focused addition to previous reviews by Wagemann and Muir (1984) and Law (1996) on metals in all species of marine mammals. The paper is intended to provide an overview of bioaccumulation rates, concentrations and effects of metals in cetacean species. Firstly, the major routes of uptake and site specificity of metal concentrations will be examined. A comparison will then be made between concentrations of metals on three levels: at an individual animal level; an intra-species level; and at the sub-order level between *Odontoceti* and *Mysticeti*. Known associated physiological effects of these concentrations on cetaceans will then be analysed following a review of the reported detoxification mechanisms.

However, difficulties arise in attempting to interpret data, particularly regarding the significance and possible effects of levels recorded. Firstly, data tend to be limited to certain species and certain metals. Although data are presented on levels of lead, cadmium, zinc, selenium, manganese, iron and copper, the majority of research has focused on mercury (see

[1] A version of this paper was presented to the IWC Scientific Committee as SC/46/O 20.

Appendix 1). Moreover, research has concentrated on particular species, such as toothed cetaceans, and in particular regions, for instance the Northwest Pacific and North Atlantic Oceans (Appendix 1). Secondly, it is problematic to compare data which have used different sampling, measuring and analytical techniques, and whose results are presented in different formats, such as wet or dry weight, original data, ranges, means or medians. Moreover, data may originate from samples of both freshly killed and stranded cetaceans, the latter likely to present altered levels of metal burdens. Thirdly, differences may occur in concentrations of metals due to factors such as the species' diet, age and sex, but this information is often lacking, particularly on the age of the sampled animal. Finally, as research on the effects of metal burdens on cetacean species is fairly recent, there are few long-term databases that can be used to depict trends and effects of metals on cetaceans.

As top predators, cetaceans can be affected by metals in two distinct ways. Firstly, they may suffer the direct effects from bioaccumulation through the marine food chain and, secondly, they may be indirectly affected by a reduction in prey availability caused by metal toxicity in species at lower trophic levels. This study will consider only the former.

Finally, the pattern of geographical variation in the metal levels found in cetaceans from different regions is examined.

OVERVIEW OF METALS

Two broad groups of metals are recognised: 'essential' and 'non-essential' metals. Essential metals are those which have a clearly documented and defined function in the body and life of a species. Species require relatively low levels of these metals as an integral part of certain biological and biochemical processes, and a deficiency in them will result in negative effects. Disease and other negative effects may also develop if the concentration of the metal exceeds the level that the species requires or is able to store. Essential metals tend to show less variation in concentrations and burdens within and between species because organisms are able to regulate them. Research has established that copper, iron, selenium and zinc all fulfil vital functions and can therefore be defined as essential metals (Thompson, 1990; Law, 1996).

Non-essential metals are those which have little or no known recorded biological function in a species. These metals, which include mercury, lead and cadmium, are often toxic even at relatively low concentrations.

Metal contamination occurs from natural and anthropogenic sources. The former include geological weathering and volcanic activity, e.g. high levels of mercury are expelled from sub-marine volcanic activities (Piotrowski and Coleman, 1980). Man-made sources of metals are predominantly from waste disposal, leakage from mining operations, the production, use and disposal of chemicals including pesticides, the burning of fossil fuels and the use of anti-fouling paints on shipping.

CONCENTRATION DIFFERENCES AT INTRA- AND INTER-SPECIES LEVELS

Levels of metals in cetaceans are the net difference between uptake of the metal and any subsequent loss, such as excretion of the metal. A number of factors affect rates of uptake and bioaccumulation. These include: the subject metal, its specification, inter-metal competition for available body sites, inter-metal synergistic effects; and the subject organism and its biological characteristics, particularly sex, age and diet.

There are three routes for the uptake of metals into cetacean bodies. The principal one is dietary, but accumulation has also been reported through the skin and into the lungs (Augier *et al.*, 1993; Law, 1996). The skin is probably of little importance as cetacean skin is an effective barrier and direct bioaccumulation by the cutaneous route probably only occurs

when lesions are present (André *et al.*, 1990). The pulmonary route is also not that significant although direct incorporation of metals may also occur from those present in the atmosphere (André *et al.*, 1990; Augier *et al.*, 1993). The bioaccumulation and biomagnification of metal compounds in cetaceans primarily occurs through diet, and will vary on a species-specific and metal-specific basis.

Bioaccumulation rates

Muir *et al.* (1992) calculated bioaccumulation rates for mercury and cadmium from a relatively simple food web in the Arctic as shown in Table 1.

The data displayed in Table 1 show that only mercury has a consistently high biomagnification factor (BMF) throughout the marine food chain. BMFs from seawater to cetaceans for mercury have been reported in only two cases. These are from seawater to the liver of minke whales (4.3×10^4) in the Antarctic (Honda *et al.*, 1987), and from seawater to the livers of narwhals (3.5×10^5) from the Arctic (Muir *et al.*, 1992). However, Viale (1974) and Augier *et al.* (1993) calculated a lower mercury BMF of between 100-1,000 times in aquatic food chains, and their figure of a BMF of 4-8 times between fish and cetaceans is also considerably lower than that reported by Muir *et al.* (1992) who quote a value of 305 for fish to narwhal. The majority of mercury concentrations reported in marine species are of the organic form, methylmercury (Lindberg *et al.*, 1987; André *et al.*, 1990), which is highly lipophilic, therefore readily accumulated in the fatty tissues of fish and cetacean species, and consequently easily transferred along the food chain.

Cadmium BMFs have been reported from seawater to the liver of minke whales in Antarctica (5.5×10^5; Honda *et al.*, 1987) and to the liver of narwhals in the Arctic (4×10^6; Muir *et al.*, 1992). These high values are due to the species' reliance on prey which are rich in cadmium. However, as Table 1 shows, a high BMF does not occur at every stage in the food chain. This confirms other reports that the highest concentrations of cadmium are recorded at the phyto- and zooplankton trophic level (Furness and Rainbow, 1990).

There are few published BMFs for other metals, although Muir *et al.* (1992) stated that the BMF for lead was low between fish and small cetaceans (Table 1).

Table 1

Biomagnification factors for certain metals in the Arctic food chain as calculated by Muir *et al.*, 1992.

	Water-algae	Algae-copepods	Amphipods-fish	Fish-small cetacean
Cadmium	2.4×10^5	1.1	0.04	80
Mercury	-	-	163.0	305
Lead	-	-	-	0.07

Site specificity of burden levels

As diet is the main source of uptake of metals in cetaceans, this will affect the pattern of site distribution of these concentrations recorded in the cetacean body. Metals ingested will be transported via the blood system to the soft tissues, so it is expected that these tissues would contain higher concentrations (André *et al.*, 1990).

Table 2 presents data recording the tissue or organ for fourteen species where the highest mean concentration of a particular metal was recorded. It shows that in the majority of baleen and toothed species, the liver consistently contained the highest concentrations of mercury,

Table 2

Tissue specificity for seven metals and thirteen species, showing the tissue containing the highest mean concentration. Li: Liver, Ki: Kidney, Sk: Skin, Bo: Bones, Bl: Blood, Mus: Muscle, ND: No data reported.

Species	Hg	Cd	Se	Zn	Cu	Fe	Pb
Spotted dolphin *Stenella attenuata*	Li[1]	ND	ND	ND	ND	ND	ND
Striped dolphin *Stenella coeruleoalba*	Li[2,6]	Ki[6,13]	Ki[11]	Li[6,13] Sk/Bo[17]	Li[6]	Li[6]	Li[6]
White whale *Delphinapterus leucas*	Li[9,14]	Ki[14,19]	Li[14,19]	Mus[14] Ki[19]	Li[19]	ND	Ki[19]
Narwhal *Monodon monoceros*	Li[3,14]	Ki[9,14]	Li[3,14]	Ki[14]	Li[3]	ND	Li[9]
Fransiscana *Pontoporia blainvillei*	Li[4]	Ki[4]	ND	Li[4]	Li[4]	ND	ND
Ganges river dolphin *Platanista gangetica*	ND	Ki[5]	ND	Li[5]	Li[5]	Li[5]	Ki[5]
Dall's porpoise *Phocoenoides dalli*	Li[10]	Ki[10]	ND	Sk[10]	Li[10]	Bl[10]	Sk/Bo[10]
Long-finned pilot whale *Globicephala melas*	Li[8]	Ki[8,12]	Li[8,12]	Li[8,12]	Li[12] Ki/Li[8]	ND	Li[8]
White-beaked dolphin *Lagenorhynchus albirostris*	Li[8]	Ki[8]	Li[8]	Li[8]	Li[8]	ND	Li/Ki[8]
Pygmy sperm whale *Kogia breviceps*	Li[4]	Ki[4]	ND	Ki[4]	Li[4]	ND	ND
Minke whale *Balaenoptera acutorostrata*	Li[14]	Ki[14]	Ki[14]	Li[14]	ND	ND	ND
Cuvier's beaked whale *Ziphius cavirostris*	ND	Li/Ki[18]	ND	Li/Ki[18]	Li[18]	Li[18]	ND
Fin whale *Balaenoptera physalus*	Li[7]	ND	ND	ND	ND	ND	ND
Harbour porpoise *Phocoena phocoena*	Li[15,16]	Ki[15,16]	Sk[16]	Sk[16]	Li[16]	ND	ND

[1]Andre *et al.*, 1990. [2]Augier *et al.*, 1993. [3]Wagemann *et al.*, 1983. [4]Marcovecchio *et al.*, 1990. [5]Kannan *et al.*, 1993. [6]Honda *et al.*, 1983. [7]Sanpera *et al.*, 1993. [8]Muir *et al.*, 1988. [9]Wagemann *et al.*, 1984. [10]Fujise *et al.*, 1988. [11]Itano *et al.*, 1984a. [12]Caurant *et al.*, 1993. [13]Honda and Tatsukawa, 1983. [14]Hansen *et al.*, 1990. [15]Teigen *et al.*, 1992. [16]Paludan-Muller *et al.*, 1993. [17]Honda *et al.*, 1986a. [18]Knap & Jickells, 1983. [19]Wagemann *et al.*, 1990.

iron and copper. In many of these species the second highest concentration was reported in the kidney.

André *et al.* (1990) and Augier *et al.* (1993) report that mercury concentrations in the striped dolphin (*Stenella coeruleoalba*) declined in the following order: liver ≥ spleen ≥ blubber, kidney, pancreas ≥ stomach, lungs ≥ skeletal muscles, intestine, heart, brain, skin ≥ melon fat, blood.

Differences in concentrations between tissues can be high. In a study of mercury levels in spotted dolphins (*Stenella attenuata*) in the Pacific Ocean, André *et al.* (1990) reported that 95% of the burden analysed in 18 different tissues and organs was in the liver, skeletal muscle and blubber; levels in the liver (62 $\mu g.g^{-1}$ the highest concentrations) were 170 times higher than those in the blood, which contained the lowest concentrations (0.36 $\mu g.g^{-1}$) and were six times higher than those in the spleen (which contained the second highest concentration). Similar differences have been reported for other species. In long-finned pilot whales (*Globicephala melas*) hepatic concentrations were ten times higher than those in the kidney, which contained the second highest concentration, and in white-beaked dolphins

(*Lagenorhynchus albirostris*) the concentations were three times higher than those in the kidney (Muir *et al.*, 1988). Similar differences in concentrations have been recorded between other body sites. For instance, mercury concentrations were 25 times lower in the melon than in the muscle of striped dolphins from the Mediterranean (André *et al.*, 1991).

The kidney contained the highest cadmium concentrations in all species recorded in Table 2. These concentrations were also significantly higher than those reported in the liver, which generally contained the second highest concentrations. For instance, mean renal cadmium concentrations were four times higher than those in the liver in striped dolphins from the west Pacific Ocean (Honda *et al.*, 1983), four times higher in harbour porpoises (*Phocoena phocoena*) from Greenland (Paludan-Muller *et al.*, 1993) and three times higher in narwhals (*Monodon monoceros*) from Greenland (Hansen *et al.*, 1990). The high concentration of cadmium in the kidney may be connected to the presence of certain storage mechanisms using cadmium metallothionein protein (Fujise *et al.*, 1988; Marcovecchio *et al.*, 1990). This will be discussed further under the section on detoxifying strategies.

Table 2 also shows that high concentrations of certain metals are reported in the skin, blood and bones. Zinc concentrations were highest in the skin and bones in studies where all the organs and tissues were analysed, and highest in the liver where only soft tissues were analysed. Only in white whales (*Delphinapterus leucas*) sampled around Greenland were the highest concentrations found in the muscle (Hansen *et al.*, 1990). Large differences were also reported between the organ or tissue containing the highest and second highest concentrations. In two Dall's porpoises (*Phocoenoides dalli*) from the western Pacific Ocean, over half the total body burdens of zinc recorded were in the skin and similarly zinc concentrations in the skin of harbour porpoises from Greenland were seven times higher than those found in the liver (Paludan-Muller *et al.*, 1993).

There are few comparative data between tissues for other metals (Table 2). High lead concentrations (40% of total burdens) were recorded in the skin and bones of Dall's porpoises (Fujise *et al.*, 1988), and high iron concentrations were reported in the blood and lungs of the same species. Selenium accumulation varies. Only one study has analysed selenium concentrations in all organs and tissues (Paludan-Muller *et al.*, 1993), and this found that the highest concentration occurred in the skin, five times greater than those in the kidney. Other studies, solely of soft tissues, recorded the highest concentrations to be in the liver, unsurprising in view of the strong correlation selenium displays with mercury concentrations.

Intra-species differences in concentrations

Two examples of intra-species differences in concentrations are given in Tables 3 and 4. Hepatic concentrations of mercury are several factors higher in minke whales from the Arctic than the Antarctic, the maximum concentrations in minke whales from the former region being 20 times higher than the maximum recorded in the Antarctic region. However, cadmium concentrations showed the reverse relationship. Maximum concentrations in Antarctic minke whales were about 20 times those recorded in the Arctic (Table 3).

The major difference between minke whale populations in the Arctic and Antarctic is their diet. Arctic minke whales feed principally on sand eels, *Ammodytes*, (Hansen *et al.*, 1990) whereas those in the Antarctic feed primarily on krill, *Euphasia* spp. (Honda *et al.*, 1987). Higher concentrations of cadmium and lower ones of mercury are found in krill when compared to fish (Honda *et al.*, 1987) and these differences are reflected in the concentrations outlined in Table 3. Honda *et al.* (1987) also stated that differences in the length of the food chain between the two regions may be relevant. The food chain in the Antarctic is short, providing less opportunity for biomagnification.

Table 3

Range and means of hepatic concentrations of cadmium and mercury in the minke whale population from three different areas (ng g^{-1} wet weight). ND: No data recorded. *The figure is the median, not the mean.

	Greenland[1]	Arctic[2]	Antarctic[3]
Cadmium	ND	500-1,450 900*	2,200-33,000
Mercury	70-410 180	140-2,680 390*	20-129 46.5*

[1]Johansen *et al.*, 1980 (*n*: 6). [2]Hansen *et al.*, 1990 (*n*: 24). [3]Honda *et al.*, 1987 (*n*: 135).

Table 4

Cadmium and mercury concentrations in the livers of harbour porpoises from two different areas (μg g^{-1} wet weight).

	Greenland[1]	UK coast[2]
Mercury	range=0.48-20.7 mean=6.23	range=0.6-150 mean=13.8
Cadmium	range=0.06-11.7 mean=4.29	range=0.03-1.2 mean=0.18

[1]Paludan-Muller *et al.*, 1993 (*n*: 43). [2]Law *et al.*, 1991 (*n*: 20).

Table 4 shows differences in mercury and cadmium concentrations in two discrete populations of harbour porpoises. Cadmium levels in those from Greenland were significantly higher than those found around the UK (by more than 20 times) but mercury concentrations were about half those from UK porpoises. The reason for this is less apparent than the minke whale example shown above, but may be due to higher mercury levels found in the environment around the UK and the fact that porpoises from Greenland feed on fish which contain higher cadmium levels than fish species found around UK coasts (Paludan-Muller *et al.*, 1993).

Differences in concentration levels between toothed and baleen cetaceans

Table 5 shows differences in the cadmium and mercury bioconcentration factors between odontocetes and mysticetes. Both in the Arctic and Antarctic they were higher in odontocetes than in mysticetes. This is clearly reflected in the concentration of metals in tissues. Honda *et al.* (1983) recorded a range of mercury concentrations in the liver of striped dolphins from the western Pacific Ocean (Table 6) of 1.7 μg^{-1} to 485 $\mu g.g^{-1}$ (mean: 205 $\mu g.g^{-1}$) whereas Honda *et al.* (1987) reported concentrations in the livers of minke whales in the Antarctic from 0.02 $\mu g.g^{-1}$ to 1.3 $\mu g.g^{-1}$ (mean: 0.4 $\mu g.g^{-1}$), a difference of over 500 times. Other baleen whales, such as bowhead whales sampled in the Arctic, show similarly low mercury levels (Byrne *et al.*, 1985). Indeed generally, the minimum concentration of mercury in the liver of toothed cetaceans is higher than the maximum concentration recorded in baleen whales.

Three principal reasons explain these differences (Honda *et al.*, 1987): (1) diet: toothed cetaceans' greater reliance on fish as a prey species; (2) geography: toothed cetaceans' predominance in coastal areas; and (3) length of food chain: toothed cetaceans' position as a top predator in longer food chains than those found in baleen whales.

Table 5

Bioconcentration factors for three metals from sea water to cetaceans (concentrations measured in the livers of narwhals and minke whales). ND: No data recorded.

	Cadmium	Mercury	Lead
Odontoceti[1]	4.0×10^6	3.0×10^5	1.9×10^3
Mysticeti[2]	5.5×10^5	4.3×10^4	ND

[1]Arctic ecosystem: Muir *et al.*, 1992. [2]Antarctic ecosystem: Honda *et al.*, 1987.

Table 6

Tentative trends in the relationship of the concentrations of nine metals with age category in the liver of striped dolphins from the west Pacific Ocean. (data taken from Honda *et al.*, 1983.) +ve: increase in concentration in this age bracket; -ve: decrease in concentration in this age bracket; -: no discernible change in concentration recorded; ND: no data recorded.

Age of cetacean	Fe	Zn	Pb	Mn	Ni	Cd	Hg	Se	Cu
Gestation period	+ve	+ve	+ve	+ve	ND	-	+ve	ND	+ve
Suckling (calf)	-ve	+ve	+ve	+ve	+ve	+ve	+ve	ND	+ve
Up to 8 years	+ve	-ve	-	-ve	-	-	+ve	+ve	-ve
Adult	-	-	+ve	-	+ve	+ve	+ve	+ve	-

Focardi *et al.* (1992) reported levels of mercury in baleen and toothed cetaceans from the Mediterranean Sea, the geographical region where the highest burden of mercury in a small cetacean has been recorded. Levels of mercury and cadmium were on average 5-20 times lower in baleen whales and three times lower than those recorded in toothed cetacean species in the same locality (Focardi *et al.*, 1992). Indeed small cetaceans have recorded levels of mercury and selenium which are higher than in any other organism (Koeman *et al.*, 1973; André *et al.*, 1991).

Although accumulation rates and concentration levels for most metals are generally lower in baleen whales, as reported earlier, species-specific differences can occur as a result of diet. Cadmium levels are usually higher in krill than in fish (Thompson, 1990; Hapke, 1991) and this explains the comparatively higher cadmium levels present in the krill-eating minke whales from the Antarctic than in the fish-eating cetaceans from the Pacific Ocean (Honda *et al.*, 1987).

However, when cephalopod-eating odontocetes are compared with krill-eating mysticetes, concentrations are much higher in the former, again reflecting dissimilar cadmium richness in their diets. Caurant *et al.* (1993) recorded cadmium concentrations in the kidney of long-finned pilot whales in the North Atlantic to be up to 30 times higher than those recorded for minke whales. They reported a range of concentrations in the kidney from one school of pilot whales 1.4-158 $\mu g.g^{-1}$ (mean: 93.1 $\mu g.g^{-1}$) which compares with a range of 1.7-5.6 $\mu g.g^{-1}$ (median: 3.7 $\mu g.g^{-1}$) for cadmium concentrations in the kidneys of minke whales in the Arctic (Hansen *et al.*, 1990) and 2.2-33 $\mu g.g^{-1}$ in hepatic tissues of minke whales from the Antarctic (Honda *et al.*, 1987).

The greater reliance of baleen whales on krill may also be responsible for higher levels of nickel found in these species than in toothed whales (Honda *et al.*, 1987), although data on nickel levels in toothed cetaceans are sparse, making comparison difficult.

PHYSIOLOGICAL EFFECTS

Toxicity occurs in a species when the accumulation of a metal is not matched by the body's storage, excretory, metabolic and detoxification mechanisms (Underwood, 1977; Piotrowski and Coleman, 1980). Once this stage is reached, spill-over of the metal occurs to other cells, particularly in soft tissues such as the liver and kidney (Underwood, 1977; Piotrowski and Coleman, 1980).

A number of factors will determine the actual toxic effects on a species. These will include the levels of metal ingested; the period of ingestion; any storage, metabolic, excretory or detoxifying mechanisms; synergistic interactions with concentrations of other metals; the tissue site; relationships and effects resulting from failure at other different tissue sites (Langston, 1990). Synergistic effects from high concentrations of other pollutants such as polyaromatic hydrocarbons have also been reported (George, 1990).

Little research has been undertaken on the effects of metals in cetacean species. The capacity for excretion of mercury appears to be low, so most ingested mercury remains in the animal (Nigro and Leonzio, 1993). Probably because mercury occurs naturally in the environment, to compensate for poor excretory mechanisms, storage and detoxifying strategies have evolved in many cetaceans. These allow metals to be stored in an inert state in tissues. Thus, the presence of high concentrations of a metal is not necessarily correlated with toxicity.

Detoxifying strategies

In many cetacean species, correlations have been reported between metal concentrations in the tissues and organs analysed (Table 7). Some of these relate to detoxification mechanisms.

Mercury

Most mercury available in the ecosystem is inorganic, but is converted by micro-organisms present in freshwater and marine sediments to methylmercury, a more toxic and readily bioaccumulative form (Law, 1996). The effect of detoxification can be seen in the high values of inorganic mercury recorded in cetaceans, despite the fact that most mercury is ingested in its organic form.

A decline in the ratio of methylmercury to total mercury has been recorded in the soft tissues of harbour porpoises (Joiris *et al.*, 1991), pilot whales (Julshamn *et al.*, 1987; Caurant *et al.*, 1993), narwhals (Wagemann *et al.*, 1984), striped dolphins (Itano *et al.*, 1984b) and common dolphins (Joiris *et al.*, 1992b) and in the hard tissues of striped dolphins (Honda *et al.*, 1986b), confirming that a de-methylating process occurs within the tissues of the animal. In one adult narwhal examined by Wagemann *et al.* (1984), methylmercury only represented 7% of total mercury values in the liver and 11% in the kidney.

Joiris *et al.* (1992b) interpreted mercury detoxification in common dolphins as follows: methylmercury concentrates in the fatty areas of the animal, where it is mineralised and re-mobilised to accumulate as inorganic mercury in the liver. Here it is detoxified by binding to selenium or metallothionein proteins.

The binding of mercury to selenium can be seen in the high levels of selenium that have been widely reported in conjunction with high levels of mercury in a number of cetacean species (Table 6), but appears to only occur on specific tissues in certain species and only after a certain age. It has been reported in the bone, kidney, liver and muscle of striped dolphins (Itano *et al.*, 1984b; Honda *et al.*, 1986b; Leonzio *et al.*, 1992), and the livers of pilot whales (Muir *et al.*, 1988; Caurant *et al.*, 1993), narwhals (Wagemann *et al.*, 1990) and harbour porpoises (Paludan-Muller *et al.*, 1993). However, no relationship was found in the

Table 7

Inter-metal correlations reported in cetaceans. Key = Ki: Kidney, Li: Liver, Mu: Muscle, Bo: Bone, Blu: Blubber; +ve: positive correlation recorded; -ve: negative correlation recorded.

	Tissue	Species	Reference
Mercury-selenium +ve	Li; Ki; Mu	White whale	Wagemann *et al.*, 1990
	Li; Ki	Narwhal	Wagemann *et al.*, 1983
	Li	Common dolphin	Joiris *et al.*, 1992b
	Li	Bottlenose dolphin	Nigro & Leonzio, 1993
	Li; Bo	Striped dolphin	Nigro & Leonzio, 1993
			Honda *et al.*, 1986a
	Li	Harbour porpoise	Teigen *et al.*, 1992
	Li	Minke whale	Hansen *et al.*, 1990
	Li; Ki	Pilot whale	Caurant *et al.*, 1993
	Li	Cuvier's beaked whale	Martoja & Viale, 1977
Cadmium-selenium +ve	Ki	Beluga	Wagemann *et al.*, 1990
	Li; Mu	Pilot whale	Caurant *et al.*, 1993
	Li	White whale	
		Minke whale	Hansen *et al.*, 1990
		Narwhal	
Cadmium-mercury -ve	Ki	Beluga	Wagemann *et al.*, 1990
Cadmium-mercury +ve	Li; Blu	Narwhal	Wagemann *et al.*, 1983
	Li; Ki; Mu	Pilot whale	Caurant *et al.*, 1993
Cadmium-zinc +ve	Li; Ki	Striped dolphin	Honda & Tatsukawa, 1983
	Li; Ki	Beluga	Wagemann *et al.*, 1990
	Li; Ki	Narwhal	Wagemann *et al.*, 1983
	Ki	Harbour porpoise	Paludan-Muller *et al.*, 1993
	Li	Minke whale	Honda *et al.*, 1987
	Li; Ki	Pilot whale	Caurant *et al.*, 1993
Lead-cadmium +ve	Ki	Narwhal	Wagemann *et al.*, 1983
Zinc-mercury -ve	Ki	Beluga	Wagemann *et al.*, 1990
Zinc-mercury +ve	Mu	Beluga	
	Li	Minke whale	Honda *et al.*, 1986b
	Ki	Pilot whale	Caurant *et al.*, 1993
Zinc-selenium +ve	Ki	Beluga	Wagemann *et al.*, 1990
Silver-mercury +ve	Li	Beluga	Becker *et al.*, 1995
		Pilot whale	

muscle of harbour porpoises (Schnapp, 1993) or in the liver of long-finned pilot whale foetuses (Caurant and Navarro, 1994).

The interactions between selenium and mercury are still poorly understood. Detoxification could occur due to competition for binding sites, or a formation of a less toxic and more easily storable complex such as mercury selenide (Koeman *et al.*, 1973; Augier *et al.*, 1993). The occurrence of mercury selenide granules within phagocytic cells reported by Nigro and Leonzio (1993) in bottlenose dolphins suggests that the production of mercury selenide, and thus the detoxification of methylmercury, is performed by phagocytosis. As mercury selenide granules have been reported in the liver (Martoja and Viale, 1977; Nigro and Leonzio, 1993), lungs (Augier *et al.*, 1993) brain and muscle (Nigro and Leonzio, 1993) of cetacean species, it appears that storage and detoxification of mercury occurs at different sites. However, cetaceans cannot excrete mercury selenide (Martoja and Berry, 1980; Caurant *et al.*, 1994), so particles will accumulate in their cells.

By binding mercury to metal-binding proteins, damage is reduced and the storage of certain metals regulated. The actual toxic effects of the metal will only occur once the binding capacity of the metallothionein becomes saturated and a spillover of excess ions

occurs to other cells (Langston, 1990). Metallothioneins have been found in long-finned pilot whales (Caurant *et al.*, 1993), narwhals (Wagemann *et al.*, 1984) and common dolphins (Joiris *et al.*, 1992b).

Other metals

The sequestration of free ions of metals by metallothionein has been recorded in a number of species for other metals, including, in descending order of binding affinity, copper, cadmium and zinc; no sequestration of lead has yet been reported (Eisler, 1984; Quarterman, 1986; Tohyama *et al.*, 1986; Law, 1996).

Paludan-Muller *et al.* (1993) reported that the relationship between zinc and cadmium concentrations in the kidneys of harbour porpoises was due to cadmium binding to zinc-metallothionein. This correlation has also been recorded in the liver of narwhals and white whales (Hansen *et al.*, 1990) and in the liver and kidney of other marine mammals (Wagemann and Stewart, 1994). Wagemann *et al.* (1984) reported that in the liver of a narwhal, a high percentage of both cadmium and copper were thionein-bound, whereas for mercury it was lower. Similarly, in the livers of common dolphins, Joiris *et al.* (1992b) reported that 50% of inorganic mercury was not thionein or selenium-bound and was thus potentially toxic.

Other synergistic effects have been reported. A deficiency in levels of iron and zinc, for example, can increase the absorption rate of lead in certain species (Honda and Tatsukawa, 1983; Kostial, 1986; Quarterman, 1986). Honda and Tatsukawa (1983) also reported that cadmium accumulation may inhibit detoxification rates for zinc and copper in striped dolphins. Other variables can increase the toxic potential of a metal. High water temperature and low salinity have been reported to react with metals such as cadmium, mercury and zinc to result in an increase in the metal's toxic potential (Langston, 1990).

Effects of metals in cetacean species

Mercury

The high toxicity, long biological half-life, lipophilicity and biomagnification of mercury in the food chain make this metal one of the most threatening. In their review, Wagemann and Muir (1984) proposed that tolerance limits for mercury in mammals may be in the range of 100-400 $\mu g.g^{-1}$ in hepatic tissue, although the evidence for this is unclear. Table 8 shows that seven studies of three species have reported concentrations above this limit.

Despite the assertion in Wagemann and Muir (1984), studies to ascertain the effects of these concentrations in cetaceans are rare, although in certain non-cetacean species, mercury poisoning has resulted in serious disorders in the liver, kidney and brain, and methylmercury poisoning resulted in behavioural defects, loss of coordination and loss of vision. In other marine mammal species, high hepatic and renal mercury concentrations have caused liver and kidney failure (Law, 1996). Samples of six of the seven case studies of cetaceans shown in Table 8 were taken from stranded dolphins, suggesting a possible causal link with high mercury concentrations (Augier *et al.*, 1993).

Rawson *et al.* (1993) reported toxic effects of mercury in a pod of bottlenose dolphins stranded off the USA coast. Nine of the 18 animals sampled had extensive deposits of a granular pigment within the livers' portal areas. These animals also contained the highest mercury liver concentrations which ranged from 61-433 $\mu g.g^{-1}$. Furthermore, four of the nine animals with pigmentation deposits also had active liver disease, including necrosis and fat globules among the hepatocytes adjacent to the portal areas. The presence of fat globules revealed that the animals' fat metabolism had been affected, and may have led to cell death. In the absence of any correlation with age, Rawson *et al.* (1993) suggested that the pigment accumulation was related to the toxic effect of mercury.

Table 8

Concentrations of mercury recorded in the liver of cetaceans which exceed the proposed tolerence limits suggested by Wagemann & Muir, 1984 (100-400μg.g^{-1}). All figures are μg.g^{-1} wet weight except *=dry wt.

	Range of concentrations	Mean/median concentration	Geographical area	Reference
White whale				
(*n*: 30)	1.42-756*	126*	St Lawrence, Canada[2]	Wagemann *et al.*, 1990
False killer whale				
(*n*: 38)	41-479	249	New South Wales, Australia[2]	Kemper *et al.*, 1994
Bottlenose dolphin				
(*n*: 12)	0.1-443	134.6	US[2]	Rawson *et al.*, 1993
(*n*: 4)	12.2-13,155.6*	med: 270.4*	Mediterranean[2]	Leonzio *et al.*, 1992
Striped dolphin				
(*n*: 45)	1.7-475	205	West Pacific[1]	Honda *et al.*, 1983
(*n*: 25)	1.2-1,544	346.1	Mediterranean[2]	Andre *et al.*, 1991
(*n*: 13)		med: 327		
(*n*: 19)	48-1,613	474	Mediterranean[2]	Augier *et al.*, 1993
	324.4-4,400*	med: 324.4*	Mediterranean[2]	Leonzio *et al.*, 1992

[1]Samples taken from freshly killed animal. [2]Samples taken from dead animal.

The effects of anthropogenic pollutants have also been studied extensively over a nine year period on the population of white whales in the St Lawrence River, Canada. Pathological abnormalities such as bladder cancer, severe lesions and tumours have been reported (Martineau *et al.*, 1985; 1988; 1994). Twenty-four neoplasms were found in 18 of the 45 animals necropsied in the nine year study, eight neoplasms being malignant (Béland *et al.*, 1993). The population has a high level of bacterial infections, pneumonia and tooth loss, about 2% have spinal deformities and its reproductive rate is only half that found in other white whale populations (Martineau *et al.*, 1988; Béland *et al.*, 1993). Although mercury levels are extremely high in white whales from the St Lawrence (Table 8), it is difficult to attribute specific effects to mercury poisoning as the concentrations of lead (Wagemann *et al.*, 1990) and other anthropogenic pollutants, notably organochlorines (Béland *et al.*, 1992; 1993), are also high. Wagemann *et al.* (1990) believed that the adverse effects reported in this population are likely to be a combination of all the toxic elements acting over a long time period (see also the review by Martineau *et al.* in the present volume).

So, for certain species, there is some evidence that high levels of mercury may have resulted or contributed to chronic illness in disease and mortality in die-offs (Wagemann *et al.*, 1990; Béland *et al.*, 1992; Augier *et al.*, 1993; Law, 1996). However, equally high mercury levels in mature striped dolphins from the North Pacific have apparently not resulted in any side effects (Itano *et al.*, 1984a). This difference may be due to interspecific differences in susceptibility to the effects of metals or that the rate of bioaccumulation of mercury is more important than its actual burden level.

Other metals

Wagemann and Muir (1984) were unable to suggest tolerance limits for metals other than mercury. In the absence of any specific marine mammal values for cadmium tolerance limits in the kidney, Law (1996) has used the figures suggested for humans where renal damage occurs above concentrations of 200-400 μg.g^{-1} (Piotrowski and Coleman, 1980). From research on the association of cadmium concentrations in the kidney and liver, Fujise *et al.* (1988) proposed that concentrations of cadmium higher than 20 μg.g^{-1} in the liver would

result in renal dysfunction. Taking the tolerance figure for the kidney, Law (1996) proposed that this corresponded to a liver tolerance figure in the range of 40-200 $\mu g.g^{-1}$. Maximum and mean concentrations reported in cetaceans above these proposed tolerance limits are shown in Table 9.

According to Caurant *et al.* (1994), renal cadmium levels varied considerably among schools of Faroese long-finned pilot whales, with several having levels higher than 100 $\mu g.g^{-1}$, possibly approaching critical levels. Cadmium concentrations in the blood were also higher than the minimum levels established for adverse effects in humans. Caurant *et al.* (1993), whilst suggesting that high levels might reflect an adaptive response of pilot whales, qualified this by noting that those animals which contained high cadmium concentrations had less efficient regulation of copper and zinc (cadmium is bound to available metallothionein thus reducing its function of ensuring homeostasis of copper and zinc). Wagemann *et al.* (1983) also reported high cadmium concentrations in narwhals from the Arctic. They did not examine metal damage in the narwhals' kidneys but reported that concentrations were high enough to cause renal dysfunction. In non-cetacean species, cadmium poisoning has resulted in adverse effects on reproduction, growth and bone structure (Kostial, 1986), but to date no causal relationship between cadmium and physical effects have been reported in cetaceans, although Caurant *et al.* (1994) associated gastric erosion and ulcers in pilot whales with high cadmium levels.

Little work has been done on the effects of the other metals in cetaceans. Levels of lead tend to be low although Wagemann *et al.* (1990) reported that the St Lawrence River white whale population had very high levels, reaching 2.13 $\mu g.g^{-1}$ dry weight in the liver (mean: 0.59 $\mu g.g^{-1}$ dry weight; n:30). These concentrations, about 10 times higher than those found in Arctic white whales, were attributed to high aquatic levels of lead resulting from anthropogenic sources (Wagemann *et al.*, 1990). A young bottlenose dolphin stranded along the South Australian coast had levels of 61 $\mu g.g^{-1}$ in the bone, which Kemper *et al.* (1994)

Table 9

Concentrations of cadmium in the liver and kidney which exceed the range of tolerance limits proposed by Piotrowski & Coleman (1980), Fujise *et al.* (1988) and Law (1996) (200-400 $\mu g\ g^{-1}$ in the kidney and 20-200 $\mu g\ g^{-1}$ in the liver). x: mean value. All concentrations are wet weight except [1] converted from dry weight (Law, in press) and [2] dry weight. All samples analysed from freshly killed animals.

		Hepatic concentration ≥20-200$\mu g\ g^{-1}$	Renal concentration ≥200-400$\mu g\ g^{-1}$	Geographical region	Reference
Narwhal	(*n*: 98)	0.02-73.7	-	Greenland	Hansen *et al.*, 1990
	(*n*: 38; Li)	1.28-130.8	1.0-205.4	Canadian Arctic	Wagemann *et al.*, 1983
	(*n*: 55; Ki)	x: 34.1	x: 63.5		
	(*n*: 55)	2.44-137	-	Canadian Arctic	Wagemann *et al.*, 1996
		x:29.7			
White whale	(*n*: 109)	0.03-97[2]	0.05-277[2]	Canadian Arctic	Wagemann *et al.*, 1990
		x: 12.5[2]	x: 48.2[2]		
Minke whale	(*n*: 27)	2.2-33	-	Antarctic	Honda *et al.*, 1987
False killer whale	(*n*: 27)	14.3-75.8[2]	-	Australia	Kemper *et al.*, 1994
		40.4[2]			
Pilot whale	(*n*: 52)	0.1-94	-	Atlantic Ocean	Caurant *et al.*, 1993
		x: 41			Julshamn *et al.*, 1987
		0.74-125	-		
			1.4-962		Caurant & Amiard-Triquet,
			x: 78		1995
	(*n*: 13)	0.03-118.9[1]	-	Canada	Muir *et al.*, 1988
		x: 54.2[1]			Law, 1996

attributed to contamination from a lead smelter in the area. In non-cetacean species, lead poisoning is associated with the inhibition of enzyme systems, renal damage and cardiac disease (Quarterman, 1986).

REGIONAL DIFFERENCES IN BIOCONCENTRATIONS

The regional concentration of a metal, and thus its availability to marine biota, is dependent on the source of the metal and its method of transportation to and within the marine environment. Any localised high concentrations of metals will be important in determining levels of transfer to species whose range includes such regions. Thus, metals derived from anthropogenic sources in the form of fossil fuel combustion emissions (e.g. lead) show higher concentrations in areas close to the shoreline source (Davis, 1993; Herut *et al.*, 1993). Coastal concentrations are also high for those metals deposited by riverine transportation (e.g. manganese, aluminium and copper), which tend to show high levels close to and a rapid decrease away from the source.

Only a comparison of metal concentrations from the same tissues or organs and from similar species and comparable habitats, can provide data for a preliminary analysis of geographical differences in metal levels. Even attempts to identify 'hot spots' from metal concentrations in cetaceans can only be tentative due to the other, often uncontrolled, factors that affect concentration levels, particularly the influence of diet. For instance, cadmium concentrations in narwhals from Baffin Bay were 100 times higher than those for white-beaked dolphins from Newfoundland, whilst lead concentrations were 40 times lower; dietary rather than higher background pollution levels were considered responsible for these differences (Muir *et al.*, 1988). Lima and Sequeira (1993) also showed that mercury concentrations in common dolphins from the Portuguese coast were lower than those found in the Mediterranean, where the main prey species, sardines *(Sardinus pilchardus)*, contained very low mercury levels.

Differences have also been reported in concentrations of mercury from species caught in the same place. As Table 10 shows, mean liver concentrations of mercury in adult long-finned pilot whales (*Globicephala melas*) caught in the Faroe Islands in 1977 was 280.2 $\mu g.g^{-1}$ but data from animals caught in 1978 showed a decline to a fifth of the previous years figure, 53.4 $\mu g.g^{-1}$ (Julshamn *et al.*, 1987). This decline is also reflected in the mean muscle levels of mercury which in 1978 averaged 1.8 $\mu g.g^{-1}$, almost half the mean of 3.3 $\mu g.g^{-1}$ recorded in the previous year. The cause of this discrepancy may be due to discrete populations of pilot whales feeding on different prey items rather than a change in the background pollutant levels of mercury (Julshamn *et al.*, 1987). Further differences were reported by Caurant *et al.* (1993) on two schools of pilot whales caught in the same place both in 1986. These showed large differences in mean mercury concentrations in the liver (52.1 $\mu g.g^{-1}$ compared to 84.1 $\mu g.g^{-1}$). Differences in mercury concentrations from the same geographical area have also been reported for striped dolphins (Itano *et al.*, 1984b).

Despite recognising these sources of variation, Table 10 presents data on the hepatic concentrations of mercury in seven cetacean species from eight regions in an attempt to tentatively identify certain 'hot spots' where reported concentrations of mercury are significantly higher.

Extremely high levels of mercury have been recorded in stranded striped (1,544 $\mu g.g^{-1}$ ww) and bottlenose (3,828 $\mu g.g^{-1}$ ww) dolphins from the Mediterranean Sea (André *et al.*, 1991; Leonzio *et al.*, 1992; Law, 1996). The reasons for the high levels recorded in cetaceans from the Mediterranean Sea is likely to be a combination of anthropogenic causes and the high level of background geological mercury levels present in the area (Augier *et al.*, 1993).

Table 10

A geographical comparison of mercury concentrations in the liver of eight toothed cetacean species ($\mu g.g^{-1}$). * = median value, ND: no data reported. All values are dry weight except [1] which has been converted from dry weight and [2] which is dry weight.

Species	Region	Range (where known)	Mean	Study
Striped dolphin (*n*: 35)	Mediterranean	48-1,613	474.0[1]	Augier *et al.*, 1993
Striped dolphin (*n*: 45)	NW Pacific	1.7-485	205.0	Honda *et al.*, 1983
Pantropical spotted dolphin (*n*: 44)	ET Pacific	0.18-218	62.7	André *et al.*, 1990
Long-finned pilot whale (*n*: 14)	Faroe islands	ND	280.0	Julshamn *et al.*, 1987
Narwhal (*n*: 98)	Arctic	0.01-42.8	5.26*	Hansen *et al.*, 1990
Harbour porpoise (*n*: 36)	Irish Sea	0.6-190	20.5	Law *et al.*, 1992
Bottlenose dolphin (*n*: 1)	SW Atlantic	ND	86.0	Marcovecchio *et al.*, 1990
White-beaked dolphin (*n*: 27)	NW Atlantic	0.13-1.6	3.0[2]	Muir *et al.*, 1988

Similarly there appears to be a relationship between the high levels recorded and anthropogenic sources in the western North Pacific. Mercury input into the ocean from Japanese chlor-alkali production in 1970 alone amounted to 650 tons, and a correlation has been reported between the increasing amount of industrial waste inputs into the marine environment and the levels of metals present in the sediments (Goto, 1973; André *et al.*, 1991).

Law *et al.* (1992) have identified another 'hot spot' in the Irish Sea. Inputs from a variety of industrial sources, particularly from local phosphate plants, raised levels of cadmium in seawater in the area to about 50 times higher than that found in the open ocean, and levels of zinc and lead to ten times higher (Forstner, 1980). This explains the relatively high concentrations of metals in cetaceans from the eastern Irish Sea, for example those found in harbour porpoises (Table 10), which continue to remain high despite a reduction in inputs in the past 10 years (Law *et al.*, 1991; 1992).

CONCLUSIONS

In the first published overview of metals in marine mammals, Wagemann and Muir (1984) reviewed 16 different studies on 14 cetacean species. In the ten years subsequent to this review this database has increased to over 70 studies on 26 different species (the major ones are shown in Appendix 1). Some of the difficulties outlined at the start of this review continue to apply. Studies still tend to be limited to odontocetes, mercury concentrations and soft tissues. Comparison of concentrations between different species is still difficult because of the number of uncontrolled variables that can affect the levels reported. Different analytical techniques increase the difficulty, although there have been calls to establish a more standardised and coordinated approach (Kuiken and Hartmann, 1991; Kemper *et al.*, 1994) and several long-term studies using consistent techniques have recently been published (Law, 1994; Marcovecchio *et al.*, 1994; Miyazaki, 1994).

The increase in data has resulted in definite trends being established for the accumulation of many metals within the cetacean body. There is a large database on the site specificity of metal concentrations within the animal. Most metals accumulate in the soft tissues, particularly in the liver and kidney. Since 1984, new information has shown that the highest concentrations of zinc and lead have been found in hard tissues such as the skin and bone, and that concentrations of certain metals are transferred between the female and her young. New data have also been recorded on bioaccumulation rates throughout the food chain. Although this is limited to certain metals, studies to date show that only mercury biomagnifies at each level of the food chain.

Wagemann and Muir (1984) stated that systematic differences were not apparent in metal concentrations but, since then, new data have revealed both regional and species differentiation in concentrations of metals. Many of these are due to differences in background levels of metals and diet. Baleen whale species have lower concentrations of the majority of metals due to a shorter food chain and the fact that they feed lower in the trophic chain than odontocetes (O'Shea and Brownell, 1994).

In the past decade, information on detoxification has improved. It is now known that the positive correlation between mercury, probably the most toxic metal, and selenium results in a detoxification of the organic mercury into a storable compound. Metallothioneins can also reduce damage to cells by binding the toxic metals. Toxicity will occur in a species when the accumulation rate is greater than the combined detoxification, excretion or storage rates, but the actual levels of concentration needed for toxicity to occur are still unknown for most metals or species. Tentative ranges have been proposed for tolerance levels of hepatic and renal mercury and cadmium concentrations, and there are several examples of species which have concentrations exceeding these limits. A possible causal link between high mercury levels and liver disease has been suggested in two study groups of animals (Rawson *et al.*, 1993; Caurant *et al.*, 1994). There may also be a causal link between high levels of metals and 'die-offs' (André *et al.*, 1991; Béland *et al.*, 1992; 1993). Although the effects of these concentrations according to variables such as the species, age and sex, have yet to be established, the available data suggests that high levels of metals have an impact on at least some cetacean species.

ACKNOWLEDGEMENTS

Dr S. Pullen, WWF-UK and Dr K. Brown, University of Durham, gave invaluable assistance in the preparation of this review. Dr R. Law, Directorate of Fisheries Research, UK Ministry of Agriculture, Fisheries and Food and M. Simmonds, University of Greenwich, UK, provided useful comments on an earlier draft of this paper. Two anonymous referees also gave comments on the paper. Finally, thanks are due to the IWC Secretariat for their help in the final preparation of this paper and for providing answers to endless questions.

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Editors' note: The paper of Henry and Best (pp. 177-94 in this volume) arrived too late for inclusion in this review.

Appendix 1

Summary of species, metals and geographical areas covered in this review.

Species	Metal	Tissue/Organ	Area	No.
MYSTICETI				
Minke whale,	Hg, Cd, Zn, Cu, Pb, Fe, Mn	Li	Ant	1
Balaenoptera acutorostrata	Hg, Cd, Se, Zn	Li, Ki, Mu	GD	2
	Cd	Li, Ki, Blu	GD	32
	Hg	Li, Mu	GD	6
	Hg	Li, Ki, Mu	GD	34
	Hg, Cd, Zn, Cu, Pb, Cr	Li	UK	11
	Hg	Mu	NWP	49
	Hg	Mu	NWP	50
Sei whale,	Hg	Mu	SP	5
Balaenoptera borealis	Hg	Mu	NWP	49
Bowhead whale,	Hg, Cd, Se, Zn, Cu, Pb, Ni, Ag	Li, Ki, Mu, Blu	AS	17
Balaena mysticetus	Hg	Li, Ki, Mu, Blu	AS	62
	Hg, Cd, Se, Zn, Cu, Pb, Fe	Li, Ki, Mu, Blu	AS	71
	Ag	Li	AS	75
Bryde's whale,	Hg	Mu	NWP	50
Balaenoptera edeni				
Fin whale,	Hg	Li, Ki, Mu	NEA	3
Balaenoptera physalus	Hg, Cd, Pb	Sk	Med	4
	Hg	Mu	SP	5
Pygmy right whale,	Hg, Cd, Pb	Li, Mu, Blu, Bo	Aust	69
Caperea marginata				
Gray whale,	Hg, Cd, Se, Zn, Cu, Pb, Fe, Ni, Ag	Li, Ki, Sto, Br	AS, ETP	71
Eschrichtius robustus				
ODONTOCETI				
Sperm whale,	Hg	Mu	Sp	5
Physeter macrocephalus	Hg	Mu	Ant	5
	Hg	Li, Mu	NS	25
	Hg	Mu	NWP	49
	Hg	Mu	NWP	50
	Hg, Cd	Li	NWP	63
	Hg, Cd, Pb	Li, Ki, Mu, Blu	Aust	72
	Hg	Mu	Aust	73
	Hg,Cd, Se, Zn, Cu, Pb, As, Ni, Cr	Li	UK	76
Pygmy sperm whale,	Hg, Cd, Zn, Cu	Li, Ki, Mu, Blu	SWA	8
Kogia breviceps	Hg, Cd, Pb	Li, Mu, Blu, Bo	Aust	72
Ganges river dolphin,	Hg, Cd, Zn, Cu, Pb, Fe, Ni, Cr	Li, Ki, Mu	ID	7
Platanista gangetica				
Franciscana,	Hg, Cd, Zn, Cu	Li, Ki, Mu, Blu	SWA	8
Pontoporia blainvillei				
White whale,	Hg, Cd, Se, Zn	Li, Ki, Mu	GD	2
Delphinapterus leucas	Hg, Cd, Pb	Li, Ki, Mu	GD	34
	Hg	Li, Ki, Mu	CA	32
	Hg	Li, Ki, Mu	CA	42
	Hg	Li, Mu, Blu	CA	44
	Hg, Cd, Se	Li	SLA	27
	Hg	Li, Ki, Mu	SLA	60
	Hg, Cd, Se, Zn, Cu, Pb	Li, Ki, Mu	SLA/C	33
	Hg, Se, Ag	Li	A	75
	Hg, Cd, Se, Zn, Cu, Pb	Li, Ki, Mu, Sk	AS CA	78

Species	Metal	Tissue/Organ	Area	No.
Narwhal,	Hg, Cd, Se, Zn,	Li, Ki, Mu	GD	2
Monodon monoceros	Hg	Li, Ki, Mu	GD	34
	Hg, Cd, Se, Zn, Cu, Pb, As	Li, Ki, Mu, Blu	CA	20
	Hg	Li, Ki, Mu	CA	42
	Hg, Cd, Zn, Cu	Li, Ki	CA	64
	Hg, Cd, Se, Zn, Cu, Pb	Li, Ki, Mu, Sk	CA	78
Harbour porpoise,	Hg	Li, Mu	CA	19
Phocoena phocoena	Hg	Li, Ki, Mu	CA	24
	Hg, Cd, Se, Zn, Cu	Li, Ki, Mu, Sk	GD	10
	Hg, Cd, Zn, Cu, Pb, Cr	Li	UK	11
	Hg, Cd, Zn, Cu, Pb, Ni, Cr	Li	UK	15
	Hg, Cd, Se, Zn, Cu, Pb, Ni	Li, Ki	UK	66
	Hg, Cd, Zn, Cu, Pb	Li, Ki, Br	UK	18
	Hg, Cd, Zn, Cu, Pb, Ni, Cr	Li, Mu, Blu	UK	35
	Hg, Cd, Pb	Li, Ki, He, Sp, Br	UK	22
	Hg, Se	Mu	UK	53
	Hg	Li, Ki, Mu	NS	25
	Hg	Li, Mu	NS	43
	Hg, Cu, Pb, Zn	Li, Mu,Blu	NS	45
	Hg, Cd, Zn, Cu, Pb	Li, Ki, Mu	NS	46
	Hg, Cd, Zn	Li, Ki, Blu	NS	54
	Hg, Se	Li, Ki	NY	14
	Ag	Li	AS	75
	Hg, Cd	Ki	NEA	79
	Hg	Li, Ki	NEA	80
	Hg, Cd, Se, Zn, As	Li, Br	NEA	81
	Hg	Li, Ki, Mu, Blu, Br	UK	82
	Hg, Cd, Cu, Zn, Pb, Ni, Mn	Li	UK	83
Dall's porpoise,	Hg, Cd, Zn, Cu, Pb, Fe, Mn	Li, Ki, Mu, Sk, Bl	NWP	13
Phocoenoides dalli				
White beaked dolphin,	Hg, Cd, Se, Zn, Cu, Pb, Cr	Li, Ki	UK	66
Lagenorhynchus albirostris	Hg, Cd, Zn, Cu, Pb, Cr	Li	UK	11
	Hg, Cd, Se, Zn, Cu, Pb	Li, Ki, Mu	AC	9
White sided dolphin,	Hg, Cd, Se, Zn, Cu, Pb, Cr	Li	UK	66
Lagenorhynchus acutus				
Bottlenose dolphin,	Hg, Cd, Se, Zn, Cu, Pb, Cr	Li, Ki	UK	66
Tursiops truncatus	Hg, Cd, Zn, Cu, Pb, Cr	Li	UK	11
Tursiops gephyreus	Hg, Cd, Zn, Cu	Li, Mu, Blu	UK	35
	Hg	Li, Ki, Mu	NS	25
	Hg	Li	WA	26
	Cu	Li, Ki, Mu	WA	57
	Hg, Cd, Zn, Cu, Pb	Bo	WA	59
	Hg, Cd, Zn, Cu, Pb, Ni, Cr	Li, Ki, Mu, Blu	SWA	8
	Hg, Cd, Se, Zn, Pb	Li, Ki, Mu	Med	37
	Hg, Se	Li, Ki, Mu, Br	Med	51
	Hg, Cd, Pb	Li, Ki, Mu, Blu, Bo	Aust	72
Pantropical spotted dolphin,	Hg	Li, Ki, Mu, Sk, Bo, Blu	ETP	39
Stenella attenuata	Hg, Se	Mu	NWP	41
Striped dolphin,	Hg, Cd	Li, Ki, Mu	NWP	12
Stenella coeruleoalba	Zn, Cu, Pb, Fe, Mn	Li, Ki, Mu, Sk, Bo	NWP	30
	Hg, Se	Li, Ki, Mu	NWP	21
	Cd, Zn	Bo	NWP	29
	Hg, Se, Zn, Cu, Pb, Fe, Mn	Li, Mu, Blu	NWP	31
	Hg, Se	Li, Ki, Mu	NWP	41
	Hg, Se	Li	UK	66

Species	Metal	Tissue/Organ	Area	No.
	Hg, Cd, Se, Zn, Cu, Pb, Cr	Li	UK	11
Striped dolphin,	Hg, Cd, Zn, Cu, Pb, Fe, Mn	Li	Med	16
Stenella coeruleoalba	Hg	Li, Ki, Mu, Sk, Blu	Med	23
(cont.)	Hg	Sk	Med	4
	Hg, Cd, Pb	Li, Ki, Mu	Med	37
	Hg, Cd, Se, Zn, Pb	Li, Ki	Med	47
	Hg, Se	Li, Ki, Mu, Br	Med	51
	Hg, Se	Li, Ki, Mu, Sk	Med	55
	Hg, Cd, Pb	Li, Ki, Mu, Blu	Aust	72
Common dolphin,	Hg, Cd, Zn, Cu, Pb, Cr	Li	UK	11
Delphinus delphis	Hg, Cd, Se, Zn, Cu, Pb, Cr	Li, Ki, Sto	UK	66
	Hg	Li, Ki, Mu	NS	25
	Hg	Li, Mu, Blu	NS	65
	Hg	Li, Ki, Mu, Blu, Br	EA	52
	Hg, Cd, Zn, Cu	Li, Ki, Mu, Blu	EA	59
	Cd, Zn	Li, Ki, Mu	SWA	58
	Hg, Cd, Pb	Li, Ki, Mu, Blu, Bo	Aust	72
Risso's dolphin,	Hg, Cd, Zn, Cu, Pb, Cr	Li	UK	66
Grampus griseus				
Long-finned pilot whale,	Hg, Cd, Se, Zn, Cu, Pb	Li, Ki, Mu	CA	9
Globicephala melas	Hg, Cd, Se, Zn, Cu	Li, Ki, Mu	NEA	28
	Hg, Cd, Se, Zn, Cu	Li, Ki, Mu	NEA	38
	Hg	Blu	NEA	41
	Cd	Li, Ki, Mu, Bl	NEA	74
	Hg, Se	Mu	NWP	41
	Hg, Cd, Se, Zn, Cu, Pb, Cr	Li	UK	66
Short-finned pilot whale,	Hg, Cd, Se	Li, Ki	USA	32
Globicephala macrorhynchus	Hg	Li, Ki	WI	40
	Hg, Pb	Li, Ki, Mu	Aust	72
Killer whale,	Hg, Cd, Pb	Li, Ki	Aust	72
Orcinus orca				
False killer whale,	Hg, Cd, Pb	Li, Ki, Mu, Blu	Aust	72
Pseudorca crassidens				
Cuvier's beaked whale,	Hg, Se	Li	NEA	67
Ziphius cavirostris	Hg, Cd	Li, Ki, Mu, Blu	WA	36
	Hg, Zn, Cu, Pb,, Fe, Mn, Ni	Li, Ki, Mu	SWA	68
Beaked whale,	Hg, Cd, Pb	Li, Ki, Mu, Blu, Bo	Aust	72
Mesoplodon spp				
Bottlenose whale,	Hg	Li, Mu	NS	43
Hyperoodon ampullatus				

SITES

Li: Liver; Ki: Kidney; Mu: Muscle; Blu: Blubber; Bl: Blood; Sk: Skin; Bo: Bone; Br: Brain; Sp: Spleen; He: Heart; Sto: Stomach

GEOGRAPHICAL AREAS

Ant: Antarctic; AS: Alaska; Aust: Australia; CA: Canada; EA: East Atlantic Ocean; ETP: Eastern Tropical Pacific Ocean; GD: Greenland; ID: India; Med: Mediterranean Sea; NEA: North East Atlantic Ocean; NS: North and Baltic Seas; NWP: North West Pacific Ocean; NY: Norwegian coast; SLA: St Lawrence Seaway, Canada; SP: South Pacific Ocean; SWA: South West Atlantic Ocean; UK: UK coastline; WA: West Atlantic Ocean; WI: West Indies

STUDIES

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J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 149–155, 1999

Summary of temporal trends in pollutant levels observed in marine mammals*

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ABSTRACT

The present paper reviews reported time trends in concentrations and relative abundance of pollutants in marine mammals. Available information refers only to pinnipeds and cetaceans, mainly covers the period 1969-1988 and focuses on DDTs, PCBs and mercury. Although data are limited, there are indications that in the Canadian Arctic, mercury levels in marine mammals have increased in recent decades. By contrast, during the late 1970s and the 1980s, concentrations of DDTs and PCBs in marine mammals from highly polluted areas have tended to decrease. While this trend is likely to continue for DDTs in the future, it is foreseen that until at least the first decades of the next century, PCB levels will stabilise as degradation is compensated by new inputs caused by the recycling of the fraction currently present in non-marine compartments.

KEYWORDS: REVIEW; TRENDS; POLLUTION-METALS; POLLUTION- ORGANOCHLORINE; POLLUTION-PESTICIDES; CETACEANS-GENERAL; PINNIPEDS

INTRODUCTION

The history of the production, use and release into the environment of the different chemicals now considered as pollutants is complex and extremely variable from one compound to another. This fact, together with their different persistence and dispersal rates, makes it extremely difficult to assess historic time trends as well as to predict future trends in the level of exposure of cetaceans to these compounds. For synthetic chemicals such as organochlorines and organobrominates, it is obvious that present concentrations are higher than in preindustrial times, although variations over time in recent years are not easy to determine. In the case of trace elements, radionuclides and polyaromatic hydrocarbons, compounds that are also naturally occurring, there is some controversy as to whether the levels detected in the environment today have always been there. The general opinion is that pre-industrial levels were lower and that an increasing temporal trend is superimposed on the geochemical baseline levels (Wagemann *et al.*, 1990).

It is the intention of this summary paper to delineate observed trends in pollutant levels in marine mammals to assess probable future trends on a global scale.

HISTORIC TRENDS

For synthetic chemicals, there is a paucity of data on levels in pre-industrial or early industrial times. This is largely due to the fact that most compounds of this type presently found in marine mammals were only introduced in the 1930s or later.

* A version of this paper was originally submitted to the IWC Scientific Committee as SC/M95/P7.

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In the case of trace elements, the situation is different. Glacial records show that heavy metals have been in the marine environment since prehistoric times (Murozumi *et al.*, 1969; Weiss *et al.*, 1971). The natural cycling of mercury for example, has usually exceeded that of anthropogenic origin. For this reason, in many cases, trace element tissue concentrations in wildlife can be assumed to be natural, with the exception of certain localised cases, mainly in enclosed seas or riverine systems However, diverse sources, ranging from geological registers and the composition of polar ice to historic autopsy studies, suggest that the levels of exposure to at least some heavy metals may have increased markedly in modern times This holds particularly for concentrations of mercury, lead and cadmium, which have increased since the beginning of the present century in both marine mammals and man (Wagemann *et al.*, 1990). A noteable example is the increase in the lead content of Greenland ice, which is today 200-times that considered 'natural' some thousand years ago (Goyer, 1991).

TRENDS SINCE 1965 AND PROSPECTS

Table 1 summarises our literature survey of pollutant trends observed in marine mammals (usually refereed publications have been considered and the results as presented by the authors have been considered to be valid). About 60% ot the studies refer to Pinnipedia and the rest to Cetacea. The period covered ranges from 1965 to 1994, although most data correspond to the period 1969-1988. The apparent decrease in information after 1988 may reflect the time-lag for publication of more recent surveys. Almost all information is for the Northern Hemisphere. Only two surveys (bottlenose dolphins and common dolphins in South Africa) were carried out during the period 1980-1987 in the Southern Hemisphere.

Data on pollutants other than PCBs and tDDT are insufficient to evaluate trends on a global scale. However, an interesting example of a local trend is the increase in liver mercury levels reported for white whales in the western and eastern Canadian Arctic between the early 1980s and 1994, in ringed seals in the western Canadian Arctic and in narwhals in the eastern Canadian Arctic (Wagemann *et al.*, 1996). Apparently, there was no temporal change in cadmium levels in these species during this period.

Results for PCBs and tDDT for the period prior to 1977 are highly variable and include increases in some areas, although in most cases no significant trends were observed.

Since 1977, all studies have reported either no trend or a decrease in tissue pollutant levels. This may point to a continuing reduction of exposure to contaminants in most marine mammal populations. In relative terms, a decrease in concentrations was first noticed for DDT and later for PCBs, a fact which is consistent with the history of the production, use and restriction of these two groups of organochlorine compounds and their persistence in the ecosystem (de Voogt and Brinkman, 1989; Peterle, 1991). The decrease in DDT levels caused by the discontinuation in its use in pesticides has been associated in many areas with a parallel increase in the relative abundance of its metabolised forms. This has led to the use of various ratios between metabolised and parental compounds of DDT, to assess the time passed since the last inputs of the pesticide were introduced into the ecosystem. In particular, during the 1970s and 1980s, the ratio DDE/tDDT was found to progressively rise in odontocetes and pinnipeds in the North Atlantic (Aguilar, 1984) and in grey and harp seals from eastern Canada (Addison *et al.*, 1984). The decrease of PCBs was particularly apparent in areas close to sources such as Lake Ontario, the Baltic Sea, the Wadden Sea and the North Sea (OECD, 1980; Addison *et al.*, 1986; Olsson and Reutergård, 1986; Reijnders, 1996a). However, concentrations in marine biota from the Baltic and the North Seas levelled off in the 1980s (de Boer, 1988; 1994; Bignert *et al.*, 1993) and similar observations were made by Norstrom *et al.* (1988) for polar bears in the Canadian Arctic, and by Tanabe *et al.* (1994b)

Table 1(a)

Temporal trends in pollutant levels in pinnipeds. i: increase, d: decrease, nt: no trend.

Area	Species	Period	PCB	tDDT	HCB	HCHs	Diel	Hg	Reference
BALTIC									
Baltic Sea	*H. grypus*	1969-88	nt	d					Blomkvist *et al.*,1992; Olsson *et al.*,1994
Aland Sea	*H. grypus*	1970, 1971-72, 1973	nt	nt					Olsson *et al.*,1974
Baltic Sea	*P. hispida*	1969, 1973-80, 1988	d	d					Blomkvist *et al.*,1992; Olsson *et al.*,1994
Gulf of Bothnia	*P. hispida*	1969, 1971-72, 1973	nt	nt					Olsson *et al.*,1974
Gulf of Bothnia	*P. hispida*	1972-77	i	nt					Helle,1981
Gulf of Bothnia	*P. hispida*	1977-80	d	d					Helle,1981
Gulf of Bothnia	*P. hispida*	1980-83	nt	nt					Helle and Stenman, 1984
Lake Saimaa	*P. hispida*	1970-1977, 1981	d	d					Helle *et al.*.,1983
Gulf of Finland	*P. hispida*	1977, 1980-83	d	d					Helle *et al.*,1985
NORTH SEA									
Farne Islands	*H. grypus*	1968-75	nt	d					Holden,1978
Scottish coast	*H. grypus*	1965-71	nt	d			d		Holden,1975
Dutch coast	*P. vitulina*	1973-81	d						Van der Zande and de Ruiter,1983
Dutch coast	*P. vitulina*	1969, 1970-75, 1976						nt	Koeman *et al.*,1972; Reijnders,1980
Dutch coast	*P. vitulina*	1973, 1975-88	d						Reijnders,1980; Reijnders,1996a
E. CANADA									
Sable Island	*H. grypus*	1974, 1976-82	nt	d					Addison *et al.*,1984
North Baffin	*P. hispida*	1972, 1976-84	d	d		nt	nt		Muir *et al.*,1988
St Lawrence Gulf	*P. groenlandicus*	1971-73	d	d			nt		Jones *et al.*,1976
St Lawrence Gulf	*P. groenlandicus*	1971-78	d	d			i		Ronald *et al.*,1984
St Lawrence Gulf	*P. groenlandicus*	1971-82	nt	d					Addison *et al.*,1984
St Lawrence Gulf	*P. groenlandicus*	1982-89	d	nt					Beck *et al.*,1993
NORTHEAST USA									
New York	*P. vitulina*	1980-90, 1992	d	d (DDE)	nt			nt	Lake *et al.*,1995
CANADIAN ARCTIC									
Holman Island	*P. hispida*	1972-81	d	d					Addison *et al.*,1986
Western Arctic	*P. hispida*	1987-93						i	Wagemann *et al.*,1996
Eastern Arctic	*P. hispida*	1989-94						nt	Wagemann *et al.*,1996
N. NORTH PACIFIC									
Japan	*C. ursinus*	1971-76	i	i		i			Tanabe *et al.*,1994b
Japan	*C. ursinus*	1976-88	d	d		d			Tanabe *et al.*,1994b

Table 1(b)

Temporal trends in pollutant levels in cetaceans. i: increase, d: decrease, nt: no trend.

Area	Species	Period	PCB	tDDT	HCB	HCHs	Diel	Hg	Reference
E. CANADA									
W. Hudson Bay	*D. leucas*	1966, 1967-86	nt	d	nt	i			Muir *et al.* (1990)
Bay of Fundy	*P. phocoena*	1969-73		d					Gaskin *et al.* (1982)
Bay of Fundy	*P. phocoena*	1971-77	nt						Gaskin *et al.* (1983)
Bay of Fundy	*P. phocoena*	1969-73						d	Gaskin *et al.* (1979)
Bay of Fundy	*P. phocoena*	1974-77						i	Gaskin *et al.* (1979)
CANADIAN ARCTIC									
Western Arctic	*D. leucas*	1981, 1984-93, 1994						i	Wagemann *et al.* (1996)
Eastern Arctic	*D. leucas*	1984-93, 1994						i	Wagemann *et al.* (1996)
Western Arctic	*M. monoceros*	1978, 1979- 92, 1994						i	Wagemann *et al.* (1996)
N. NORTH PACIFIC									
Japan	*S. coeruleoalba*	1978, 1979-86	nt	nt	d	d			Loganathan *et al.* (1990)
SOUTHERN AFRICA									
East coast	*T. truncatus*	1980, 1983-84, 1987	nt	nt			nt		Cockroft *et al.* (1989)
East coast	*T. truncatus*	1980-87	nt	d					Kock *et al.* (1994)
East coast	*D. delphis*	1980-85	nt	nt					Kock *et al.* (1994)
MEDITERRANEAN									
N.W. coast	*S. coeruleoalba*	1987-94	d	d					Borrell *et al.* (1996)
NORTH SEA									
Scottish coast	*P. phocoena*	1965-71	nt	nt			d		Holden (1975)
ANTARCTIC									
	B. acutorostrata	1984-91	i	nt	nt	nt			Tanabe *et al.* (1995)

for northern fur seals in the Pacific. Apart from the apparent levelling off of the decrease of PCBs in local populations, Tanabe (1988) concluded that the global PCB levels are unlikely to decline in the near future due to the fact that only 30% of all the PCBs produced have been dispersed into the environment. In this context, we can also consider the estimation of Bletchly (1984), that disposal of PCBs will peak at the end of the 1990s.

With respect to future trends in levels in marine mammals, Tateya *et al.* (1988) predicted, based on studies in striped dolphins, that levels of PCBs in marine mammals would be at their highest between 2000 and 2030.

Given the fact that of the *ca* 2,000,000 tonnes of PCBs produced, only 1% has reached the ocean (Reijnders, 1996b), slow dispersal will continue and it is expected that on a global scale, no apparent reduction in the exposure of marine mammals to PCBs will occur until the turn of the 21st century.

In view of the presumed global change in the distribution of organochlorine residue levels, the Arctic waters and adjacent seas and oceans are expected to become the major sink for these contaminants (Tatsukawa, 1993). This holds to a much lesser extent for the Southern Hemisphere (Tanabe *et al.*, 1994a). It is important that monitoring programmes for pollutant trends in marine mammals take this into account.

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J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 157–167, 1999

Polychlorinated dibenzo-p-dioxins, dibenzofurans and polychlorinated biphenyls in New Zealand cetaceans*

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ABSTRACT

Limited information is available on the concentrations of halogenated aromatic hydrocarbons (HAHs) in cetaceans from the Southern Hemisphere. This paper presents data on blubber concentrations of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in Hector's dolphins, dusky dolphins, southern right whale dolphins, blue whales, minke whales, Gray's beaked whales, Cuvier's beaked whales and pygmy right whales stranded in New Zealand. Both HAH concentrations and toxic equivalents (TEQs) are found to be higher in Hector's dolphins, a species with an inshore distribution, than in other odontocetes, which are more oceanic. Baleen whales, which are oceanic and feed at lower trophic levels, present the lowest levels of pollutants, with PCDD and PCDF concentrations usually below detection limits. The PCB profiles of the various species suggest that they are exposed to different PCB sources. Overall, HAH levels detected are lower than those reported for comparable species in the Northern Hemisphere. The relative abundance of low chlorinated PCB congeners in New Zealand cetaceans, as compared to those from northern waters, suggests that the origin of these compounds is mostly atmospheric deposition.

KEYWORDS: POLLUTION; SOUTHERN HEMISPHERE; SOUTH PACIFIC; AREA-NEW ZEALAND; HECTOR'S DOLPHIN; DUSKY DOLPHIN; SOUTHERN RIGHT WHALE DOLPHIN; BLUE WHALE; MINKE WHALE; PYGMY RIGHT WHALE; GRAY'S BEAKED WHALE; CUVIER'S BEAKED WHALE

INTRODUCTION

Limited information is available on the occurrence and distribution of halogenated aromatic hydrocarbons (HAHs) such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in Southern Hemisphere cetaceans (Tanabe *et al.*, 1983). HAHs are known to bioaccumulate and biomagnify, with recent studies linking these compounds to reproductive deficiencies in some wildlife species (Reijnders, 1986; Colborn and Clement, 1992; Giesy *et al.*, 1994) and other adverse biological effects in cetaceans (Béland *et al.*, 1993). Cetaceans have been shown to bioaccumulate high concentrations of some HAHs (Tanabe *et al.*, 1988; Muir and Norstrom, 1991) and are, therefore, at risk from the effects of these contaminants. This ability to accumulate high levels of some HAHs also makes cetaceans potential indicator species for

* A version of this paper was originally submitted to the IWC Scientific Committee as SC/46/O12.

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monitoring the contamination of the marine environment (Muir and Norstrom, 1991). We present data on concentrations of HAHs in a number of cetaceans collected around the coast of New Zealand.

MATERIALS AND METHODS

PCDD, PCDF and PCB congeners were analysed following the methodology of Buckland *et al.* (1990) and Hannah *et al.* (1993). $^{13}C_{12}$ dioxin, furan and PCB congeners were added to each sample, before extraction, to act as surrogate internal standards for the calculation of 'native' congener concentration. Samples were then extracted four times by blending with 30ml 2:1 acetone:hexane. Extracts were dried by passage through anhydrous Na_2SO_4, concentrated to near dryness and redissolved in 50ml of hexane. A 5.0ml portion of the extract was removed for gravimetric lipid determination. The remaining extract, after any sub-sampling, was transferred to a separating funnel and washed eight times with concentrated H_2SO_4 followed by three washes with H_2O. The extract was again dried through Na_2SO_4 before being chromatographed sequentially on columns of H_2SO_4/silica:NaOH/silica, Al_2O_3, and Carbopac C dispersed on Celite. PCB congeners pass through the Carbopac column while PCDDs and PCDFs are retained and eluted into a separate fraction. The PCB fraction was chromatographed on Florisil to isolate the three non-*ortho* substituted congeners (Lazar *et al.*, 1992). All analytes were determined by HRGC/HRMS on a VG 70S mass spectrometer.

Toxic Equivalency Factors (TEFs) express the potency of individual HAH congeners relative to 2,3,7,8-TCDD, the most potent HAH congener. The concentration of each congener in the extracts was multiplied by its TEF (Ahlborg *et al.*, 1988; 1994) and the sum of these values gives the total toxic equivalents (TEQs) concentration in the extract.

QUALITY ASSURANCE

The dioxin laboratory maintains World Health Organisation (WHO) and TELARC (Testing Laboratory Registration Council of New Zealand) accreditation for the analysis of PCDD, PCDF and PCB congeners in a variety of environmental matrices. Laboratory blanks were run with each batch of samples. All data analysis was subject to strict quality assurance procedures as previously described (Buckland *et al.*, 1990).

SAMPLES AND SPECIES

All samples analysed in this study were obtained from dead stranded cetaceans.

Hector's dolphin (*Cephalorhynchus hectori*) is an inshore dolphin with a small home range feeding on a variety of fish species (Slooten and Dawson, 1988). In contrast, the other odontocetes studied, i.e. the common dolphin (*Delphinus delphis*), the dusky dolphin (*Lagenorhynchus obscurus*), the southern right whale dolphin (*Lissodelphis peronii*), Gray's beaked whale (*Mesoplodon grayi*) and Cuvier's beaked whale (*Ziphius cavirostris*) are open ocean species and are believed to feed on a variety of fish, squid and crustacea (e.g. see summary in Martin, 1990).

All of the mysticetes (baleen whales) examined are open ocean filter feeders. The minke (*Balaenoptera acutorostrata*) and blue whale (*B. musculus*) feed largely on krill (e.g. Kawamura, 1994) and the rare pygmy right whale (*Caperea marginata*), the smallest of the baleen whales, appears to feed mainly on copepods (Best *et al.*, 1992).

Numbers of specimens and available biometric data for the individuals analysed are provided in Table 1. As these specimens were collected by several people over several years

not all biometric data are available, in particular data on age and other biological information was often lacking. As contaminant concentrations in cetaceans are known to vary with age and sex, the data are presented and discussed primarily as 'group' averages. Groups are defined in Table 1. Individual data are given in Appendix 1.

Table 1
Biometric data for cetacean specimens analysed.

Sample type	Common name	Age	Sex
Oceanic dolphins	Common dolphin	Mature	Male
	Common dolphin	Mature	Male
	Dusky dolphin	Mature	Male
	S. right whale dolphin	1yr	Male
Baleen whales	Minke whale	Mature	Female
	Minke whale	Mature	Male
	Blue whale	Sub-adult	Male
	Pygmy right whale	< 1yr	Female
	Pygmy right whale	< 1yr	Female
Beaked whales	Gray's beaked whale	Mature	Male
	Gray's beaked whale	Mature	Female
	Gray's beaked whale	Mature	Female
	Cuvier's beaked whale	Mature	Male
	Gray's beaked whale	Mature	Male
	Gray's beaked whale	Mature	Female
Hector's dolphin	Hector's dolphin	1yr	Male
	Hector's dolphin	1yr	Male
	Hector's dolphin	10yrs	Male
	Hector's dolphin	11yrs	Male
	Hector's dolphin	< 1yr	Female
	Hector's dolphin	8yrs	Female

RESULTS AND DISCUSSION

PCB congeners were detectable in all samples analysed. The 'group'-average sum of PCB congener concentrations was lowest (< 50ng/g wet weight) in the open ocean mysticetes (minke, blue and pygmy right whales), intermediate (100 to 500ng/g wet weight) in open ocean odontocetes (beaked whales and open ocean dolphins) and highest (750 to > 1,000ng/g wet weight) in the inshore Hector's dolphin (Table 2). PCB profiles from the open ocean baleen and beaked whale species show an abundance of lower chlorinated PCB congeners relative to the inshore Hector's dolphin (Fig. 1). The principle source of these congeners is believed to be atmospheric deposition. However, the difference is much less apparent when comparing inshore and offshore dolphins.

PCDD and PCDF congeners were only commonly detected in the inshore feeding Hector's dolphin (Table 2). The concentrations of almost all PCDD and PCDF congeners were below detection limits in the baleen whale species. In open ocean dolphins and beaked whales hepta- and octa-chlorinated PCDD and PCDF congeners were the most commonly detected congeners.

Toxic Equivalency Factors (TEFs) can be used to calculate the biological potency of HAH mixtures relative to 2,3,7,8-TCDD, the most potent HAH congener (Ahlborg *et al.*, 1988; 1994). Using this method, the total concentration of TCDD-Equivalents (TEQs) were

calculated for the different cetacean groups (Table 2). TEQs were lowest in the baleen whales, higher in the open ocean odontocetes and highest in the inshore Hector's dolphin. By calculating the TEQ contributed by specific compounds analysed it is possible to assess their relative toxicological significance (Fig. 2). The contribution of PCDD and PCDF to the TEQ

Table 2

Mean chlorinated hydrocarbon congener concentrations in blubber of different groups of southern ocean cetaceans (PCBs in ng/g wet weight; PCDD and PCDF in pg/g wet weight). Where values less than the detection limit occurred, one half of that detection limit was used to calculate the mean. TEFs from Ahlborg *et al.*, 1988; Ahlborg *et al.*, 1994. na = not analysed. nd = not detected. * PCDD and PCDF data from Buckland *et al.*, 1990.

Analyte	TEF	Pygmy right	Baleen whales	Oceanic dolphins	Beaked whales	Hector's dolphin*
PCB #28	0	0.25	0.03	0.93	0.95	3.78
PCB #52	0	0.34	1.30	14.5	4.90	9.93
PCB #77	0.0005	0.005	0.002	0.08	0.06	0.09
PCB #101	0	0.42	1.87	60.1	19.7	32.5
PCB #99	0	0.23	1.41	44.0	23.3	56.9
PCB #118	0.0001	0.20	0.79	37.5	14.7	56.1
PCB #105	0.0001	0.11	0.23	10.9	4.25	28.7
PCB #126	0.1	0.003	0.01	0.06	0.08	0.43
PCB #153	0	0.58	2.89	267	64.5	330
PCB #138	0	0.85	2.64	232	48.7	240
PCB #169	0.01	0.002	0.02	0.14	0.07	0.09
PCB #187	0	0.28	0.80	46.8	24.8	77.7
PCB #183	0	0.02	0.23	16.3	6.97	29.4
PCB #180	0.00001	0.14	0.77	68.1	21.1	86.2
PCB #170	0.0001	0.17	0.34	22.6	13.7	64.3
PCB #202	0	0.001	0.03	5.46	1.43	1.6
PCB #194	0	0.001	0.02	8.50	1.64	na
Congener sum (ng/g)		3.61	12.9	833	251	1,018
2,3,7,8-TeF	0.1	0.06	0.10	0.10	0.15	9.12
non-2,3,7,8-TeF	0	0.11	0.18	3.70	1.30	nd
2,3,7,8-TeD	1.0	0.10	0.08	0.10	0.06	7.83
non-2,3,7,8-TeD	0	0.18	0.08	0.10	0.10	nd
1,2,3,7,8-PeF	0.05	0.06	0.05	0.10	0.12	0.77
2,3,4,7,8-PeF	0.5	0.13	0.10	0.02	0.22	24.6
non-2,3,7,8-PeF	0	0.18	0.20	6.44	1.79	nd
1,2,3,7,8-PeD	0.5	0.13	0.10	0.10	0.09	9.08
non-2,3,7,8-PeD	0	0.23	0.13	0.15	0.09	nd
1,2,3,4,7,8-HxF	0.1	0.13	0.08	0.10	0.10	nd
1,2,3,6,7,8-HxF	0.1	0.10	0.12	0.10	0.09	0.56
2,3,4,6,7,8-HxF	0.1	0.13	0.12	0.10	0.17	0.51
1,2,3,7,8,9-HxF	0.1	0.18	0.15	0.10	0.06	0.27
non-2,3,7,8-HXF	0	0.20	0.20	2.96	0.82	nd
1,2,3,4,7,8-HpD	0.1	0.15	0.12	0.04	0.07	nd
1,2,3,6,7,8-HpD	0.1	0.15	0.17	0.04	0.15	2.83
1,2,3,7,8,9-HpD	0.1	0.18	0.12	0.04	0.08	0.23
non-2,3,7,8-HpD	0	0.30	0.20	0.10	0.15	nd
1,2,3,4,6,7,8-HpF	0.01	0.28	0.43	0.05	0.19	0.30
1,2,3,4,7,8,9-HpF	0.01	0.18	0.15	0.04	0.05	0.08
non-2,3,7,8-HpF	0	0.43	0.85	0.20	0.10	nd
1,2,3,4,6,7,8-HpD	0.01	0.65	0.98	0.15	0.53	3.15
non-2,3,7,8-HpD	0	0.68	0.76	0.50	0.27	nd
OCDF	0.001	0.52	2.33	0.15	0.26	1.54
OCDD	0.001	2.75	10.2	0.50	3.91	12.5
TEQ (pg/g)		0.77	1.9	15.7	12.5	81.4

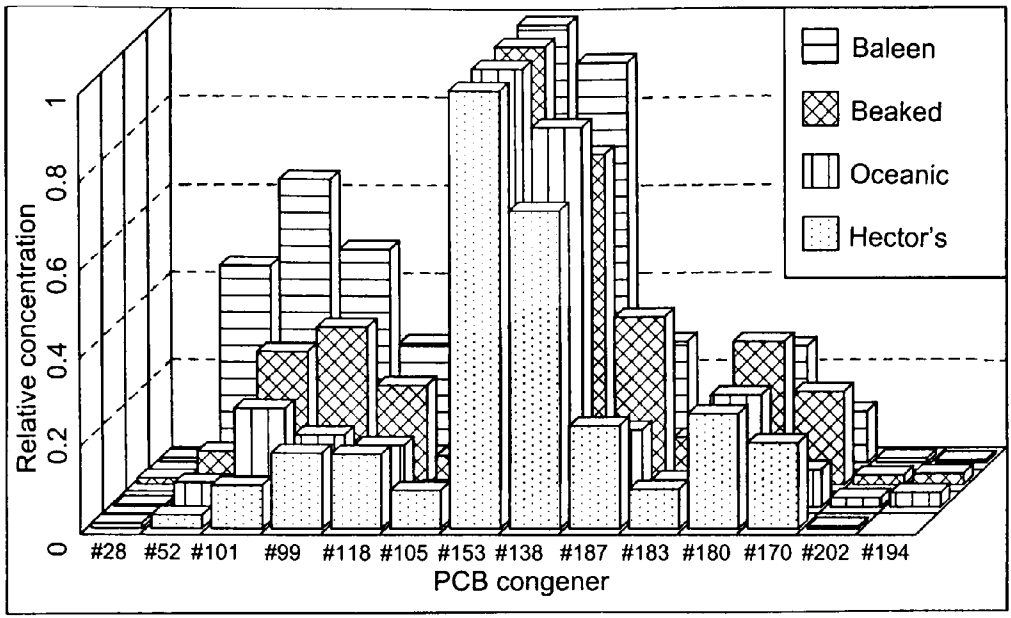

Fig. 1. PCB congener profiles for different cetacean groups. Baleen = baleen whales, Beaked = beaked whales, Oceanic = oceanic dolphins, Hector's = Hector's dolphin. Concentrations are expressed relative to CB153.

was less than 20% of the total TEQ in all open ocean groups except the pygmy right whale. The higher contribution of PCDD and PCDF in this group can be explained by the low levels of TEQs found (< 1pg/g wet weight) which resulted in a high number of 'non-detect' values. For calculation of TEQs, half the detection limit is taken for these non-detect values. Therefore, at low contaminant levels TEQ can be overestimated because of the high number of non-detect values. The levels of TEQs detected in Hector's dolphin are higher than in the other groups and the contribution of PCDD and PCDF to total TEQs is also greater.

The correlation between average 'group' PCB congener sums and TEQs in different cetacean species (Fig. 3) supports the hypothesis that these groups are exposed to different PCB sources. Any single source of HAHs will have a relatively fixed profile and therefore a fixed potency expressed as TEQ per mass of PCB. As the ratio of TEQ accumulated per mass of PCB is higher in Hector's dolphin than in the open ocean species, this suggests that this species is exposed to an HAH source of higher potency. This is also evident from the higher contribution of PCDD and PCDF to total TEQ in Hector's dolphin, previously mentioned.

A cluster analysis (Fig. 4) was performed using the PCB congener data only. PCDD and PCDF congener concentrations were not used in this statistical analysis due to the high number of non-detect values for these congeners in the open ocean cetaceans. This analysis demonstrated the similarity among the individuals of the various cetacean groups, particularly for Hector's dolphin and Gray's beaked whale. Interestingly, the Gray's beaked whale cluster is quite distinct from the open ocean dolphin cluster which also includes the single Cuvier's beaked whale. Little information is available on the dietary habits of Gray's beaked whale. However, this analysis may indicate a diet distinct from the open ocean dolphins and more similar to that of Hector's dolphin (e.g. Slooten and Dawson, 1988). Cuvier's beaked whale appears to be a catholic feeder on deep sea-fish and squid (Nishiwaki and Oguro, 1972).

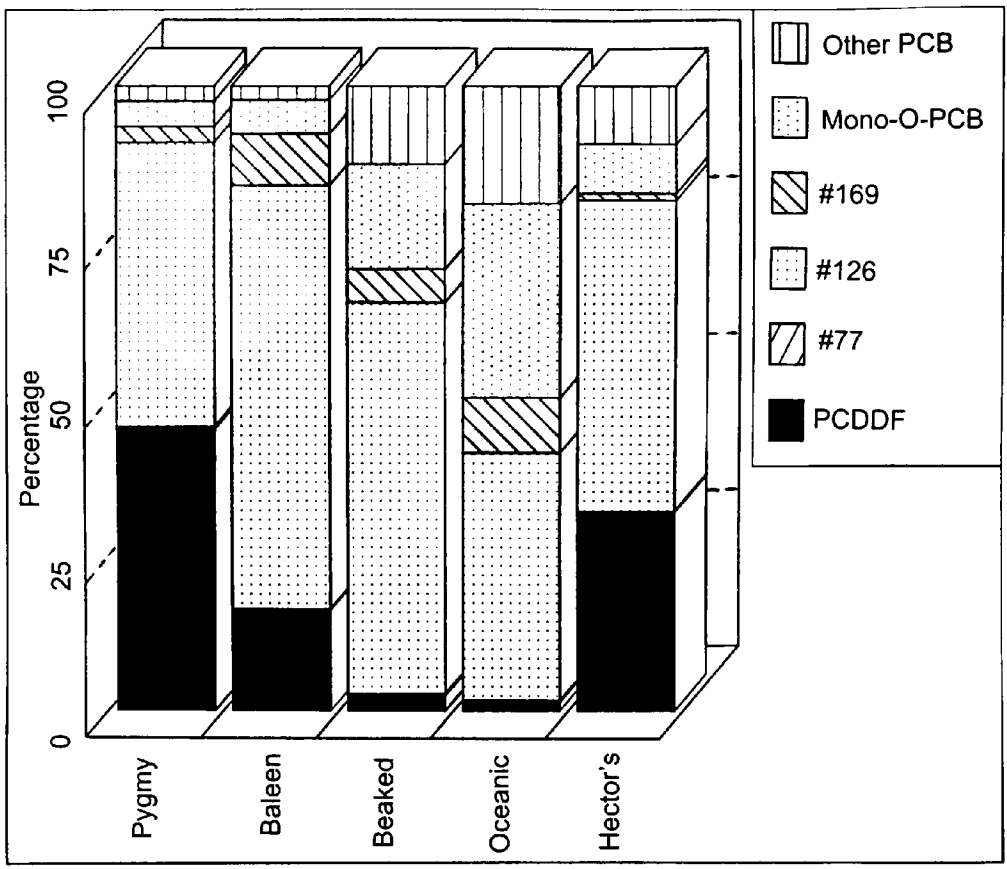

Fig. 2. Percent contribution of HAH classes and individual PCB congeners to total 2,3,7,8-TCDD toxic equivalents (TEQ).

CONCLUSIONS

The relative concentrations of chlorinated hydrocarbons in the different species examined indicates that their accumulation is related to both food habit and proximity to the coast. For example, total PCB concentrations are greater in beaked whales than in baleen whales - both are open ocean species but the latter feed lower in the food web. Higher concentrations of PCBs in Hector's dolphin as compared to common dolphins indicate higher exposure in inshore species.

Concentrations of PCBs detected in common dolphins, beaked whales and baleen whales in New Zealand are lower than those reported for similar species in the Northern Hemisphere (Table 3). This observation is to be expected considering the relative remoteness of the area from the major sources of PCB contamination that are mainly located in the Northern Hemisphere.

PCB profiles from open ocean marine mammals show an abundance of lower chlorinated PCB congeners. This abundance is most noticeable in the baleen whales which feed near the bottom of the food chain. However, the pattern is still detectable in other open ocean species. The abundance of the lower chlorinated, therefore more volatile, PCBs suggests that the principle source of these congeners is atmospheric deposition.

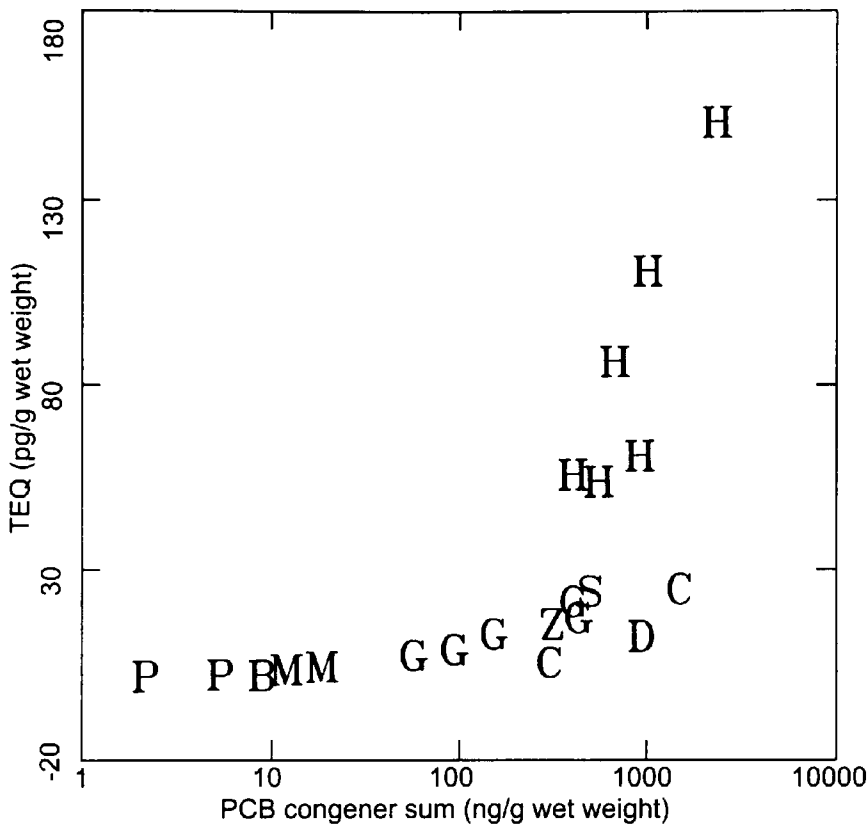

Fig. 3. Concentration of 2,3,7,8-TCDD equivalents as a function of the sum of PCB congeners for southern ocean cetaceans. H = Hector's dolphin, C = common dolphin, D = dusky dolphin, S = southern right whale dolphin, G = Gray's beaked whale, M = minke whale, B = southern blue whale, P = pygmy right whale, Z = Cuvier's beaked whale.

With the exception of the Hector's dolphin, PCDD and PCDF did not contribute a significant level of TEQs. In all the southern ocean cetaceans analysed to date PCBs contribute the major portion of TEQ calculated using the TEF values of Ahlborg *et al.* (1994). This situation may arise due to the ability of cetaceans to metabolise PCDD and PCDF congeners (Muir and Norstrom, 1991; Norstrom *et al.*, 1994) but, mostly, because of the limited atmospheric transport and deposition of these HAHs into the southern oceans.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to the numerous Department of Conservation field officers who provided samples for this research. This research was funded by contributions from ESR:Environmental, the Department of Conservation and the Foundation for Research Science and Technology.

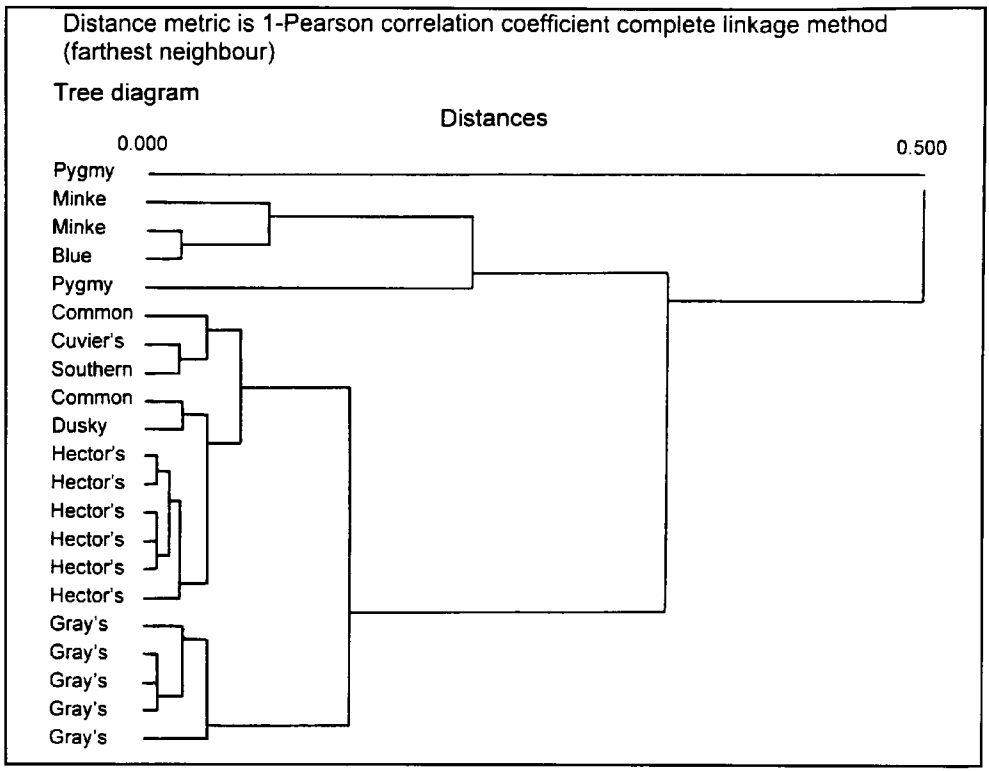

Fig. 4. Cluster analysis of Southern ocean cetaceans using PCB congener profiles. Hectors = Hector's dolphin, Pygmy = pygmy right whale, Minke = minke whale, Blue = blue whale, Grays = Gray's beaked whale, Common = common dolphin, Dusky = dusky dolphin, Cuviers = Cuvier's beaked whale, Southern = southern right whale dolphin.

Table 3

Comparison of total PCB concentrations in cetaceans.

Species	Location	PCBs (μg/g)	Reference
Bottlenose dolphin	South Africa	13.8	Cockroft *et al.*, 1989
Dall's porpoise	North Pacific	8.6	Tanabe *et al.*, 1983
White-sided dolphin	Japan	37.6	Tanabe *et al.*, 1983
Bottlenose dolphin	East USA	81.4	Kuehl *et al.*, 1991
Common dolphin	East USA	36.5	Kuehl *et al.*, 1991
White-sided dolphin	East USA	50.1	Kuehl *et al.*, 1991
Harbour porpoise	UK	55.5	Morris *et al.*, 1989
Dusky dolphin	South of NZ	1.4	Tanabe *et al.*, 1983
Baleen whales	NZ	< 0.05*	This study
Minke whales	West USA	3.3	Varanasi *et al.*, 1993
Beaked whales	NZ	0.1 - 0.5*	This study
Baird's beaked whales	Japan	3.0	Subramanian *et al.*, 1988
Common dolphin	NZ	0.75 - >1.0*	This study
Hector's dolphin	NZ	0.4 - 4.5*	This study

*Measured as the sum of 16 predominant and biologically active congeners.

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APPENDIX 1a

Concentrations of PCB congeners in individual marine mammal blubber samples. Concentrations are ng/g wet weight. n.a. = not analysed; Ma = mature adults, exact age unknown; SA = Sub-adult; and CS = Congener Sum. Sample 8729 was analysed in duplicate.

Specimen	Age (yr)	Sex	PCB# 28	52	77	101	99	118	105	126	153	138	169	187	183	180	170	202	194	CS (ng/g)
Pygmy right whale																				
NMNZ 204	<1	F	0.46	0.46	0.009	0.52	0.3	0.28	0.15	0.004	0.86	1.38	0.002	0.14	0.03	0.25	0.32	0	n.a	5
G152	<1	F	0.05	0.22	0.0003	0.32	0.16	0.13	0.08	0.003	0.3	0.31	0.002	0.42	0.01	0.04	0.02	0.001	0.001	2
Hector's dolphin																				
8733	<1	F	6.08	6.71	0.13	30.3	30.3	25.3	12	0.25	187	153	0.03	44.3	15.1	39.1	28.9	0.83	n.a.	579
8729	1	M	3.35	7.7	0.08	33.8	32.2	31.4	16.7	0.41	223	166	0.1	61	22.3	66.2	49.7	1.14	n.a	715
8729	1	M	4.04	8.63	0.1	35.3	33.2	34.8	19.5	0.49	243	180	0.13	65.9	24.7	73	58.2	1.31	n.a.	782
8731	11	M	3.77	17	0.04	35.2	178	167	82.9	0.68	881	600	0.12	162	69.1	212	161	3.39	n.a.	2,573
8615	1	M	0.63	13.9	0.09	51.8	50.5	49.7	25.5	0.55	346	258	0.11	93.5	33.6	90.2	72.6	1.96	n.a.	1,089
8617	8	F	2.55	7.77	0.07	19.8	53.7	60.7	32.7	0.32	295	229	0.09	82.5	29.3	91.3	57.6	1.81	n.a.	964
8503	10	M	6.04	7.82	0.15	21.3	20.2	23.7	11.9	0.28	133	95	0.06	34.8	12	31.9	22.4	0.6	n.a.	421
Dusky dolphin																				
DD1	Ma	M	0.35	13.96	0.08	44.2	37.5	26.2	8.28	0.03	331	281	0.11	43.6	16.8	113	23.4	8.49	13.1	961
Common dolphin																				
G149	Ma	M	0.1	4.16	0.007	8.78	16.5	4.9	2.58	0.005	92.9	81.6	0.03	31.5	10.8	34.3	16.9	1.22	2.06	308
CD1	Ma	M	1.04	23.4	0.06	155.9	86.2	96.4	14.5	0.07	515	436	0.19	65.8	12.9	87.5	31.8	11	10.4	1,548
Southern right whale dolphin																				
SRWD	1	M	2.22	16.5	0.17	31.4	35.8	22.7	18.1	0.14	131	129	0.22	46.4	24.7	37.3	18.5	1.19	n.a.	515
Gray's beaked whale																				
E174/1	Ma	M	0.95	3.9	0.03	13.8	16.9	9.2	4	0.08	40.3	28.7	0.08	14	4	11	8.5	0.72	0.89	157
E168/1	Ma	F	0.49	2.3	0.09	9.7	10.1	5.1	2.2	0.05	23.2	16.5	0.06	9.56	2.6	7.3	5.6	0.52	0.55	96
E198	Ma	F	0.36	1.6	0.07	5.3	5.5	2.1	0.98	0.04	13	8.7	0.06	7.5	1.9	5.7	3.7	0.66	0.61	58
G101	Ma	M	1.7	9	0.12	32.5	44.7	27.4	5.7	0.13	113	75.1	0.09	42.2	11.3	33.1	21.7	2.5	2.8	423
E197	Ma	F	1	5.4	0.03	25.9	41.9	24.1	6.9	0.09	117	85.3	0.05	50.5	14.4	42.6	29.3	2.4	2.7	450
Cuvier's beaked whale																				
G151	Ma	M	1.2	7.2	0.02	30.7	20.5	20.3	5.7	0.09	80.6	78	0.06	24.8	7.6	26.8	13.5	1.79	2.31	321
Minke whale																				
E208	Ma	M	0.06	1.19	0.004	2.4	1.73	1.06	0.33	0.02	4.23	3.87	0.02	1.22	0.37	1.2	0.59	0.05	0.04	18
G103	Ma	F	0.01	1.4	0.002	2.28	1.28	0.59	0.18	0.01	2.31	2.13	0.02	0.67	0.18	0.55	0.21	0.03	0.001	12
Blue whale																				
G111	SA	M	0.03	n.a.	0.002	0.93	1.21	0.73	0.19	0.003	2.14	1.9	0.003	0.5	0.14	0.56	0.24	0.02	0.02	9

APPENDIX 1b

Concentrations of PCDD and PCDF congeners in individual marine mammal blubber samples. Concentrations are pg/g wet weight. n.a. = not analysed. Values in italics are less than the method detection limit, the values provided are half of the method detection limit. Data for specimens 8733, 8729, 8731, 8615, 8617 and 8503 are from Buckland *et al.*, 1990.

	NMNZ 204	G152	8733	8729	8731	8615	8617	8503	DD1	G149	CD1
2,3,7,8-TeF	*0.1*	*0.025*	15.4	12.6	1.6	6.7	7.1	11.3	n.a.	*0.1*	n.a.
non-2,3,7,8-TeF	*0.2*	*0.025*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3.7	n.a
2,3,7,8-TeD	*0.15*	*0.05*	6.2	8.5	11	10.4	4	6.9	n.a.	*0.1*	n.a.
non-2,3,7,8-TeD	*0.3*	*0.05*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	*0.1*	n.a.
1,2,3,7,8-PeF	*0.1*	*0.025*	1.1	1.4	0.2	1.1	0.1	0.7	n.a.	*0.1*	n.a.
2,3,4,7,8-PeF	*0.1*	*0.15*	15.6	31.1	43.9	37.5	6.1	13.2	n.a.	*0.02*	n.a.
non-2,3,7,8-PeF	*0.2*	*0.15*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	6.44	n.a.
1,2,3,7,8-PeD	*0.15*	*0.1*	7.9	11.7	8.1	13.4	5.5	7.9	n.a.	*0.1*	n.a.
non-2,3,7,8-PeD	*0.35*	*0.1*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	*0.15*	n.a.
1,2,3,4,7,8-HxF	*0.15*	*0.1*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	*0.1*	n.a.
1,2,3,6,7,8-HxF	*0.15*	*0.05*	0.9	*0.95*	*0.05*	*0.65*	*0.25*	*0.55*	n.a.	*0.1*	n.a.
2,3,4,6,7,8-HxF	*0.15*	*0.1*	0.6	0.8	0.3	0.9	*0.15*	*0.3*	n.a.	*0.1*	n.a.
1,2,3,7,8,9-HxF	*0.25*	*0.1*	*0.1*	*0.35*	*0.05*	*0.25*	*0.3*	*0.55*	n.a.	*0.1*	n.a.
non-2,3,7,8-HxF	*0.3*	*0.1*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	2.96	n.a
1,2,3,4,7,8-HxD	*0.2*	*0.1*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	*0.035*	n.a.
1,2,3,6,7,8-HxD	*0.2*	*0.1*	4.3	4.6	0.4	5.3	*0.5*	1.9	n.a.	*0.04*	n.a.
1,2,3,7,8,9-HxD	*0.25*	*0.1*	0.3	0.2	0.2	*0.2*	*0.15*	*0.35*	n.a.	*0.04*	n.a.
non-2,3,7,8-HxD	*0.45*	*0.15*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	*0.1*	n.a.
1,2,3,4,6,7,8-HpF	*0.15*	0.4	0.3	*0.1*	0.7	*0.1*	0.4	*0.2*	n.a.	*0.05*	n.a.
1,2,3,4,7,8,9-HpF	*0.3*	*0.05*	0.05	*0.05*	*0.15*	*0.05*	*0.05*	*0.1*	n.a.	*0.035*	n.a.
non-2,3,7,8-HpF	*0.5*	0.36	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	*0.2*	n.a.
1,2,3,4,6,7,8-HpD	*0.3*	0.99	3.3	3	4.5	1.6	2	3.5	n.a.	*0.15*	n.a.
non-2,3,7,8-HpF	*0.5*	0.85	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	*0.5*	n.a.
OCDF	*0.5*	0.54	2.5	*2.1*	*0.5*	*1.05*	2.9	*2.1*	n.a.	*0.15*	n.a.
OCDD	*1.5*	4	14.6	15	7.1	7.5	15.8	15	n.a.	*0.5*	n.a.

	SRWD	E174/1	E168/1	E198	G151	G101	E197	E208	G103	G111
2,3,7,8-TeF	n.a.	*0.25*	*0.1*	*0.2*	*0.1*	*0.2*	*0.15*	*0.15*	*0.1*	*0.04*
non-2,3,7,8-TeF	n.a.	*0.6*	0.75	0.76	1.04	6.56	0.44	*0.15*	*0.3*	*0.1*
2,3,7,8-TeD	n.a.	*0.1*	*0.05*	*0.045*	*0.04*	*0.05*	*0.05*	*0.1*	*0.1*	*0.05*
non-2,3,7,8-TeD	n.a.	*0.1*	*0.05*	*0.045*	*0.1*	*0.05*	*0.05*	*0.1*	*0.1*	*0.05*
1,2,3,7,8-PeF	n.a.	*0.05*	*0.1*	0.2	*0.1*	0.35	*0.16*	*0.05*	*0.05*	*0.05*
2,3,4,7,8-PeF	n.a.	*0.15*	*0.1*	0.38	*0.15*	*0.2*	*0.15*	*0.15*	*0.05*	*0.1*
non-2,3,7,8-PeF	n.a.	*0.15*	*0.15*	0.88	*0.25*	4.52	*0.15*	*0.25*	*0.25*	*0.1*
1,2,3,7,8-PeD	n.a.	*0.1*	*0.05*	*0.1*	*0.1*	0.49	*0.1*	*0.1*	*0.1*	*0.1*
non-2,3,7,8-PeD	n.a.	*0.1*	*0.05*	*0.1*	*0.1*	*0.2*	*0.1*	*0.1*	*0.2*	*0.1*
1,2,3,4,7,8-HxF	n.a.	*0.1*	*0.1*	*0.1*	*0.15*	*0.2*	*0.1*	*0.05*	*0.15*	*0.05*
1,2,3,6,7,8-HxF	n.a.	*0.1*	*0.1*	*0.045*	*0.1*	*0.35*	*0.1*	*0.1*	*0.2*	*0.05*
2,3,4,6,7,8-HxF	n.a.	*0.15*	*0.1*	*0.22*	*0.15*	*0.2*	*0.1*	*0.1*	*0.2*	*0.05*
1,2,3,7,8,9-HxF	n.a.	*0.05*	*0.045*	*0.04*	*0.1*	*0.1*	*0.04*	*0.15*	*0.15*	*0.15*
non-2,3,7,8-HxF	n.a.	*0.1*	*0.1*	*0.15*	*0.3*	8	0.83	*0.05*	*0.5*	*0.05*
1,2,3,4,7,8-HxD	n.a.	*0.1*	*0.05*	*0.05*	*0.1*	*0.1*	*0.03*	*0.1*	*0.15*	*0.1*
1,2,3,6,7,8-HxD	n.a.	*0.2*	*0.15*	*0.05*	*0.3*	*0.25*	*0.1*	*0.15*	*0.25*	*0.1*
1,2,3,7,8,9-HxD	n.a.	*0.1*	*0.1*	*0.045*	*0.1*	*0.2*	*0.03*	*0.1*	*0.15*	*0.1*
non-2,3,7,8-HxD	n.a.	*0.15*	*0.1*	*0.1*	*0.3*	*0.35*	*0.1*	*0.1*	*0.4*	*0.1*
1,2,3,4,6,7,8-HpF	n.a.	*0.15*	*0.2*	0.16	*0.15*	*0.1*	*0.1*	*0.15*	1.04	*0.1*
1,2,3,4,7,8,9-HpF	n.a.	*0.05*	*0.03*	*0.04*	*0.05*	*0.03*	*0.03*	*0.15*	*0.15*	*0.15*
non-2,3,7,8-HpF	n.a.	*0.1*	*0.1*	0.09	*0.1*	2.6	*0.1*	*0.15*	2.29	*0.1*
1,2,3,4,6,7,8-HpD	n.a.	*0.4*	*0.4*	*0.35*	0.88	*0.3*	*0.4*	*0.35*	2.34	*0.25*
non-2,3,7,8-HpF	n.a.	*0.25*	*0.2*	*0.2*	0.5	*0.1*	*0.2*	*0.15*	1.87	*0.25*
OCDF	n.a.	0.46	0.27	*0.15*	*0.15*	*0.15*	0.36	*0.35*	6.25	*0.4*
OCDD	n.a.	5.32	4.59	3.96	4.7	3.66	5.33	*1*	27.7	*2*

J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 169–176, 1999

Organochlorine levels in cetaceans from South Africa: A review

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ABSTRACT

Publications on levels of organochlorines in cetaceans from South Africa are reviewed. Organochlorine contamination in cetaceans off South Africa is similar to those in Australian waters, but generally low compared to the Northern Hemisphere. An exception is the coastal dolphins inhabiting the South African east coast waters. In these animals levels are similar to Northern Hemisphere coastal cetaceans. Levels are generally higher in coastal dolphins, compared to dolphins living in deeper waters. It is suggested that these differences are directly related to the levels of industrialisation and cultivation of the surrounding area. Too few samples of either baleen whales or toothed whales are available to investigate the differences in organochlorine levels between these two groups. Similarly, even for species with the highest sample sizes – common and bottlenose dolphins – the data are insufficient to investigate trends in contaminant levels.

KEYWORDS: POLLUTION-ORGANOCHLORINES; SOUTH ATLANTIC; INDIAN OCEAN; CETACEANS-GENERAL; REVIEW

INTRODUCTION

The accumulation of toxic contaminants in marine mammals is of increasing concern (e.g. Reijnders *et al.*, 1999). Marine mammals are long-lived, have large lipid reserves in proportion to their body size and are therefore ideal repositories for high concentrations of the lipophilic chlorinated hydrocarbons. In addition, they appear to lack certain enzymes for the metabolism of some organochlorines; marine mammals appear to accumulate these compounds to a greater degree than other mammals and may therefore be more susceptible to their toxic effects (e.g. Tanabe, 1988; Tanabe *et al.*, 1988).

In common with most of the Southern Hemisphere (Kemper *et al.*, 1994; Marcovecchio *et al.*, 1994), there is little information on the toxic contaminants in the seas surrounding South Africa. Those most frequently studied and reported are the synthetic chlorinated hydrocarbons, the polychlorinated biphenyls (PCBs) and DDT and its metabolites, DDE and DDD. As a result of the intense agricultural usage of land along the eastern coastal zone, particularly in the north (Natal), quantities of DDT entered the marine system prior to 1976, when its use in agriculture was largely discontinued. However, DDT may still be in use, particularly in state managed malaria control procedures in northern Natal (Van Dyk *et al.*, 1982), implying that some may still enter the marine environment. PCBs have never been manufactured in South Africa and their input into the marine environment is, apart from aerial transport, probably limited to leaching from products containing them dumped near to the industrialised areas of Richards Bay and Durban in Natal, East London and Port Elizabeth in the Eastern Cape and Cape Town in the Western Cape.

There are some published data for organochlorine residues in coastal birds (De Kock and Randall, 1984) and seals (Cockcroft *et al.*, 1991), although most of this emanates from samples collected and processed in the 1970s and 1980s. More recently, samples have been

routinely collected from cetaceans stranded and incidentally captured, however, the high analytical costs have limited the analyses. This paper reviews the data available in published papers and technical reports.

STUDY AREA

South Africa has a coastline of about 3,000km and is bounded by two major current systems. To the west, the cold and intermittent Benguela Current runs northwards, while to the east, the warm, strongly flowing Agulhas Current runs in a southerly direction (Fig. 1). Other than in the south, around Cape Town, the west coast is mostly arid, human population density is low and industrialisation and agriculture limited. In contrast, the coastal belt of the east coast is generally moist, fairly heavily cultivated and has several large cities (>500,000 inhabitants), all of which have an active industrial base.

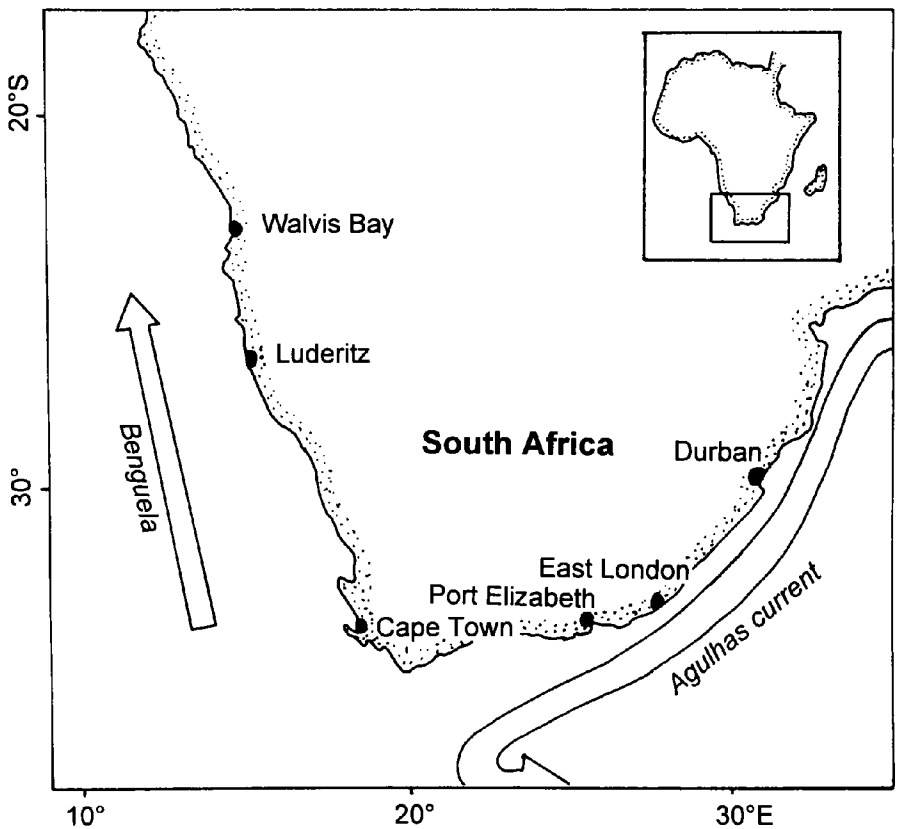

Fig. 1. South Africa is bounded by two current systems, the cold Benguela to the west and the warm, strongly flowing Agulhas to the east. The primary centres of industrialisation, agriculture and habitation are either on, or drain to, the east coast, east of longitude 20°E.

For the purpose of this review, the west coast of South Africa is taken to be that part of the coastline west and north of the southern tip of Africa (about 20°E). The east coast is taken to be that part of the coast east of this point.

METHODOLOGY

For animals sampled from the west coast, both stranded and taken by scientific permit, blubber was taken dorsally, anterior to the dorsal fin, wrapped in aluminium foil and stored at −20°C until analysis. For the east coast, using both strandings and incidental captures, blubber samples were taken just anterior to either pectoral flipper, wrapped in aluminium foil and frozen at −20°C for analysis. Analyses for all west coast samples and samples from incidentally caught dolphins on the east coast followed the methodology described by Cockcroft *et al.* (1989) and De Kock (1990) .

For east coast animals, sample extraction, clean up and analyses followed the methodology of Watling (1981) and Henry and Best (1983). Briefly, lipid from thawed samples (5-10g) was extracted by ultrasonic maceration with hexane. This was cleaned up using a silica gel and sodium sulphate column. Two fractions, the first containing the PCBs and DDTs, the second Dieldrin and most of the Lindane, were subsequently obtained. Samples were analysed using a PYE gas liquid chromatograph (GLC - tuned to approximately $250g \times 10^{-12}/\mu l$ sensitivity) with an electron capture detector. Compounds were quantified by frequent calibration of the GLC with external standards (Environmental Protection Agency, USA). Occasionally, further confirmation was done by ultraviolet photolysis.

RESULTS

West coast

De Kock *et al.* (1994) provide total PCB, HCB and DDT concentrations for 72 cetaceans of 15 species stranded or taken by scientific permit off the west coast between 1977 and 1987 (Table 1). Overall, levels of DDT and PCB were low and Dieldrin was present in only a small proportion of the samples.

East coast

The concentrations of tDDT in 36 rorquals (6 fin, 1 sei and 29 minke) landed at Durban during the 1974 whaling season were all exceptionally low ($< 1\mu g/kg$ wet weight), while PCB levels were too low to be detectable (Henry and Best, 1983). Similarly, concentrations of tDDT in 12 sperm whales landed in the same year, were also extremely low ($< 1.3\mu g/kg$ wet weight), with no detectable level of PCBs.

A study of organochlorines (Cockcroft *et al.*, 1991) in the blubber of 41 odontocete cetaceans stranded off the east coast of South Africa between 1976 and 1981, showed highly variable results (Table 2). Generally, cetaceans from the Eastern Cape showed lower tDDT, PCB, Dieldrin and Lindane levels than did those from Natal. Additionally, those inhabiting coastal waters displayed higher levels of all contaminants than did animals from pelagic waters.

The results of pollutant studies of common and bottlenose dolphins incidentally captured in shark nets off Natal (Cockcroft, 1990) were similar to those of other studies of contaminants in cetaceans (Cockcroft *et al.*, 1989; 1990). For both species, contaminant levels were considerably higher in the blubber than in the liver. Contaminant concentrations in males increased with age (based on growth layer group - GLG - counts), whereas concentrations in females showed a marked and rapid decline at sexual maturity (Tables 3 and 4). Most interesting was the finding that, in both species, this decline occurred subsequent to the females' first or second ovulation, implying that primiparous females rid themselves of the major portion of their contaminant burden to either the first and/or the second born calf, probably via lactation (Cockcroft *et al.*, 1989; 1990).

Table 1

Organochlorine residue levels (μ/g wet weight) in the blubber of cetaceans from the west coast of South Africa (1977-1987) (from de Kock *et al.*, 1994).

Species	n	Year	Sex	Length (m)	tDDT	PCB	HCB
Southern right whale	2	1984	M	9.25	0.01	0.01	0.02
(*Eubalaena australis*)		1986	M	4.85	0.01	0.01	0.01
Pygmy right whale	1	1987	F	6.5	0.05	0.12	0.01
(*Caperea marginata*)							
Minke whale	1	1984	F	3.3	0.08	0.01	0.1
(*Balaenoptera acutorostrata*)							
Sperm whale	1	1986	F	16	0.39	0.13	0.01
(*Physeter macrocephalus*)							
Pygmy sperm whale	5	1978	F	2.93	0.51	0.26	0.06
(*Kogia breviceps*)		1984	F	3.01	0.3	0.12	0.05
		1986	F	3.21	1.02	1.1	0.06
		1986	M	2.18	1.11	0.29	0.08
		1987	M	2.33	0.3	0.26	0.03
Dwarf sperm whale	4	1976	M	1.67	1.43	0.18	0.04
(*Kogia simus*)		1984	M	2.11	0.37	0.15	0.03
		1984	M	2.3	0.54	0.29	0.03
		1984	M	1.78	0.65	0.38	0.03
Blainville's beaked whale	6	1984	F	3.46	1.47	0.88	0.15
(*Mesoplodon densirostris*)		1984	F	4.54	0.29	0.3	0.02
		1984	M	2.25	1.86	0.29	0.16
		1984	F	4.54	0.38	0.51	0.03
		1984	F	4.25	0.36	0.27	0.03
		1986	M	3.76	0.39	0.26	0.02
True's beaked whale	2	1986	F	3.19	0.91	1.22	0.09
(*Mesoplodon mirus*)		1986	F	4.17	1.37	0.75	0.11
Layard's beaked whale	2	1978	M	2.9	0.54	0.15	0.13
(*Mesoplodon layardi*)		1985	M	5.2	1.22	1.93	0.27
Risso's dolphin	2	1984	M	3.14	7.51	2.72	0.02
(*Grampus griseus*)		1986	F	1.29	1.29	0.39	0.03
Striped dolphin	2	1984	F	2.3	1.02	1.28	0.02
(*Stenella coeruleoalba*)		1986	M	2.01	0.74	1.11	0.03
Bottlenose dolphin	6	1976	M	2.69	4.14	0.34	0.03
(*Tursiops truncatus*)		1980	M	2.86	0.17	0.16	0.01
		1984	M	1.35	2.52	1.93	0.01
		1985	M	1.72	3.6	2.33	0.03
		1985	M	2.61	12.29	8.29	0.03
		1987	F	2.88	1.75	1.62	0.01
Common dolphin	17	1984	M	2.08	9.63	6.94	0.4
(*Delphinus delphis*)		1984	M	2.22	0.48	0.43	0.02
		1984	M	2.3	5.73	3.39	0.02
		1984	M	1.97	0.07	0.07	0.02
		1984	M	1.49	13.69	12.27	0.16
		1984	M	1.55	25.25	15.51	0.18
		1984	F	2.15	3.02	2.61	0.04
		1986	M	2.07	9.83	2.88	0.02
		1986	M	1.97	9.58	5.75	0.05
		1986	M	2.07	5.49	5.41	0.01
		1986	M	2.43	7.73	4.18	0.01
		1986	F	2.11	2.96	2.96	0.01
		1986	F	1.95	0.96	1.12	0.05
		1986	F	2.2	2.68	5.07	0.05
		1987	F	2.17	0.57	0.59	0.02
		1987	F	1.62	2.88	5.03	0.08
		1987	F	2.29	0.71	2.41	0.05

continued

Table 1 continued

Species	n	Year	Sex	Length (m)	tDDT	PCB	HCB
Heaviside's dolphin	9	1977	F	1.68	1.17	0.11	0.04
(*Cephalorhynchus heavisidii*)		1982	F	1.58	4.21	0.65	0.06
		1984	M	1.47	4.77	1.76	0.06
		1984	M	1.56	2.71	0.75	0.01
		1984	M	1.56	1.96	0.65	0.08
		1985	NA	NA	0.26	1.09	0.02
		1985	F	1.18	0.25	0.07	0.01
		1985	M	1.51	3.61	1.13	0.04
		1987	M	1.53	0.78	0.27	0.03
Dusky dolphin	12	1977	F	0.85	2.74	0.64	0.01
(*Lagenorhynchus obscurus*)		1977	M	1.69	16.15	2.29	0.02
		1984	M	1.66	13.74	5.59	0.14
		1984	F	1.69	1.49	2.37	0.06
		1986	F	1.61	1.69	1.17	0.01
		1986	F	1.71	1.31	0.56	0.03
		1986	F	1.87	1.04	0.76	0.02
		1986	M	1.84	8.17	4.24	0.06
		1986	M	0.89	0.49	0.38	0.02
		1987	F	1.78	5.82	1.71	0.04
		1987	M	1.76	2.21	1.58	0.05
		1987	M	0.92	1.21	1.89	0.07

CONCLUSIONS

Although conclusions from available data are confounded by the variety of cetacean species sampled and differences in the sex and level of maturity of individuals, it appears that organochlorine contamination in cetaceans off South Africa is similar to that in Australian waters (Kemper *et al.*, 1994), but generally low in comparison with the Northern Hemisphere. Nevertheless, coastal dolphins, particularly those inhabiting South African east coastal waters, appear to be an exception to this pattern and show levels similar to Northern hemisphere coastal cetaceans (Wagemann and Muir, 1984).

On both the west and east coasts of South Africa, coastal dolphins (common, bottlenose, Heaviside's and dusky dolphins) generally show higher contaminant levels than do those cetaceans inhabiting deeper waters. As an example, Indo-Pacific hump-backed dolphins (*Sousa chinensis)* from Natal are never seen in waters deeper than 20m and show the highest organochlorine concentrations of any marine mammal in South African waters (Gardner *et al.*, 1983; Cockcroft, unpublished data). Regardless of the influences of dolphin prey preferences and movement patterns, differential hinterland development of industry and agriculture, or the effect of unknown factors, these data imply that inshore waters are generally more contaminated than deeper waters, even within the coastal belt out to 200m depth.

Despite the occurrence of one mature male bottlenose dolphin from the west coast with PCB levels similar to those for east coast males, there is strong evidence that contaminant levels in cetaceans are related to the level of agricultural and industrial development of the surrounding area. Coincident with agricultural and industrial development, bottlenose dolphins captured in northern Natal, from Durban northwards, display significantly higher levels of PCBs, DDT and Dieldrin than do those from the south (Cockcroft *et al.*, 1989). This clearly suggests that coastal contaminant levels are directly related to the levels of industrialisation and cultivation of adjacent land areas.

Table 2

Chlorinated hydrocarbon residue concentrations (µg/g wet weight) in the blubber of cetaceans stranded on the east coast of South Africa (tDDT=DDT+DDE+TDE, JD = just detectable <0.01, ND = not detectable).

Species	Length (cm)	Weight (kg)	Sex	PCB	DDE	TDE	DDT	Diel	Lind	tDDT	DDE/ TDDT
Eastern Cape											
Grampus griseus	2120	84.8	F	ND	0.86	0.54	1.79	ND		1.40	0.61
G. griseus	2540		F	ND	0.61	0.37	1.07	ND		0.98	0.62
G. griseus	2980	304	M	ND	4.37	0.07	0.40	ND	ND	4.44	0.98
G. griseus	2640	185	M	ND	1.93	ND	ND	ND	ND	1.93	1.00
Tursiops truncatus	1075	14.5	F	ND	0.42	0.02	0.11	0.03	ND	0.44	0.96
T. truncatus	1160	46.5	F	4.23	5.37	ND	1.27	0.06		9.60	0.56
T. truncatus	950	9.5	F	ND	0.32	0.59	ND	0.04		0.91	0.35
T. truncatus	2250		M	10.02	2.40	ND	0.97	2.95		12.42	0.19
T. truncatus	1050	17.7	M	2.57	23.68	ND	0.50	0.03		26.25	0.90
T. truncatus	1040	30.3	M	ND	6.86	ND	ND	0.09		6.86	1.00
T. truncatus			M	ND	0.87	1.16	1.00	0.09		2.03	0.43
Stenella coeruleoalba				ND	1.21	ND	ND	ND		1.21	1.00
S. coeruleoalba				ND	2.49	0.82	1.74	0.04		3.31	0.75
S. coeruleoalba	2280	128	F	ND	6.54	ND	ND	ND		6.54	1.00
S. coeruleoalba	2150	78.5	M	ND	1.82	ND	ND	0.16		1.82	1.00
Orcinus orca	6050	3067	M	ND	7.84	3.70	8.45	0.04		11.54	0.68
Mesoplodon densirostris	2300	157	F	ND	ND	ND	ND	ND		ND	
M. densirostris	3510	468	F	1.71	2.79	ND	0.43	JD		4.50	0.62
M. densirostris	4500		F	ND	ND	ND	ND	ND		ND	
M. densirostris	4650		M	0.45	1.71	ND	0.32	0.02		2.16	0.79
Delphinus delphis	2280	107	M	ND	5.61	ND	1.04	0.43	ND	5.61	1.00
D. delphis	2210	112	M	ND	ND	0.17	0.34	0.02		0.17	JD
D. delphis	1385	27.5	M	ND	0.48	0.02	JD	0.02	ND	0.49	0.99
D. delphis	1210	37.5	M	4.33	10.77	ND	1.88	0.08		15.10	0.71
D. delphis	2280	112	M	6.82	12.90	JD	0.51	ND	JD	19.72	0.65
Kogia simus	2650			ND	0.61	ND	ND	ND	ND	0.61	1.00
K. simus				ND	0.07	ND	ND	ND	ND	0.07	1.00
K. simus	2200	156	F	ND	0.32	0.14	0.44	ND		0.45	0.70
K. simus	1470	61.5	F	ND	0.04	ND	0.07	ND		0.04	1.00
K. simus			M	1.77	3.09	ND	0.25	0.02	ND	4.86	0.64
K. breviceps	2560	177	M	ND	1.01	ND	ND	0.04		1.01	1.00
Physeter macrocephalus	3720		F	ND	0.09	0.04	0.08	ND		0.13	0.69
P. macrocephalus	4080		M	ND	0.20	ND	ND	ND	ND	0.20	1.00
Natal											
G. griseus	2033	81.5	M	ND	ND	ND	ND	0.11	ND	ND	
Stenella attenuata	1880	51.4	F	30.60	32.00	1.27	0.52	0.04	ND	63.87	0.50
S. attenuata	1740	45.5	M	48.30	16.90	ND	3.45	0.07	ND	65.20	0.26
S. attenuata	1820	55.5	M	8.73	8.83	1.60	3.37	ND		19.16	0.46
S. attenuata	2200	84.1	M	4.78	2.65	0.17	ND	ND		7.60	0.35
T. truncatus	1020		M	22.90	13.60	0.71	4.95	0.19	0.03	37.21	0.37
S. coeruleoalba	1860	76	M	ND	2.30	1.17	2.85	ND		3.47	0.66
Hyperoodon planifrons	2910	228	M	ND	1.20	0.40	2.99	0.07		1.60	0.75

Despite the lack of direct cause and effect information for cetaceans, there is sufficient indirect information for the high levels of contaminants in coastal dolphins to warrant concern (see Reijnders *et al.*, 1999). Not only are levels in mature males possibly high enough to impair reproductive capacity (Cockcroft *et al.*, 1989), but the build up and

reproductive transfer to offspring of contaminants in primiparous females could have important implications. With respect to build-up, high concentrations of organochlorines in pregnant females can potentially cause developmental problems in offspring (e.g. Colborn and Clement, 1992). Interpolation from a study of the milk consumption of a captive bottlenose dolphin calf (Cockcroft and Ross, 1990) suggests that primiparous females transfer, via lactation, the major portion of their contaminant load within six to eight weeks *post partum*. The implications of the rapid mobilisation and metabolism of such quantities of contaminants on the primiparous female are unknown. Similarly, the ingestion of the majority of the female's organochlorine load over a short period exposes first born calves to massive doses of contaminants and raises concern for the calf's survival. Both aspects warrant further investigation and should be considered in the conservation and management of coastal dolphins.

Table 3

Mean concentrations (µg/g wet weight) of PCBs and tDDT in different common dolphin sex and age classes (from Cockcroft *et al.*, 1990).

	Sample size	PCB	tDDT
Immatures (<10 GLGs)	42	4.8	4.0
Resting females	11	3.9	3.8
Lactating females	18	1.9	0.9
Pregnant females	9	1.7	1.5
Mature males	17	7.9	6.4

Baleen whales in general show significantly lower levels of contaminants than odontocetes. Although this may be related to their diet, their predominantly offshore occurrence and the fact that most make seasonal migrations to high latitudes are also probable factors (see review by O'Shea and Brownell, 1994). Too few samples of any one species from the region discussed in this paper are available for an unequivocal interpretation.

Table 4

Mean concentrations (µg/g wet weight) of PCBs and tDDT in different bottlenose dolphin sex and age classes (from Cockcroft *et al.*, 1989). n = sample size.

	n	PCB	n	TDDT	n	Dieldrin
Calves	38	8.57	37	10.3cent2	33	0.51
Adolescents	18	13.5	18	11.25	15	0.38
Lactating females	14	2.5	13	1.25	8	0.04
Mature males	17	8.43	16	38.3	15	0.21

In summary, there are insufficient data from the South African region to construct a clear picture of contaminant dynamics in cetaceans. Even for the species with the highest sample sizes (common and bottlenose dolphins), the lack of data since 1987 precludes any interpretation of recent or long-term trends. Consequently, there is an urgent need for further studies, particularly of collected and archived samples, where only the lack of funding hinders analysis. Future studies should include the use of biopsy samples (e.g. Aguilar and Borrell, 1994) and innovative assessments of contaminant effects. For example, sampling of

both dead and free-ranging cetaceans should be combined with pathological examinations in order to assess the actual impact of pollutants on the health of cetacean individuals and populations.

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J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 177–194, 1999

A note on concentrations of metals in cetaceans from southern Africa

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ABSTRACT

Concentrations of zinc, copper, cadmium, mercury and lead were measured by atomic absorption spectrophotometry in samples of the brain, kidney , liver and muscle tissue from 178 individuals of 323 different cetacean species (4 right whales - *Eubalaena australis*, 2 pygmy right whales - *Caperea marginata*, 3 minke whales - *Balaenoptera acutorostrata*, 3 Bryde's whales - *B. edeni*, 1 humpback whale - *Megaptera novaeangliae*, 1 sperm whale - *Physeter macrocephalus*, 11 pygmy sperm whale - *Kogia breviceps*, 6 dwarf sperm whales - *K. simus*, 1 southern bottlenose whale - *Hyperoodon planifrons*, 1 Cuvier's beaked whale - *Ziphius cavirostris*, 9 Blainville's beaked whales - *Mesoplodon densirostris*, 5 strap-tooth whales - *M. layardii*, 2 True's beaked whales - *M. mirus*, 3 long-finned pilot whales - *Globicephala melas*, 30 Risso's dolphins - *Grampus griseus*, 12 bottlenose dolphins - *Tursiops truncatus*, 5 striped dolphins - *Stenella coeruleoalba*, 1 pantropical spotted dolphin - *S. attenuata*, 1 hump-backed dolphin - *Sousa chinensis*, 21 dusky dolphins - *Lagenorhynchus obscurus*, 1 hourglass dolphin - *L. cruciger*, 12 Heaviside's dolphins - *Cephalorhynchus heavisidii* and 43 common dolphins - *Delphinus delphis*). All but the hourglass dolphin were strandings or animals taken incidental to fishing operations or under scientific permit in coastal waters of South Africa or Namibia. Highest concentrations of Zn, Cu and Hg were generally found in the liver and of Cd in the kidney. Comparisons of animals pre-and post puberty indicated accumulation of hepatic mercury in the pygmy sperm whale, Risso's dolphin, dusky dolphin and common dolphin. Loss of a metal (zinc) after puberty was only shown in the common dolphin. No individual analyses exceeded proposed (human) tolerance limits for hepatic mercury and hepatic or renal cadmium.

KEYWORDS: SOUTH AFRICA; POLLUTION; METALS; RIGHT WHALE; PYGMY RIGHT WHALE; MINKE WHALE; BRYDE'S WHALE; HUMPBACK WHALE; SPERM WHALE; PYGMY SPERM WHALE; DWARF SPERM WHALE; SOUTHERN BOTTLENOSE WHALE; CUVIER'S BEAKED WHALE; BLAINVILLE'S BEAKED WHALE ; STRAP-TOOTHED WHALE; TRUE'S BEAKED WHALE; LONG-FINNED PILOT WHALE; RISSO'S DOLPHIN; BOTTLENOSE DOLPHIN; STRIPED DOLPHIN; SPOTTED DOLPHIN; HUMPBACK DOLPHIN; DUSKY DOLPHIN; HOURGLASS DOLPHIN; HEAVISIDE'S DOLPHIN; COMMON DOLPHIN.

INTRODUCTION

This note stems from a general survey of the incidence of metals in marine organisms in South African waters carried out between 1982 and 1990. Cetaceans were considered especially interesting because of their position at the top of the food chain and the consequent expectation that contaminant levels might be relatively high. However, legal and logistical constraints meant that access to material was mainly opportunistic depending on the natural

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occurrence of strandings and the accidental entanglements of individuals in fishing gear. As a consequence, sample sizes vary greatly among species, and for no one species were sufficient individuals analysed for a detailed examination of the effects of sex, age and locality on contaminant levels (Aguilar *et al.*, 1999). Nevertheless, this represents the first such survey for cetaceans in the sub-region, and has value as a baseline study against which future developments might be measured.

Five metals have been analysed in this paper; zinc, copper, cadmium, mercury and lead. The first two are usually classified as essential metals and the remainder as non-essential metals (Bowles, 1999).

MATERIALS AND METALS

Samples for metal analysis were taken from 178 cetaceans of 23 species (Table 1 and Appendix Table 1). All but three animals were obtained between 1982 and 1990. Of the specimens, 127 were stranded on the coastline, 24 entangled in fishing gear and 5 collected at sea under special permit in the waters of South Africa or Namibia. An hourglass dolphin specimen was also collected at sea under special permit in the southwestern Atlantic, and one common dolphin was found unlabelled in the freezer.

Most samples of brain tissue ($n = 170$) were taken through the foramen magnum, and so probably consisted of cerebellum. Muscle tissue ($n = 173$) was removed from the core of one of the dorsal muscle fillets, liver tissue ($n = 167$) from the apex of one of the lobes and kidney tissue ($n = 166$) from the centre of one of the kidneys. All samples were placed in acid-washed polystyrene containers and kept frozen at −20°C until analysis.

Individual cetaceans were assigned into relative age categories, as follows:

(1) calf - still suckling;
(2) juvenile - weaned but sexually immature;
(3) sub-adult - sexually mature but physically immature;
(4) adult - both sexually and physically mature.

Criteria for sexual maturity (and thus immaturity) in females included the presence of an active lactating mammary gland, a corpus luteum or corpus albicans in the ovaries or a foetus. Sexual maturity in males was determined from the size of the testes. An individual was judged physically mature if the epiphyses of the fourth or fifth anterior thoracic vertebrae were fused to their centra: otherwise they were adjudged to be physically immature.

In total, 21 calves, 67 juveniles, 55 sub-adults and 35 adults were sampled. To increase the sample size for analyses of trend in metal concentration with age, calves and juveniles have been combined as 'immature' and sub-adults and adults as 'mature'.

Analyses of metal concentrations were carried out by atomic adsorption spectrophotometry. For the analysis of copper, zinc, cadmium and lead, 2g wet weight of tissue were added to 25ml of concentrated nitric acid and allowed to stand overnight. The resulting digest was gently evaporated almost to dryness. A further 25ml of a 4:1 mixture of concentrated nitric acid and perchloric acids was added to the residue of the first digestion and again slowly evaporated almost to dryness. The final residue was dissolved in 10% nitric acid. This solution was aspirated into a *Varian Spectra* 10 atomic absorption spectrophotometer (AAS) which was set up for each metal according to the instrument manufacturer's recommendations.

The analytical method used for mercury was that of Evans *et al.* (1986) in which wet tissue was treated with concentrated nitric acid and allowed to stand overnight. The acid was then slowly heated to a temperature of 125°C over a period of three hours and then reflexed for

Table 1

Summary of cetaceans from southern Africa sampled for metal analysis.
Key: F = female, M = male, ? = unknown; I = incidental catch, P = special permit catch, S = stranding, C = calf, J = juvenile, SA = subadult, A = adult.

Common name	Latin name	Sample				Origin				Age category				Weight		Length	
		F	M	?	Total	I	P	S	?	C	J	SA	A	Yes	No	Yes	No
Southern right whale	*Eubalaena glacialis*	2	2	0	4	0	0	4	0	4	0	0	0	1	3	4	0
Pygmy right whale	*Caperea marginata*	1	1	0	2	0	0	2	0	0	1	0	1	2	0	2	0
Minke whale	*B. acutorostrata*	2	1	0	3	0	0	3	0	1	1	1	0	1	2	3	0
Bryde's whale	*B. edeni*	1	2	0	3	0	0	3	0	1	2	0	0	1	2	3	0
Humpback whale	*Megaptera novaeangliae*	1	0	0	1	0	0	1	0	1	0	0	0	0	1	1	0
Sperm whale	*Physeter macrocephalus*	0	1	0	1	0	0	1	0	0	0	0	1	0	1	1	0
Pygmy sperm whale	*Kogia breviceps*	7	4	0	11	0	0	11	0	2	2	2	5	9	2	11	0
Dwarf sperm whale	*Kogia simus*	3	3	0	6	0	0	6	0	0	2	3	1	5	1	6	0
Southern bottlenose whale	*Hyperoodon planifrons*	1	0	0	1	0	0	1	0	0	0	1	0	0	1	1	0
Cuvier's beaked whale	*Ziphius cavirostris*	1	0	0	1	0	0	1	0	0	1	0	0	0	1	1	0
Blainville's beaked whale	*Mesoplodon densirostris*	5	4	0	9	0	0	9	0	1	1	0	7	4	5	9	0
Strap-toothed whale	*Mesoplodon layardii*	4	1	0	5	0	0	5	0	1	0	0	4	4	1	5	0
True's beaked whale	*Mesoplodon mirus*	1	0	1	2	0	0	2	0	0	2	0	0	1	1	2	0
Long-finned pilot whale	*Globicephala melas*	2	1	0	3	0	0	3	0	0	1	1	1	1	2	3	0
Risso's dolphin	*Grampus griseus*	14	16	0	30	0	0	30	0	3	6	19	2	21	9	30	0
Bottlenose dolphin	*Tursiops truncatus*	4	8	0	12	0	0	12	0	4	3	2	3	10	2	11	1
Striped dolphin	*Stenella coeruleoalba*	2	3	0	5	0	0	5	0	0	2	2	1	5	0	5	0
Pantropical spotted dolphin	*Stenella attenuata*	1	0	0	1	0	0	1	0	0	0	1	0	1	0	1	0
Hump-backed dolphin	*Sousa chinensis*	1	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0
Dusky dolphin	*Lagenorhynchus obscurus*	14	7	0	21	13	2	6	0	2	10	7	2	20	1	20	1
Hourglass dolphin	*Lagenorhynchus cruciger*	1	0	0	1	0	1	0	0	0	1	0	0	1	0	1	0
Heaviside's dolphin	*Cephalorhynchus heavisidii*	4	7	1	12	2	7	3	0	1	4	5	2	11	1	11	1
Common dolphin	*Delphinus delphis*	21	22	0	43	9	16	17	1	1	27	11	4	43	0	43	0

a further four hours. The final digest was diluted with water and analysed using a *Varian* vapour generation accessory (VGA-76) linked to the *Varian Spectra* 10 AAS and fed by a *Varian* PSC-55 sample changer. The reducing agent, 25% w/v stannous chloride in 20% w/v hydrochloric acid was added at a rate and under conditions only slightly modified from those recommended by Evans *et al.* (1986). The limit quantification LOQ, defined in Zak *et al.* (1983) as the concentration above which quantitative results can be obtained with a specified confidence, in this case ±30% in the measured value at 99% confidence level, was calculated for copper as 0.1μg/g, zinc as 0.05μg/g, cadmium as 0.2μg/g, lead as 0.4μg/g, and mercury as 0.1μg/g.

All metal concentrations quoted are in μg/g wet mass. Figures following ± (and all error bars on the Figures) refer to one standard deviation of the mean.

A total of 2,354 analyses was carried out, 491 of brain, 655 of kidney, 644 of liver and 564 of muscle tissue (Table 2). Analyses for zinc, copper and mercury were similar in number (534, 656 and 577 respectively), whereas fewer analyses were completed for cadmium (355) and lead (132). Three lead analyses from brain tissue have been excluded from the results presented because they originated from animals killed by a gunshot to the head: lead concentrations recorded for these individuals (25.9, 95.2 and 164μg/g) were 7-46 times higher than the next highest concentration recorded for the brain.

Table 2

Numbers of analyses of each heavy metal carried out for each tissue, in cetaceans from southern Africa.

	Metal					
Tissue	Zinc	Copper	Cadmium	Mercury	Lead	Total
Brain	131	165	34	130	31	491
Kidney	163	164	142	147	39	655
Liver	168	165	134	151	26	644
Muscle	172	162	45	149	36	564
Total	634	656	355	577	132	2,354

RESULTS

Distribution between tissues

Given the high individual variability in contaminant levels, only individuals in which all four tissues were analysed have been examined in order to determine the relative contaminant loads between tissues. The results for zinc, copper, cadmium and mercury are shown in Table 3.

The highest concentrations of zinc were found in the liver of 84.9% of the individuals for which all four tissues were examined. Some 13% had their highest concentration in the kidney, and very few (0.6 and 1.5% respectively) in the brain or muscle.

The liver was also the site of the highest concentrations of copper in most (85.7%) individuals, followed by the kidney (8.2%) and the brain (6.1%). The two *Kogia* species formed an exception, in that the highest copper concentration most frequently occurred in the brain (53.8%), followed by the liver (46.2%).

Cadmium levels were highest in the kidney of 73.9% of individuals, followed by the liver (21.7%) and muscle (4.4%).

Mercury levels were highest in the liver of 85% of individuals, with relatively few animals (3.5, 4.2 and 6.7% respectively) having their highest levels in the kidney, brain or muscle.

Table 3

Frequency with which the highest concentrations of heavy metals were found in the brain, kidney, liver or muscle of cetaceans off southern Africa.

Tissue	Zinc	%	Copper	%	Cadmium	%	Mercury	%
Brain	1.0	0.6	9.0	6.1	0.0	0.0	5.25	4.8
Kidney	21.0	13.0	12.0	8.2	17.0	73.9	3.75	3.5
Liver	137.5	84.9	126.0	85.7	5.0	21.7	91.75	85.0
Muscle	2.5	1.5	0.0	0.0	1.0	4.4	7.25	6.7
Total	162.0		147.0		23.0		108.0	

Owing to the relatively few lead analyses carried out it is not possible to present a similar analysis for this metal. The average lead concentrations recorded for each tissue, however, varied little, from 1.07 ± 0.75μg/g in brain to 1.37 ± 0.79μg/g in liver, 2.05 ± 3.10 in kidney and 2.42 ± 3.56μg/g in muscle.

Relationship between metal concentration and maturity status

There were only six species (all odontocetes) for which a sufficient number of samples had been analysed to investigate possible changes in metal concentration with the age of the individual. These were the pygmy sperm whale and the Risso's, bottlenose, dusky, Heaviside's and common dolphins. Even in these cases, the number of individuals sampled (11-40) was only sufficient for an examination on a gross scale, i.e. between immature (calves plus juveniles) and mature (sub-adults plus adults) animals of both sexes combined, and only for four metals (zinc, copper, cadmium and mercury). Comparisons were confined to those tissues in which the highest concentrations of each metal were usually found, i.e. the liver for zinc, copper and mercury and the kidney for cadmium (Table 4).

Mean values were compared using a two-tailed Mann-Whitney U test. Significantly higher hepatic values of mercury in mature individuals were found for the pygmy sperm whale and the Risso's, dusky and common dolphins. Significantly higher renal values of

Table 4

Comparison of concentrations of hepatic zinc, copper, mercury and renal cadmium in immature and mature individuals of six cetaceans in southern African waters. All concentrations are expressed in μg/g wet weight.

		Hepatic zinc		Hepatic copper		Renal cadmium		Hepatic mercury	
Species	Maturity	No.	Mean ±SD	No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD
Pygmy sperm whale	Immature	4	28.5 ± 13.7	4	18.3 ± 21.9	3	2.4 ± 1.2	3	0.9 ± 0.8
	Mature	7	21.9 ± 4.7	7	3.0 ± 1.2	7	15.1 ± 10.7	6	6.1 ± 5.6[1]
Risso's dolphin	Immature	9	34.6 ± 40.9	9	4.2 ± 1.7	6	9.2 ± 1.9	8	2.3 ± 4.6
	Mature	20	28.1 ± 13.0	20	5.7 ± 2.7	20	24.1 ± 16.1[2]	20	45.6 ± 32.2[3]
Bottlenose dolphin	Immature	7	49.7 ± 23.2	7	19.4 ± 16.2	4	7.8 ± 7.0	7	2.5 ± 3.4
	Mature	5	39.9 ± 21.1	5	5.4 ± 1.4	5	9.1 ± 4.8	4	71.0 ± 104.3
Dusky dolphin	Immature	12	18.1 ± 8.1	12	5.1 ± 3.5	10	3.5 ± 1.8	12	1.3 ± 1.1
	Mature	8	34.4 ± 23.0	8	6.5 ± 4.1	8	8.3 ± 5.0[2]	8	7.7 ± 9.2[1]
Heaviside's dolphin	Immature	5	43.0 ± 23.3	5	9.7 ± 2.4	4	8.4 ± 8.3	4	0.6 ± 0.4
	Mature	7	45.9 ± 18.1	7	11.4 ± 4.2	7	11.0 ± 7.3	6	6.2 ± 9.7
Common dolphin	Immature	26	40.2 ± 19.7	26	7.5 ± 3.8	23	4.0 ± 3.3	25	5.3 ± 5.5
	Mature	14	26.8 ± 10.1[1]	14	6.0 ± 2.4	13	8.6 ± 5.3[3]	13	22.0 ± 16.2[3]

[1]Significant at 5% level. [2]Significant at 2% level. [3]Significant at 1% level (or less).

cadmium were found in mature Risso's, dusky and common dolphin individuals. The only significant reduction in the concentration of a metal with age occurred in the common dolphin where hepatic zinc levels were lower in mature animals.

Inter-specific variation in metal concentrations

Appendix Figs 1 - 5 (hereafter simply referred to as 'Figs 1 - 5') show the mean concentrations recorded for each metal for each tissue for each age category of each species. Comparisons between species are complicated by the small sample sizes, high individual variability and correlation with age (at least in some species and metals).

For the five species where three or more adults were examined, hepatic zinc levels averaged between about 20 and 50μg/g (Table 5), with renal levels equivalent or only slightly less. Inspection of Fig. 1 fails to suggest any consistent trend in concentration with age between species. Although there are indications of a trend for some species (minke whale, dwarf sperm whale, striped dolphin and dusky dolphin) sample sizes precluded statistical analysis apart from the significant link reported for the common dolphin in Table 4. Few adult baleen whales were examined. High zinc concentrations were recorded in the liver of right (88.1 ± 47.7μg/g) and Bryde's (87.0μg/g) whale calves and in an adult pygmy right whale (191μg/g).

Table 5

Concentrations of four metals for cases where samples for three or more physically mature specimens were analysed. Concentrations are expressed in μg/g wet mass. Figures following ± refer to one standard deviation of the mean.

Species	Hepatic Zn	Hepatic Cu	Hepatic Hg	Renal Cd
Dwarf sperm whale	22.6 ± 5.4	2.8 ± 1.3	7.2 ± 5.6	20.82 ± 8.4
Blainville's beaked whale	22.7 ± 7.5	5.0 ± 2.0	46.9 ± 26.2	32.3 ± 17.8
Strap-toothed whale	49.7 ± 20.2	8.7 ± 1.7	93.8 ± 57.1	76.9 ± 39.1
Bottlenose dolphin	39.0 ± 20.5	4.6 ± 0.4		11.0 ± 5.2
Common dolphin	19.3 ± 5.1	3.9 ± 0.9	34.4 ± 15.0	7.6 ± 2.3

Concentrations of copper in the liver of adults of the same five odontocetes averaged between about 3 and 9μg/g (Table 5). Inspection of Fig. 2 also fails to suggest any consistent age related change in concentration levels between species. As for zinc, high hepatic copper concentrations were recorded in right (169.5 ± 104.3) and Bryde's (118.0) whale calves and in an adult pygmy right whale (100μg/g).

Concentrations of cadmium in the kidneys of adults of the same species were highly variable (from around 7 to 77 μg/g) as shown in Table 5. A general tendency for concentrations to increase with age is apparent from Fig. 3 (and was demonstrated statistically for three species as shown in Table 4). Renal cadmium values for baleen whale calves and juveniles were low (0.15 – 0.5 μg/g). The value for the adult pygmy right whale however was 46.8 μg/g, similar to some of the odontocetes.

Inspection of Fig. 4 shows a clear indication for mercury concentrations in the liver to increase with age in several species, and such an increase has been statistically demonstrated in Table 4 for four species. In the four species for which there were analyses from three or more adults, average mercury concentrations were highly variable (from around 7 to 94 μg/g) as shown in Table 5. All the baleen whales examined had very low hepatic mercury levels (< 1μg/g), although only one adult (a pygmy right whale) was examined.

The data for lead concentrations are so few and so dispersed among species and ages (Fig. 5) that it is difficult to draw any meaningful conclusions. Values are generally low, less than 5μg/g across all species and tissues, with the highest concentration being 17.6μg/g in the kidney of a sub-adult pygmy sperm whale.

DISCUSSION

For the metals examined here, the site specificity recorded agrees largely with previous findings for cetaceans. In a review of the literature, Bowles (1999) lists the liver as the tissue most commonly containing the highest concentrations of mercury (12/12 species) and the liver (or kidney) as containing the highest concentrations of copper (10/10 species). The kidney (or kidney and liver) was the tissue most commonly containing the highest concentration of cadmium (12/12 species). Excluding skin and bone (which were not sampled in this study), highest concentrations of zinc were found in the liver (6/10 species) or kidney (2/10 species) or both (1/10 species); the tenth species had its highest concentration in the muscle. Site specificity for lead was not often determined (in general, lead concentrations are highest in bone which was not examined in this study). The present results extend Bowles' listing by several species, including the dwarf sperm whale, Blainville's beaked whale, strap-toothed whale, Risso's dolphin, dusky dolphin, Heaviside's dolphin and common dolphin.

Relationships between maturity status and the concentrations of zinc, copper, cadmium and mercury in the liver/kidney recorded in this paper for pygmy sperm whales, Risso's dolphins, bottlenose dolphins, dusky dolphins, Heaviside's dolphins and common dolphins generally agreed with trends found with age in other or the same species. For most essential metals there is no clear age relationship (Bowles, 1999), and the only trend found here was for hepatic zinc concentrations to decline after maturity in common dolphins. High concentrations of zinc relative to those in adults have been found in the livers of neonatal harbour porpoises (Law *et al.*, 1992; Paludan-Muller *et al.*, 1993), and in striped dolphins hepatic zinc concentrations increased during gestation and lactation but declined after weaning (Honda and Tatsukawa, 1983). These trends are not inconsistent with the pre-/post-maturity contrast seen here in common dolphins. Elevated hepatic copper levels in neonates, as evident here for right whales and Bryde's whales, have been previously recorded in several cetacean species (Bowles, 1999). Of the non-essential metals, a strong correlation with age has been demonstrated for mercury in at least eight different cetacean species; harbour porpoises, *Globicephala* spp., striped dolphins, white-beaked dolphins, narwhals, white whales, fin whales and minke whales (Bowles, 1999). To these can now be added pygmy sperm whales, Risso's dolphins, dusky dolphins and Heaviside's dolphins. Hepatic levels of cadmium showed increases with age in at least some post-natal stages of striped dolphins, *Globicephala* spp., narwhals, white whales and harbour porpoises (Bowles, 1999). The significant increases in renal cadmium levels with maturity in Risso's dolphins, dusky dolphins and Common dolphins demonstrated here are consistent with this trend.

Comparisons of the levels of metals found in cetaceans off southern Africa with those elsewhere is complicated by possible inter-specific differences in bioaccumulation rates, differing ages (and sexes) of animals in the samples, and inconsistent analytical techniques (Aguilar *et al.*, 1999). Furthermore, the high individual variability in concentration levels of non-essential metals means that adequate sample sizes must be available for a valid statistical comparison. Bowles (1999) considers that mercury is the only metal which can be readily compared between regions as it accumulates throughout life and has been widely researched. Of the seven species for which he tables values for hepatic mercury from a number of geographical regions (striped dolphins, spotted dolphins, *Globicephala* spp., narwhals, white

whales, harbour porpoises, bottlenose dolphins and white-beaked dolphins), only the bottlenose dolphin is common to the list of species for which an adequate number of adult specimens was analysed in this paper (Table 4). Unfortunately the data for this species tabled by Bowles come from a single specimen, so that a comparison is hardly meaningful. Kemper *et al.* (1994) give a range of values of 0.14-10.18 µg/g for hepatic mercury in nine bottlenose dolphins from Australian waters; four out of twelve values for hepatic mercury from southern African bottlenose dolphins exceeded this range. However, no associated ages are available for the Australian animals, so the significance of this apparent difference cannot be determined.

Although the physiological effects of the metal concentrations found were not part of the investigation, it is interesting to compare the levels recorded with proposed tolerance limits for the metals. Before doing so however, it should be noted that tolerance limits are usually based on information from a range of species and extrapolation from one species to another requires considerable caution. Wagemann and Muir (1984) suggested a range of 100-400µg/g for hepatic mercury[1], whilst Law (in Bowles, 1999), in the context of humans, proposed a range of 200-400µg/g for renal cadmium and 40-200µg/g for hepatic cadmium, as tolerance limits above which toxic effects could occur. Levels of hepatic mercury within (but not exceeding) Wagemann and Muir's tolerance limits only occurred in two out of 151 individuals analysed here, a sub-adult bottlenose dolphins (251.2µg/g) and an adult strap-toothed whales (171.0µg/g). There were no individuals (amongst 142 analysed) with renal cadmium levels that equalled or exceeded Law's proposed tolerance limits. There were six out of 134 cetaceans analysed, however, with hepatic cadmium levels that fell within (but did not exceed) Law's tolerance limits: two adults strap-toothed whales (49.5 and 78.4µg/g), a juvenile hourglass dolphin (41.2µg/g) and three sub-adult Risso's dolphins (46.7, 59.9 and 72.3µg/g). Considering the nature of the sample (dominated by stranded individuals, in which debilitated animals might be expected to feature strongly), the absence of individuals with mercury or cadmium levels exceeding the proposed tolerance ranges suggests that contamination of cetaceans with these metals is not as yet a serious problem in the region noting however, the problems associated from extrapolating from one species to another.

ACKNOWLEDGEMENTS

Numerous people assisted with the collection of material at strandings and elsewhere: these included Michael Meyer, Keiko Sekiguchi, Ken Findlay, Derek Ohland, Martin Haupt, Gus van Dyk, Peter Sims, Mike Combrinck, Kevin Smith, Peter Heather-Noon, Deon Kotze, Herman Oosthuizen and Colin Potts. Their help under sometimes unpleasant circumstances is much appreciated. PBB was supported by the Foundation for Research Development, and the South African Marine Corporation through WWF-South Africa.

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[*Appendix Table 1 and Figs 1 - 5 begin overleaf*]

Appendix

Appendix Table 1

Details of cetaceans from southern Africa sampled for heavy metal analysis. Key: C = calf, J = juvenile, SA = subadult, A = adult; S = stranding, I = incidental catch, P = special permit catch.

Field no.	Length(cm)	Sex	Weight(kg)	Age	Origin	Date	Location	
Southern right whale								
86/29	460	F	-	C	S	20 Aug. 1986	34°33'S	19°21'E
86/32	485	M	-	C	S	2 Sep. 1986	34°33'S	20°25'E
89/23	463	M	-	C	S	31 Jul. 1989	33°38'S	18°23'E
90/29	480	F	1,146	C	S	14 Aug. 1990	34°24'S	19°16'E
Pygmy right whale								
82/11	596	M	2,716	A	S	25 May 1982	34°19'S	18°28'E
89/03	332	F	408	J	S	17 Feb. 1989	22°50'S	14°31'E
Minke whale								
83/17	666	M	4,310	SA	S	30 Mar. 1983	32°43'S	17°59'E
84/34	333	F	-	C	S	20 Nov. 1984	33°54'S	18°26'E
89/01	429	F	-	J	S	3 Jan. 1989	33°58'S	18°22'E
Bryde's whale								
84/20	409	F	514	C	S	10 Jul. 1984	34°46'S	19°52'E
84/28	616	M	-	J	S	9 Nov. 1984	32°44'S	18°01'E
88/04	804	M	-	J	S	15 Feb. 1988	34°46'S	19°42'E
Humpback whale								
90/40	615	F	-	C	S	14 Dec. 1990	33°09'S	17°59'E
Sperm whale								
86/19	1605	M	-	A	S	13 May 1986	34°08'S	18°20'E
Pygmy sperm whale								
82/04	288	F	344	A	S	6 Feb. 1982	32°52'S	17°53'E
82/20	299	F	329	A	S	26 Sep. 1982	34°23'S	20°51'E
82/21	215	M	161	J	S	26 Sep. 1982	34°23'S	20°51'E
83/20	301	F	350	A	S	6 May 1983	34°23'S	20°51'E
83/21	191	M	127	C	S	6 May 1983	34°23'S	20°51'E
83/27	147	M	68	C	S	11 Jun. 1983	34°22'S	18°52'E
83/33	256	F	272	J	S	23 Sep. 1983	34°25'S	19°15'E
84/24	301	F	-	A	S	10 Aug. 1984	34°21'S	19°03'E
84/26	238	F	-	SA	S	4 Sep. 1984	22°50S	14°31'E
86/17	321	F	480	A	S	11 Apr. 1986	34°30'S	20°28'E
86/22	218	M	186	SA	S	23 May 1986	33°27'S	18°16'E
Dwarf sperm whale								
84/36	178	M	110	J	S	26 Nov. 1984	34°06'S	18°28'E
84/35	230	M	200	SA	S	26 Nov. 1984	34°06'S	18°28'S
85/02	251	F	-	A	S	8 Mar. 1985	34°30'S	20°28'E
88/02	226	F	178	SA	S	27 Jan. 1988	34°31'S	20°27'E
88/20	231	F	173	J	S	14 Jul. 1988	34°37'S	19°24'E
90/34	238	M	191	SA	S	16 Oct. 1990	32°47'S	18°04'E
Southern bottlenose whale								
90/02	655	F	-	SA	S	10 Jan. 1990	32°46'S	18°08'E
Cuvier's beaked whale								
89/21	557	F	-	J	S	17 Jul. 1989	33°38'S	18°23'E

continued

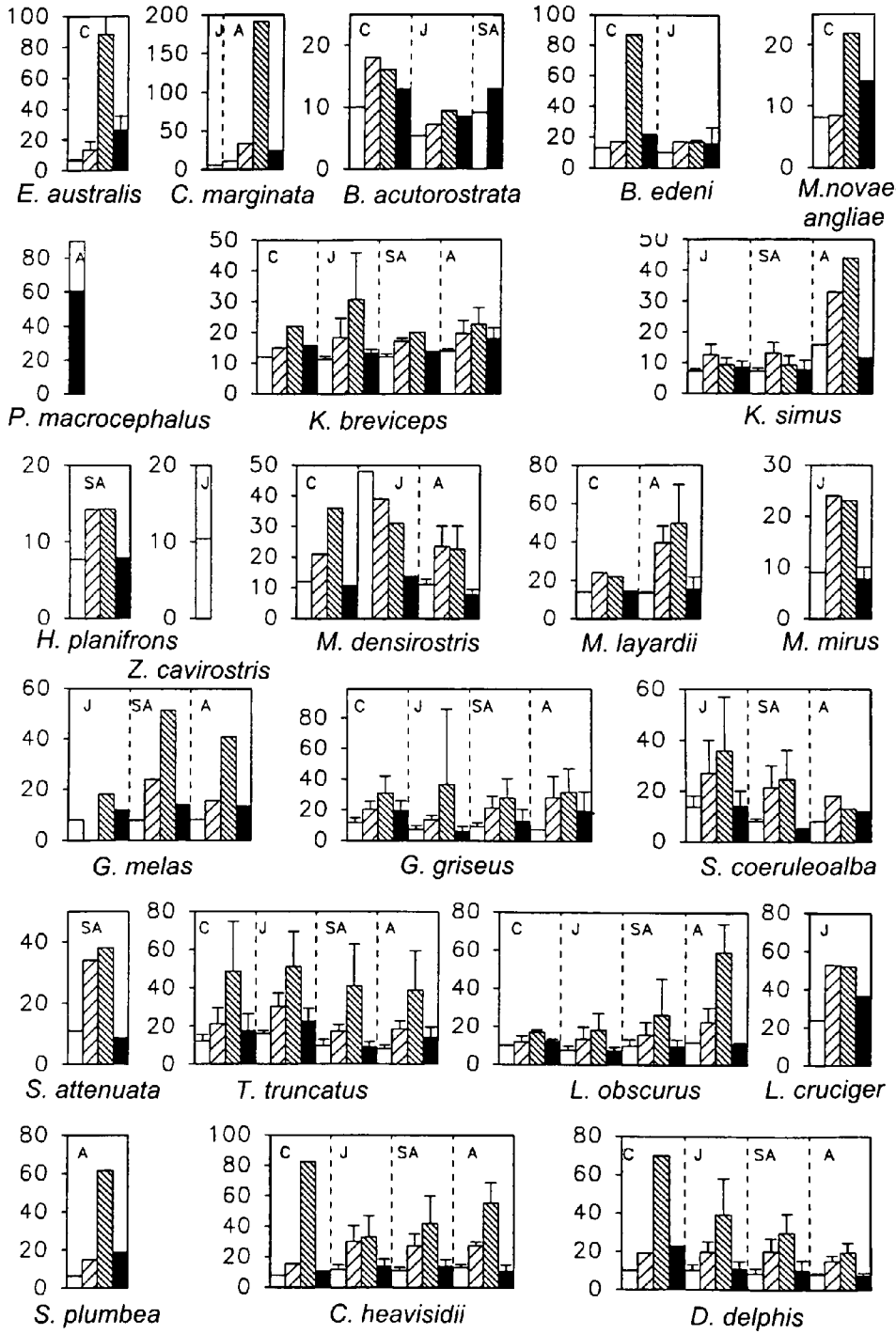
Field no.	Length(cm)	Sex	Weight(kg)	Age	Origin	Date	Location	
Blainville's beaked whale								
84/11	346	F	460	J	S	12 Mar. 1984	34°06S	18°28'E
84/12	454	F	986	A	S	15 Mar. 1984	34°50'S	20°00'E
84/15	225	M	159	C	S	30 Apr. 1984	34°49'S	19°57'E
84/16	454	F	-	A	S	30 Apr. 1984	34°49'S	19°57'E
84/23	425	F	-	A	S	31 Jul. 1984	34°28'S	20°32'E
86/23	376	M	624	A	S	7 Jun. 1986	34°46'S	19°52'E
88/09	436	M	-	A	S	5 Apr. 1988	34°46'S	19°42'E
88/25	413	M	-	A	S	18 Oct. 1988	34°46'S	18°03'E
88/27	401	F	-	A	S	25 Nov. 1988	34°46'S	18°03'E
Strap-toothed whale								
82/09	562	F	690	A	S	6 Apr. 1982	32°43'S	17°59'E
82/10	318	F	415	C	S	6 Apr. 1982	32°43'S	17°59'E
83/14	553	F	1,568	A	S	20 Mar. 1983	32°42'S	18°14'E
83/16	526	F	1,618	A	S	30 Mar. 1983	32°45'S	18°01'E
85/10	500	M	-	A	S	15 Apr. 1985	32°43'S	17°58'E
True's beaked whale								
86/30	318	F	351	J	S	22 Aug. 1986	32°33'S	18°19'E
86/38	417	-	-	J	S	2 Nov. 1986	33°47'S	18°22'E
Long-finned pilot whale								
83/44	381	M	520	J	S	19 Dec. 1983	33°24'S	20°52'E
90/25	371	F	-	SA	S	2 Jun. 1990	33°21'S	18°09'E
90/26	410	F	-	A	S	6 Jun. 1990	34°28'S	19°21'E
Risso's dolphin								
82/18	302	F	325	A	S	24 Aug. 1982	32°43'S	17°56'E
83/15	303	F	350	SA	S	23 Mar. 1983	33°48'S	18°28'E
83/18	152	F	32	C	S	30 Mar. 1983	34°07'S	22°08'E
83/25/1	297	F	327	SA	S	27 May 1983	34°19'S	18°28'E
83/25/2	341	M	486	SA	S	27 May 1983	34°19'S	18°28'E
83/25/3	315	M	404	SA	S	27 May 1983	34°19'S	18°28'E
83/25/4	331	M	450	SA	S	27 May 1983	34°19'S	18°28'E
83/25/5	327	M	468	SA	S	27 May 1983	34°19'S	18°28'E
83/25/6	292	F	292	SA	S	27 May 1983	34°19'S	18°28'E
83/25/7	301	F	345	SA	S	27 May 1983	34°19'S	18°28'E
83/25/8	284	F	304	SA	S	27 May 1983	34°19'S	18°28'E
83/30	188	M	67	C	S	18 Jun. 1983	34°01'S	18°20'E
83/34	225	M	125	J	S	4 Oct. 1983	34°12'S	18°27'E
84/25	314	M	350	SA	S	31 Aug. 1984	34°12'S	18°27'E
88/11	317	M	375	SA	S	17 Jun. 1988	34°22'S	19°51'E
88/12	290	F	313	SA	S	17 Jun. 1988	34°22'S	19°51'E
88/13	315	M	422	SA	S	17 Jun. 1988	34°22'S	19°51'E
89/05	165	F	46	C	S	21 Feb. 1989	34°16'S	18°23'E
89/11/1	304	F	-	SA	S	5 Apr. 1989	32°46'S	17°59'E
89/11/2	266	M	-	J	S	5 Apr. 1989	32°46'S	17°59'E
89/11/3	336	M	-	A	S	5 Apr. 1989	32°46'S	17°59'E
89/11/4	276	M	-	J	S	5 Apr. 1989	32°46'S	17°59'E
89/11/5	270	F	-	J	S	5 Apr. 1989	32°46'S	17°59'E
89/11/6	286	M	-	J	S	5 Apr. 1989	32°46'S	17°59'E
89/11/8	306	M	-	SA	S	5 Apr. 1989	32°46'S	17°59'E
89/11/9	282	F	-	SA	S	5 Apr. 1989	32°46'S	17°59'E
89/20	294	F	-	SA	S	17 Jun. 1989	34°18'S	18°28'E
89/22	281	F	324	SA	S	17 Jul. 1989	34°12'S	18°27'E
89/35	226	M	96	J	S	23 Feb. 1989	34°22'S	20°52'E
90/27	305	M	318	SA	S	3 Apr. 1990	34°50'S	20°01'E

continued

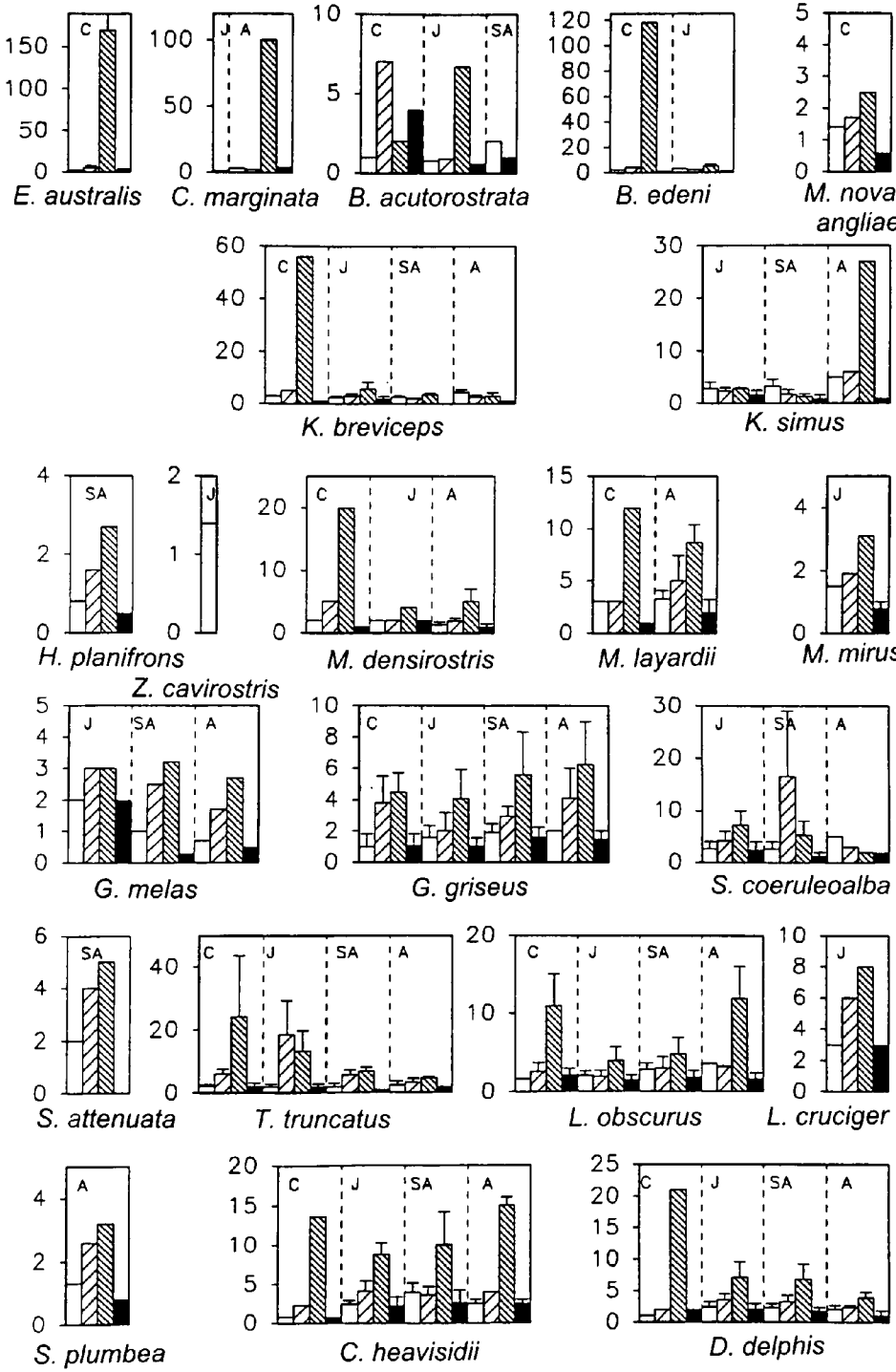
Field no.	Length(cm)	Sex	Weight(kg)	Age	Origin	Date	Location	
Bottlenose dolphin								
80/25	266	M	260	J	S	17 Oct. 1980	22°50'S	14°31'E
82/12	275	M	169	A	S	15 Jun. 1982	33°25'S	19°08'E
83/45	293	M	265	SA	S	21 Dec. 1983	34°29'S	19°21'E
89/08	259	M	166	SA	S	28 Dec. 1988	34°22'S	20°52'E
89/17	293	F	204	A	S	3 Jun. 1989	34°22'S	18°50'E
89/18	218	F	-	C	S	3 Jun. 1989	34°22'S	18°50'E
83/28/1	281	F	304	A	S	14 Jun. 1983	32°43'S	17°56'E
83/28/2	220	F	125	C	S	14 Jun. 1983	32°43'S	17°56'E
83/29	227	M	130	J	S	15 Jun. 1983	34°11'S	22°09'E
84/13	135	M	26	C	S	14 Mar. 1984	22°13'S	14°19'E
85/14	172	M	60	J	S	24 May 1985	34°08'S	18°20'E
85/19	-	M	-	C	S	12 Dec. 1985	34°13'S	22°02'E
Striped dolphin								
82/14	195	M	62	J	S	7 Jul. 1982	34°22'S	21°26'E
83/42	215	F	96	SA	S	6 Dec. 1983	34°09'S	18°51'E
84/31	230	F	116	A	S	18 Oct. 1984	34°08'S	18°20'E
87/37	199	M	77	J	S	19 Dec. 1987	34°23'S	21°12'E
89/28	216	M	104	SA	S	28 Nov. 1989	34°33'S	20°25'E
Pantropical spotted dolphin								
84/19	210	F	88	SA	S	25 Jun. 1984	34°40'S	19°29'E
Humpback dolphin								
90/35	259	F	171	A	S	24 Nov. 1990	34°22'S	21°26'E
Dusky dolphin								
82/01	165	M	52	SA	S	6 Jan. 1982	22°50'S	14°31'E
83/32	-	F	-	A	S	16 Jul. 1983	33°54'S	18°24'E
83/38	187	M	86	SA	P	22 Nov. 1983	33°22'S	18°00'E
84/10	166	M	68	SA	P	26 Feb. 1984	32°16'S	18°13'E
84/33	169	F	62	J	S	6 Jan. 1982	22°57'S	14°30'E
86/03	89	M	8	C	S	22 Jan. 1986	34°08'S	18°27'E
86/24	187	F	93	A	I	16 May 1986	32°43'S	17°58'E
87/01	178	F	55	SA	S	7 Feb. 1987	34°12'S	18°22'E
87/03	92	M	9	C	S	25 Feb. 1987	33°43'S	18°27'E
88/15	160	M	59	J	I	13 Jul. 1988	19°20'S	12°32'E
88/16	182	F	78	SA	I	13 Jul. 1988	19°20'S	12°32'E
88/17	173	F	65	J	I	13 Jul. 1988	19°20'S	12°32'E
88/18	176	F	78	SA	I	13 Jul. 1988	19°20'S	12°32'E
89/09	167	F	57	J	I	19 Mar. 1989	34°08'S	18°27'E
89/26	173	F	67	SA	I	26 Oct. 1989	30°00'S	16°01'E
89/27	176	F	66	J	I	26 Oct. 1989	30°00'S	16°01'E
89/31	170	F	69	J	I	23 Jun. 1989	24°44'S	14°28'E
89/32	176	F	62	J	I	27 Jun. 1989	18°15'S	12°04'E
89/33	169	F	64	J	I	23 Jun. 1989	24°44'S	14°28'E
89/34	156	M	56	J	I	23 Jun. 1989	24°44'S	14°28'E
90/23	170	F	68	J	I	11 May 1990	30°49'S	17°29'E
Hourglass dolphin								
82/07	164	F	74	J	P	25 Dec. 1981	55°39'S	60°41'W
Heaviside's dolphin								
77/07	168	F	64	A	P	18 Jan. 1977	23°00'S	14°22'E
80/17	166	F	68	SA	P	24 Aug. 1980	29°08'S	16°46'E
82/19	158	F	52	J	P	28 Aug. 1982	30°52'S	17°33'E
84/08	147	M	51	J	P	26 Feb. 1984	32°50'S	17°45'E
84/09	156	M	56	SA	P	28 Aug. 1982	32°45'S	17°46'E
84/30	156	M	59	A	P	29 Sep. 1984	30°25'S	17°18'E
85/07	118	F	27	J	S	9 Apr. 1985	32°20'S	18°19'E

continued

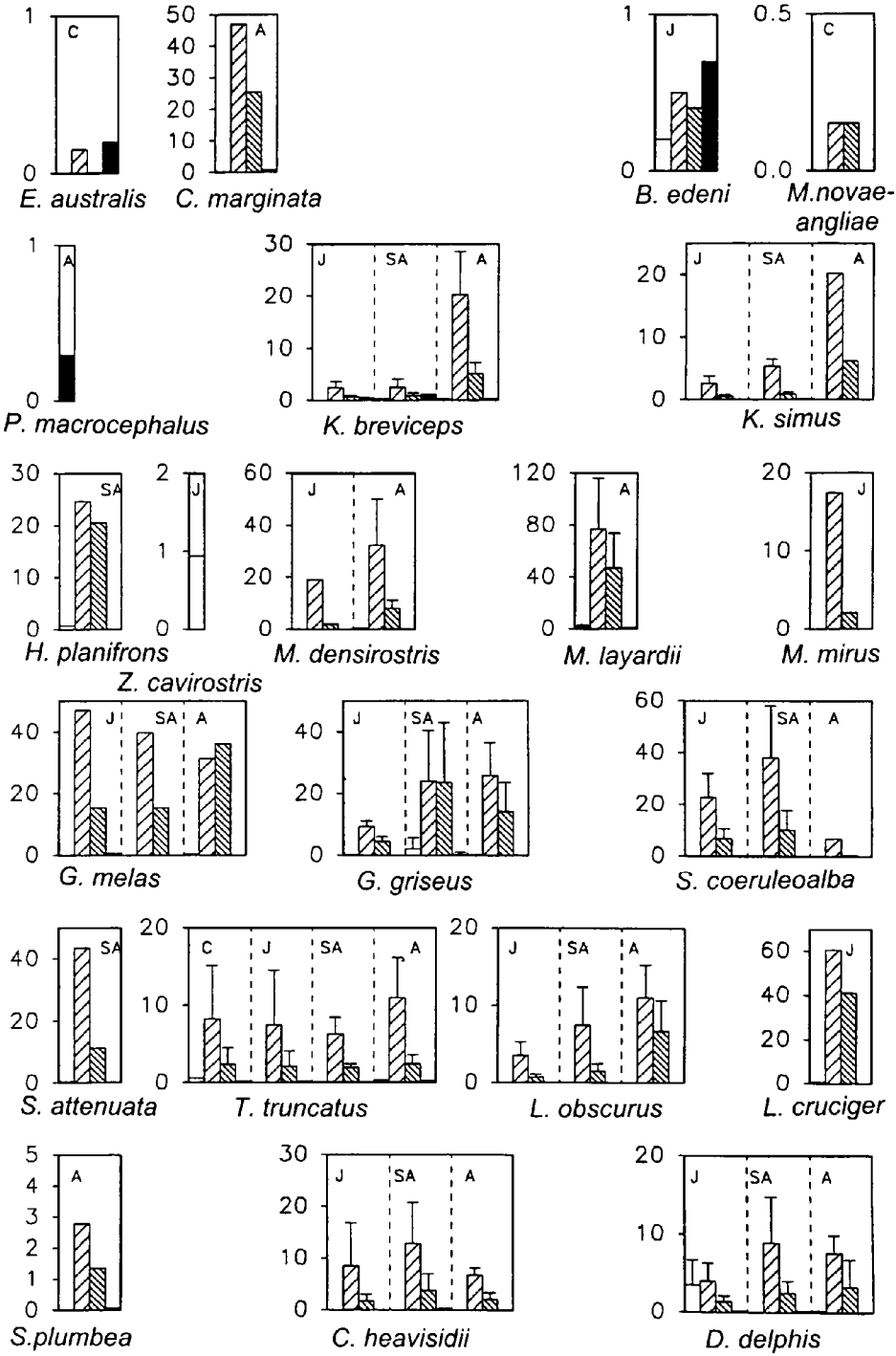
Field no.	Length(cm)	Sex	Weight(kg)	Age	Origin	Date	Location	
Heaviside's dolphin (cont.)								
85/09	-	-	-	SA	I	11 Mar. 1985	32°44'S	17°52'E
85/15	151	M	44	SA	S	19 Jul. 1985	34°03'S	18°22'E
87/15	155	M	46	J	P	17 Aug. 1987	18°48'S	12°19'E
89/29	79	M	6	C	S	18 Jan. 1989	22°07'S	14°16'E
90/21	164	M	68	SA	I	19 Apr. 1990	30°18'S	17°10'E
Common dolphin								
82/06	102	M	10	C	S	15 Feb. 1982	34°10'S	18°26'E
83/01	224	M	136	SA	P	23 Jan. 1983	34°38'S	24°38'E
83/02	208	F	87	J	P	26 Jan. 1983	33°50'S	25°50'E
83/03	221	F	136	SA	P	27 Jan. 1983	34°15'S	24°53'E
83/04	196	M	85	J	P	9 Feb. 1983	34°18'S	18°18'E
83/05	203	F	88	J	P	12 Feb. 1983	34°44'S	19°04'E
83/06	220	F	103	J	P	13 Feb. 1983	34°49'S	20°29'E
83/10	248	M	158	A	S	5 Mar. 1983	34°28'S	20°32'E
83/11	190	M	76	J	I	23 Feb. 1983	17°42'S	11°28'E
83/12	177	F	65	J	I	22 Feb. 1983	17°45'S	11°26'E
83/19	215	M	81	J	S	1 Apr. 1983	34°19'S	18°28'E
83/36	208	F	96	J	I	8 Nov. 1983	34°28'S	20°58'E
83/37	215	F	90	J	I	8 Nov. 1983	34°28'S	20°58'E
83/39	223	M	109	SA	P	29 Nov. 1983	34°14'S	18°19'E
83/40	210	F	90	J	P	29 Nov. 1983	34°27'S	18°29'E
84/01	208	M	82	J	S	26 Jan. 1984	34°08'S	18°20'E
84/03	222	M	106	J	P	22 Feb. 1984	34°48'S	19°11'E
84/04	232	M	121	SA	P	22 Feb. 1984	34°54'S	19°31'E
84/05	230	M	115	SA	P	22 Feb. 1984	35°00'S	19°58'E
84/06	210	M	102	J	P	24 Feb. 1984	34°44'S	19°04'E
84/07	215	F	98	J	P	24 Feb. 1984	34°39'S	18°59'E
84/17	149	M	27	J	S	30 Apr. 1984	34°22'S	18°52'E
84/32	155	M	38	J	S	30 Nov. 1984	34°26'S	19°14'E
85/11	216	F	92	J	P	18 Apr. 1985	34°07'S	22°31'E
85/12	210	M	93	J	P	21 Apr. 1985	34°00'S	25°08'E
85/13	254	M	158	SA	P	24 Apr. 1985	34°27'S	21°34'E
86/01	195	F	80	SA	S	3 Jan. 1986	34°39'S	20°15'E
86/07	115	M	15	J	S	10 Feb. 1986	34°05'S	18°34'E
86/09	220	F	100	SA	I	18 Feb. 1986	34°35'S	19°05'E
86/10	207	M	84	J	I	18 Feb. 1986	34°35'S	19°05'E
86/11	197	M	69	J	I	18 Feb. 1986	34°35'S	19°05'E
86/12	211	F	84	J	I	18 Feb. 1986	34°35'S	19°05'E
86/13	226	F	98	J	I	18 Feb. 1986	34°35'S	19°05'E
86/33	207	M	65	J	S	6 Sep. 1986	34°09'S	18°27'E
86/37	224	M	124	SA	S	24 Oct. 1986	34°06'S	18°31'E
86/39	243	M	129	A	S	10 Oct. 1986	34°45'S	19°36'E
87/07	184	F	63	J	S	10 Mar. 1987	34°24'S	19°16'E
87/36	217	F	96	SA	S	17 Dec. 1987	34°23'S	21°12'E
89/25	165	F	34	J	S	15 Sep. 1989	33°54'S	18°28'E
90/01	224	F	96	A	S	6 Jan. 1990	34°06'S	18°48'E
90/22	224	F	94	SA	S	25 Apr. 1990	34°43'S	20°07'E
90/24	151	F	31	J	-	-	-	-
90/31	231	F	94	A	S	4 Sep. 1990	34°47'S	19°38'E



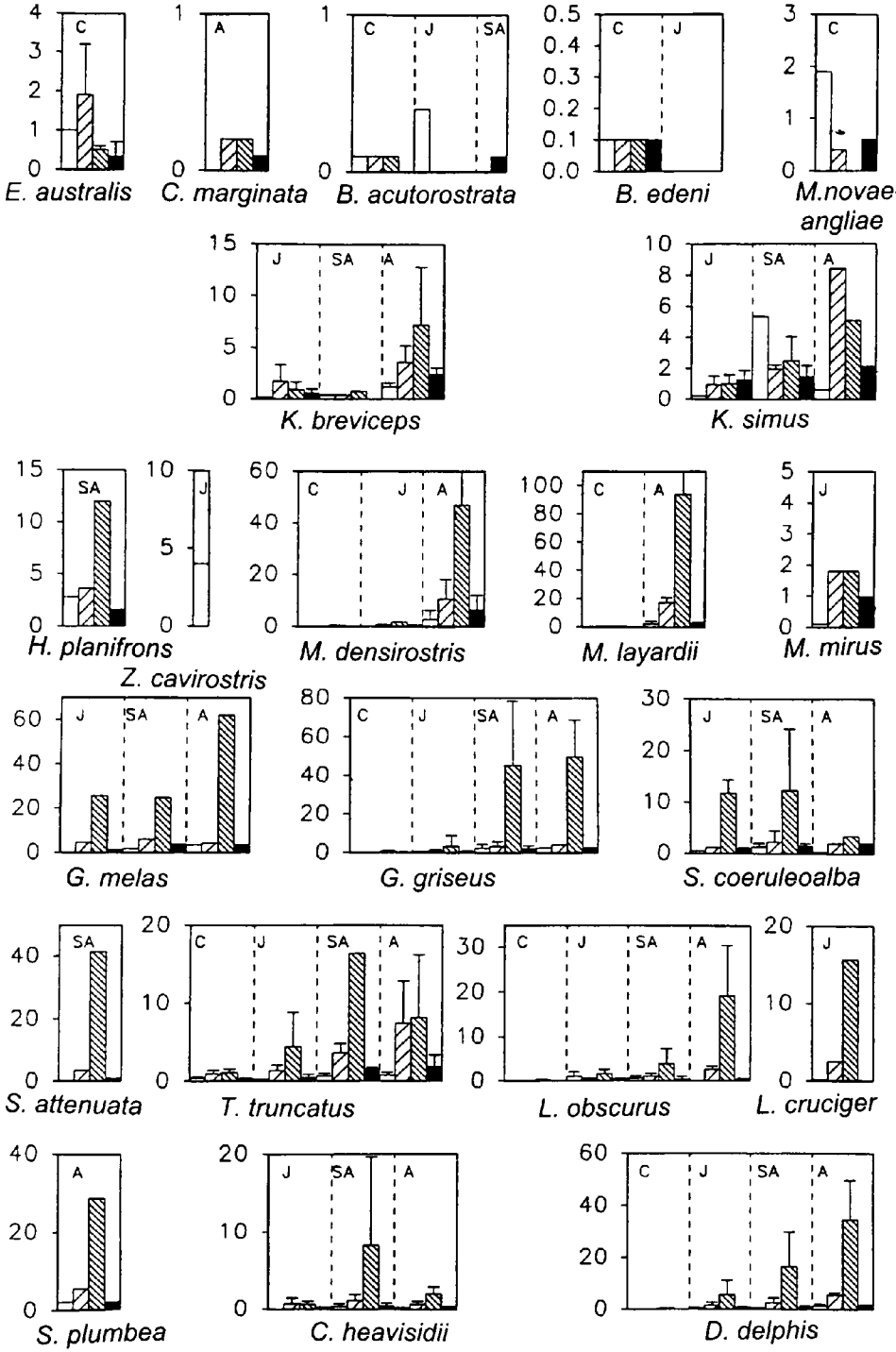
Appendix Fig. 1. Mean concentrations (in μg/g wet weight) of zinc in brain (open), kidney (rising right), liver (rising left) and muscle (solid) of different age classes of cetaceans from southern Africa (C = calf, J = juvenile, SA = sub-adult, A = adult).



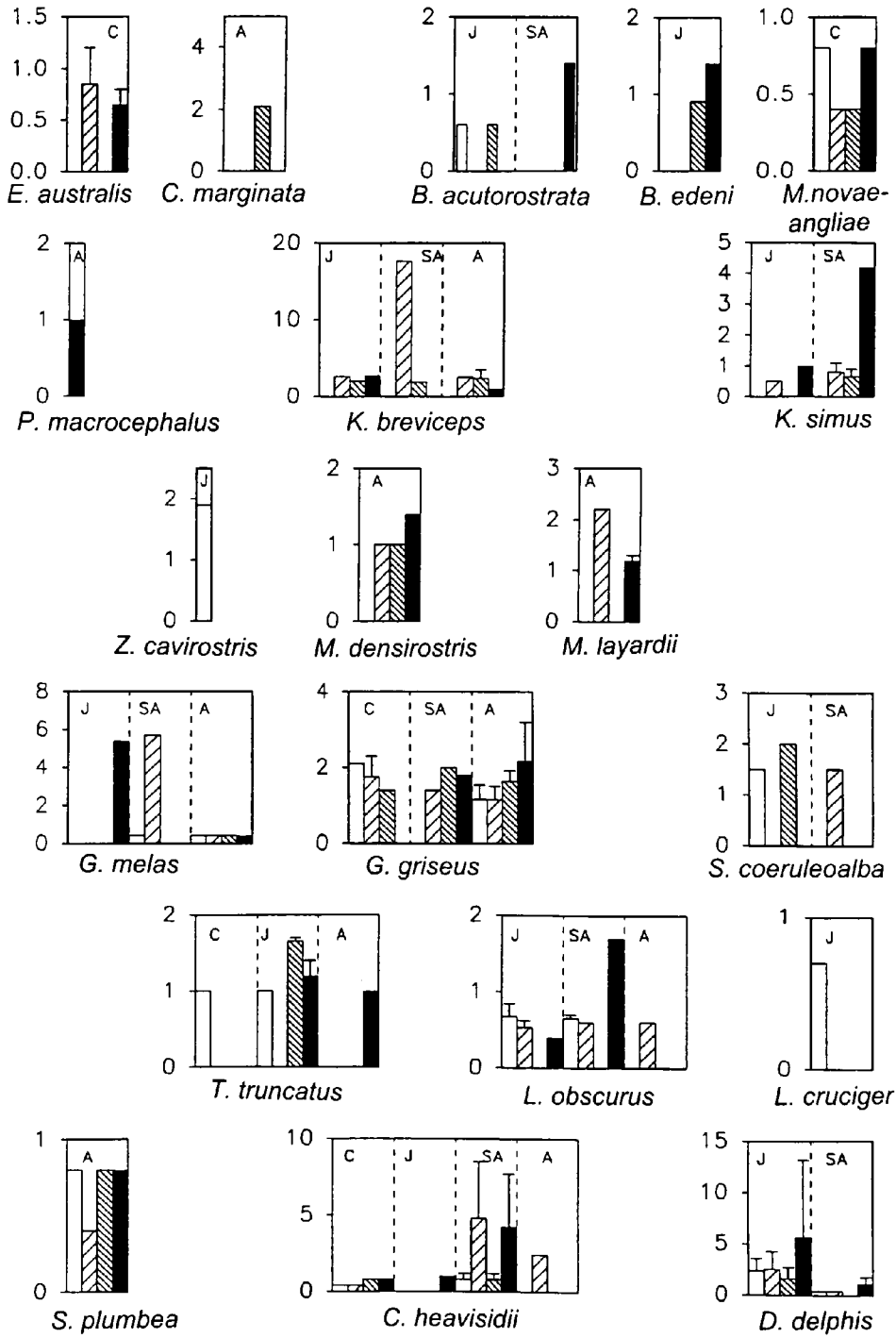
Appendix Fig. 2. Mean concentrations (in μg/g wet weight) of copper in brain (open), kidney (rising right), liver (rising left) and muscle (solid) of different age classes of cetaceans from southern Africa (C = calf, J = juvenile, SA = sub-adult, A = adult).



Appendix Fig. 3. Mean concentrations (in µg/g wet weight) of cadmium in brain (open), kidney (rising right), liver (rising left) and muscle (solid) of different age classes of cetaceans from southern Africa (C = calf, J = juvenile, SA = sub-adult, A = adult).



Appendix Fig. 4. Mean concentrations (in µg/g wet weight) of mercury in brain (open), kidney (rising right), liver (rising left) and muscle (solid) of different age classes of cetaceans from southern Africa (C = calf, J = juvenile, SA = sub-adult, A = adult).



Appendix Fig. 5. Mean concentrations (in μg/g wet weight) of lead in brain (open), kidney (rising right), liver (rising left) and muscle (solid) of different age classes of cetaceans from southern Africa (C = calf, J = juvenile, SA = sub-adult, A = adult).

J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 195–207, 1999

A review of organochlorine and metal pollutants in marine mammals from Central and South America

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ABSTRACT

Published data on pollutants found in marine mammals from Central and South America are limited. Few species have been studied (18) and sample sizes are usually too small to allow for proper assessment of trends or impacts of pollutants on the populations being studied. The only exceptions to this are the franciscana dolphin from Argentina and the spotted dolphin from the eastern tropical Pacific: the former population studied for organochlorines and the latter for heavy metals. Information on organochlorine levels, mainly on PCBs and DDTs, suggests low levels of exposure when compared to other regions of the world. The ratio DDT/PCB is higher than in other areas, which indicates the predominance of agricultural contamination over that of industrial origin. The generally low DDE/tDDT ratio, particularly in southern America, indicates a recent usage of this pesticide in the region. Levels of mercury were moderate overall, although marine mammals from the areas where contamination by this metal is likely to be higher, such as the Amazon river, have not been studied in this regard. In contrast, mean cadmium and zinc concentrations were higher overall than those in the range typical for northern marine mammals, while copper and lead levels were comparatively low, although information on these latter metals is extremely limited. The lack of comprehensive, long-term studies makes a sound evaluation of the impact of pollutants on the marine mammals from the region unfeasible.

KEYWORDS: POLLUTION-ORGANOCHLORINES; POLLUTION-METALS; MARINE MAMMALS; SOUTH ATLANTIC; SOUTH PACIFIC

INTRODUCTION

Hazardous chemicals such as organochlorine compounds and heavy metals have been dumped in large quantities in the environment for decades and have finally been deposited in the sea (Borrell and Reijnders, 1999). Some of these synthetic chemicals, such as most organochlorines, have high bioaccumulation potential and biomagnify along trophic webs. In contrast, others, such as some metals, have low potential for bioaccumulation and their transfer rate through the food web is limited (Aguilar *et al.*, 1999).

Marine mammals are threatened by these toxic contaminants because many of them occupy high trophic levels, are long-lived, and are therefore able to bioaccumulate high concentrations of these persistent contaminants. Although information on the actual impact of pollutants on marine mammals is scarce (Reijnders *et al.*, 1999), it has been demonstrated that certain organochlorines have a potential for causing reproductive failure and depression of the immune system of seals (e.g. Reijnders, 1986; De Swart *et al.*, 1994).

This review collates information on pollutant levels in marine mammals from Central and South America. In an attempt to ensure the quality of the information collated in this review, only papers published in refereed journals have been included.

REVIEW OF AVAILABLE INFORMATION

Organochlorine compounds

Organochlorine compounds are hydrocarbons with chlorine atoms in their molecules. Only two groups of these are highly resistant to biodegradation and have entered the marine food webs in significant quantities: the DDTs (dichlorodiphenyltrichloroethanes) and the PCBs (polychlorinated biphenyls). Other organochlorine compounds such as hexachlorobenzene (HCB), aldrin, dieldrin, toxaphene, heptachlor epoxide, trans-nonachlor, endrin, α-HCH, β-HCH and lindane (τ-HCH) have also been detected in the tissues of some marine mammals although their concentrations are usually quite low.

Organochlorine compounds are lipophilic and thus reach their highest concentrations in fatty tissue and, particularly, in the hypodermic fat or blubber. For this reason, and because blubber is readily accessible to sampling both in live and dead individuals, the target tissue in studies devoted to this group of pollutants has traditionally been blubber (Aguilar, 1987). Thus, although some references contain data on organochlorine concentrations in other tissues, the present review considers only those relative to blubber.

Coverage of studies

Organochlorine concentrations have been reported from 12 cetacean species in Central and South America: three mysticetes and nine odontocetes (Table 1). However, this diversity is not distributed homogeneously either temporally or geographically. Indeed, the data for many species originate solely from Central America, particularly from the Caribbean. From the six odontocete and one mysticete species collected in Central America, with the exception of three dolphin species (Fraser's dolphin, striped dolphin and spinner dolphin) surveyed in the Pacific, the rest (humpback whale, sperm whale, short-finned pilot whale, spinner dolphin and tucuxi) were all studied in the Caribbean Sea. However, even in the Antillean region sample sizes are small, consisting of 11 individuals from four different species. Moreover, most surveys reported from Central America were undertaken during the early 1970s and almost no recent information is available from this area (Fig. 1).

In South America, three odontocete and two mysticete species were examined. The odontocetes studied were the franciscana and Burmeister's porpoise sampled in Argentina and Uruguay from the mid-seventies to the early nineties, and a tucuxi from Surinam sampled in 1971. The mysticete sample was composed of fin and Bryde's whales caught off the coast of Chile by whaling operations in 1983 (Fig. 1).

Levels

Table 1 details published organochlorine residue concentrations in the blubber of marine mammals from Central and South America. Data are expressed on the basis of $\mu g.g^{-1}$ wet weight, a common basis for calculating concentrations. When data were reported on a lipid weight basis, concentrations were converted into wet weight levels through their tissue lipid content.

In most surveys, sample sizes were small and biological data from specimens studied, particularly age and reproductive status, were not stated. When this information was available, mean organochlorine concentrations were recalculated separately for males and females. In nearly all cases in which the sexes could be separated, organochlorine concentrations were higher in males (Table 1). Males have higher organochlorine concentrations than females because females transfer part of their contaminant burden to their offspring during gestation and lactation; this is the main factor producing an age-related increase in organochlorine loads and levels in males and a decrease in females (Aguilar *et al.*, 1999). However, studies on patterns of age- and sex-related variation in PCB and tDDT

levels are available only from franciscanas caught in gillnets off Argentina, in which the only significant trend observed was an increase in tDDT levels with age in males; other trends were not significant, probably as a consequence of biased representation of age classes in the sample due to selectivity of incidental capture in gillnets (Borrell *et al.*, 1996).

Overall residue levels found in cetaceans from both Central and South American waters are low. Mean PCB concentrations were in all cases lower than $10\mu g.g^{-1}$ wet weight (range $0.4\text{-}9.1\mu g.g^{-1}$) in all species. These levels fall within the lower bounds of the range commonly detected in marine mammals from other regions, and are much lower than those associated with reproductive impairment or depression of immunocompetence in seals (e.g. De Swart *et al.*, 1994). Congener specific PCB concentrations have only been reported in Burmeister's porpoises from Argentina (Corcuera *et al.*, 1995).

In general, levels of tDDT were also generally low, although remarkably high concentrations were detected in three small odontocete populations: striped dolphins from the eastern tropical Pacific, with tDDT concentrations averaging $102\mu g.g^{-1}$ in males and $28\mu g.g^{-1}$ in females; franciscanas from Uruguay, the mean tDDT concentrations of which reached $30\mu g.g^{-1}$ in males and $20\mu g.g^{-1}$ in females (O'Shea *et al.*, 1980) and tucuxi from

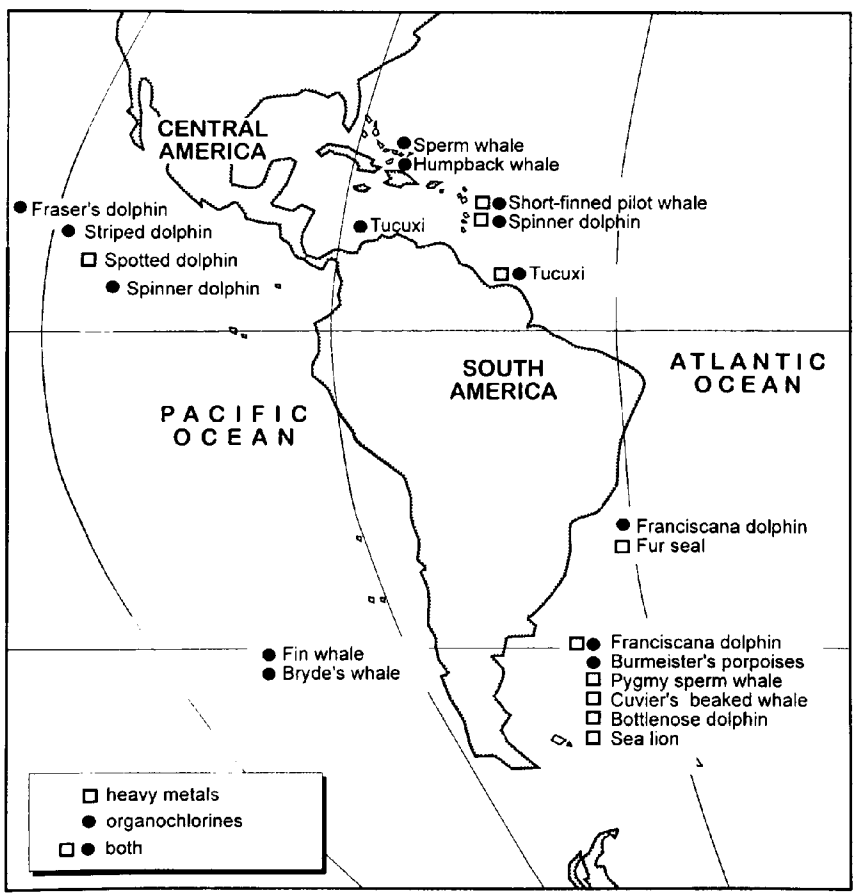

Fig. 1. General location of the marine mammal species analysed from Central and South America.

the Caribbean Sea (Colombia), in which 51.2μg.g^{-1} were detected in one male and 63.3μg.g^{-1} in a female (Duinker *et al.*, 1989). The remaining species all had mean levels below 16μg.g^{-1} (range 0.05-15.5μg.g^{-1}) (Table 1).

As one might expect, the two baleen whale species sampled in Chile had significantly lower mean levels of tDDT (0.005-0.6μg.g^{-1}) than the other species (Pantoja *et al.*, 1984). This can be explained by the fact that they feed on krill and are therefore situated low in the trophic web. Surprisingly, however, humpback whales from the Antilles presented comparatively high tDDT levels of 1.75μg.g^{-1} (Taruski *et al.*, 1975) and, despite being towards the lower bound of the overall concentration ranges, their organochlorine levels were not statistically different from those found in odontocetes in the same region.

Table 1

Organochlorine residue concentrations and relative ratios (mean ± SD) in the blubber of marine mammals from Central and South America.

Species Code	Area/Species	Sex	n	Date	Provenance of researcher	Reference
	CENTRAL AMERICA					
	ANTILLES					
1	Humpback whale, *Megaptera novaengliae*	m	2	1972	USA	Taruski *et al.*, 1975
2	Sperm whale, *Physeter macrocephalus*	m	1	1972	USA	Taruski *et al.*, 1975
		f	1	1972		
	STA. LUCIA (LESSER ANTILLES)					
3	Short-finned pilot whale, *Globicephala macrorhynchus*	m	4	1972	Canada	Gaskin *et al.*, 1974
		f	1	1972		
4	Spinner dolphin, *Stenella longirostris*	m	1	1972	Canada	Gaskin *et al.*, 1974
		f	1	1972		
	EASTERN TROPICAL PACIFIC					
5	Striped dolphin, *Stenella coeruleoalba*	m	4	1973-76	USA	O'Shea *et al.*,1980
		f	10	1973-76		
6	Fraser's dolphin, *Lagenodelphis hosei*	m	1	1973-76	USA	O'Shea *et al.*,1980
		-				
	COLOMBIA					
7	Tucuxi, *Sotalia fluviatilis*	m	1	1977	Germany	Duinker *et al.*, 1989
		f	1	1977		
	SOUTH AMERICA					
	CHILE					
8	Fin whale, *Balaenoptera physalus*	-	1	1983	Chile	Pantoja *et al.*, 1984, 1985
9	Bryde's whale, *Balaenoptera edeni*	-	2	1983	Chile	Pantoja *et al.*, 1984, 1985
	URUGUAY					
10	Franciscana dolphin, *Pontoporia blainvillei*	m	5	1974	USA	O'Shea *et al.*,1980
		f	3	1974		
	ARGENTINA					
11	Franciscana dolphin, *Pontoporia blainvillei*	m	43	1988-92	Spain	Borrell *et al.*, 1995
		f	31	1988-92		
12	Burmeister's porpoises, *Phocoena spinipinnis*	m	4	1989-90	Spain	Corcuera *et al.*, 1995
		f	4	1989-90		
	SURINAM					
13	Tucuxi *Sotalia fluviatilis*	-	1	1971	The Netherlands	Koeman *et al.*, 1972

Table 1—*continued over*

Most studies present results only for PCB and DDT residues. However, O'Shea *et al.* (1980) also documented levels of other, less ubiquitous, organochlorine compounds in a variety of odontocetes from the region. These compounds, when detected, were always found at extremely low concentrations. In franciscanas, they found mean HCB concentrations (given to ± 1 SD) were of 0.067 ± 0.047μg.g^{-1} in females and 0.08 ± 0.08μg.g^{-1} in males; the compound was not detected in striped or Fraser's dolphins. Two males out of 13 striped dolphins contained toxaphene and heptachlor epoxide at mean concentrations of 4.8 ± 0.07 and 0.28 ± 0.05μg.g^{-1} respectively. Endrin occurred at 0.22μg.g^{-1} in a male striped dolphin, but in no other individuals of that species or in Fraser's dolphin or franciscanas. Trans-nonachlor was detected in six female striped dolphins (0.19 ± 0.2μg.g^{-1}) out of 13 analysed, and in all but one female franciscana (males 0.18 ± 0.04μg.g^{-1}; females 0.073 ± 0.05μg.g^{-1}). Residues of cis-chlordane were positively

Table 1—*continued*

	ppm fresh weight in blubber								
S C	Dieldrin	opDDT	TDE	DDE	ppDDT	DDTs	PCBs	%DDE/ tDDT	%DDT/ PCB
1	0.05±0.05	-	0.2±0.1	0.95±0.05	0.60±0.30	1.75±0.35	1.40±0.10	57.1±14.0	124±16
2	0	-	0.1	0.8	0.20	1.10	0.7	72.7	157
	0	-	1.6	9.9	4.0	15.5	4.00	63.9	387
3	0.05±0.007	-	0.18±0.02	0.96±0.22	0.56±0.14	1.69±0.38	1.24±0.32	56.3±0.7	146±45.4
	0.01	-	0.12	0.83	0.35	1.30	0.69	63.8	188
4	0.007	-	0.58	6.67	0.13	7.38	5.00	90.4	148
	0.05	-	0.08	1.19	0.17	1.44	2.00	82.6	72
5	0.26±0.34	6.49±6.02	3.37±2.83	63.7±51.9	20.7±19.3	102.1±83.2	5.50±1.92	57.5±19.3	1610±1026
	0.17±0.16	0.77±0.93	1.26±1.15	16.7±21.3	5.77±9.64	28.4±36.2	3.35±4.26	60.2±11.1	754±328
6	0	0	0.72	7.2	1.80	11.02	5.20	65.3	212
7	0.17	-	2.90	41.84	6.43	51.18	7.26	81.76	704.55
	0.38	-	8.00	41.17	14.17	63.33	9.14	65.00	692.62
8	0.0206	-	0.0046	0.0481	0.0017	0.0544	-	88.4	-
9	0.053±0.042	-	0.19±0.12	0.33±0.27	0.068±0.022	0.59±0.42	-	46.3±13.4	-
10	0.52±0.25	1.24±0.40	2.56±0.79	4.48±1.24	7.68±1.20	29.62±5.82	7.88±5.20	15.7± 4.8	513±297
	0.21±0.01	2.81±3.39	1.48±0.66	2.16±0.20	4.27±1.06	20.23±6.06	3.93±0.25	11.9± 4.07	513±138
11	-	0.12±0.15	0.12±0.12	0.91±0.93	0.38±0.44	1.52±1.61	1.78±1.01	61.1± 5.7	75±41
	-	0.06±0.06	0.08±0.08	0.60±0.45	0.22±0.22	0.97±0.80	1.34±0.67	64.9±10	64.8±23.9
12	-	0.40±0.16	0.28±0.10	2.35±1.40	0.99±0.48	4.01±2.02	2.77±1.07	55.4± 9.7	126±42
	-	0.21±0.19	0.17±0.17	0.58±0.83	0.56±0.48	1.53±1.52	1.84±1.32	35.5±16.9	66.0±32.6
13	0.19	-	0.35	2.1	0.32	2.77	<0.4	75.8	693

identified in two male franciscanas ($0.53 \pm 0.41 \mu g.g^{-1}$) and in one striped dolphin female ($0.18 \mu g.g^{-1}$). Oxychlordane, mirex and cis-nonachlor were not found in any of the individuals studied by O'Shea *et al.* (1980). Tanabe *et al.* (1996) found extremely low levels of α-, β- and τ- (lindane) HCH in two spinner dolphins from the eastern tropical Pacific.

Lindane and aldrin have also been reported from Bryde's and fin whales caught off Chile, where the mean levels were always below $0.07 \mu g.g^{-1}$ (Pantoja *et al.*, 1985). Taruski *et al.* (1975) found a concentration of $0.1 \mu g.g^{1}$ of α-chlordane in one of the two humpback whales studied in the Antilles, but in none of the sperm whales from the same region. Dieldrin, the most widespread organochlorine pollutant after DDT and PCBs, was detected in several species and regions although levels were always low, below $0.5 \mu g.g^{-1}$.

The tDDT/PCB ratio commonly observed in the surveys was very high (432 ± 428 in Central America and 293 ± 249 in South America) in comparison to that usually found in marine mammals from other geographical locations (Aguilar *et al.*, 1999). This indicates a greater contribution to the organochlorine pollution in this area by agriculture than industry.

By comparison, the DDE/tDDT ratio was about mid-range in Central America (68 ± 11), and somewhat lower in South America (61 ± 17), with the exception of the franciscana from Uruguay, from which extremely low values (14 ± 4) were found. DDE is the main product of the metabolisation of commercial DDT and, in areas where the use of this pesticide has been abandoned, its relative abundance in the tissues of top predator marine mammals usually ranges from 55-70% (Addison *et al.*, 1984; Aguilar, 1984). Therefore, these comparatively low ratios suggest a recent usage of the pesticide in the region, particularly in the southern part of the continent. As mentioned above, the case of the franciscana from Uruguay was exceptional, with a mean percentage quite close to that of typical commercial formulations, clearly indicating that an input of DDT into the ecosystem had occurred just before the samples were collected (O'Shea *et al.*, 1980).

Heavy metals

Although heavy metals are naturally present in the environment in some areas, human activity has substantially increased their abundance, particularly in the last century. Some, but not all, heavy metals bioaccumulate and biomagnify through food webs, and their tissue concentrations increase progressively with age (Aguilar *et al.*, 1999). In contrast to the organochlorine pollutants, heavy metals are non-lipophilic and their distribution in tissues basically follows their chemical affinities. Mercury, copper, zinc and other heavy metals accumulate mainly in the liver, but cadmium accumulates in the kidney and lead in bone (Honda *et al.*, 1982; André *et al.*, 1990a; b).

Table 2 details published results on heavy metal concentrations in marine mammals from Central and South America. Concentrations of metals are expressed as $\mu g.g^{-1}$ wet weight. Although information is available on a wide variety of tissues, the present review collates data only on liver, muscle and kidney, the tissues considered to be most representative of metal load in mammals.

Coverage of studies

As was the case for organochlorines, few marine mammal species have been analysed for heavy metals in Central or South America (Fig. 1). In general, sample sizes have been small and, excluding the studies on spotted dolphins from the eastern tropical Pacific which were carried out on a large sample (André *et al.*, 1990a; b), the total number of animals investigated so far in this regard only comprises 36 individuals from nine species over an 18-year period.

In Central America, only three species (all odontocetes from the Caribbean region) have been studied: short-finned pilot whales and spinner dolphins from the Lesser Antilles in 1972 (Gaskin *et al.*, 1974) and spotted dolphins from the tropical Pacific waters during 1977-83 (André *et al.*, 1990a; b).

In South America, almost all surveys thus far have been carried out in Argentinean waters (Moreno *et al.*, 1984; Marcovecchio *et al.*, 1990; 1994). Four odontocete species have been examined (pygmy sperm whale, Cuvier's beaked whale, bottlenose dolphin and franciscana), all before 1990, although the specific dates of sample collection are not specified except for one bottlenose dolphin that was caught in 1982. There is also isolated information from two tucuxi dolphins collected in Surinam in 1971 (Koeman *et al.*, 1972).

In addition to cetaceans, two species of pinnipeds, fur seals from Uruguay and sea lions from Argentina, were also analysed for heavy metals in 1990 and 1983-1985, respectively (Peña *et al.*, 1988; Gerpe *et al.*, 1990).

Levels

MERCURY

The effects of mercury on man and wildlife have long been recognised and mercury has received considerable attention since the first ecotoxicological surveys. Although mercury is mostly released in its inorganic form, where it has limited toxicity, once incorporated into the biota it is transformed into organic derivatives, mainly methyl-mercury, which are readily transferred through the food web and have a much higher potential for toxicity. Mercury has been responsible for several large-scale mortalities or serious impacts both in human and

Table 2a

Heavy metal concentrations (mean ± SD) in different tissues of marine mammals from Central America. Provenance of researchers: [1]Canada [2]France.

Area/Species	sex	n	Date	Tissue	Units: ppm wet weight tHG	CH3-Hg	Cd	Reference
STA. LUCIA (LESSER ANTILLES)[1]								
Short-finned pilot whale, *Globicephala macrorhynchus*	m	4	1972	liver	105.6±56.5	3.46±0.14		Gaskin *et al.* 1974
				muscle	3.98±0.92	2.32±0.66		
				kidney	8.65±2.04	1.6		
	f	1	1972	liver	21.4			
				muscle	4	2.4		
				kidney	14			
Spinner dolphin, *Stenella longirostris*	m	1	1972	liver	13	1.33		Gaskin *et al.* 1974
				muscle	0.87			
				kidney	2.68			
	f	1	1972	liver	6	1.88		
				muscle	1.33	1.33		
				kidney	2.28			
EASTERN TROPICAL PACIFIC[2]								
Spotted dolphin, *Stenella attenuata*	m	16	1977-83	liver	59.99±53.2			André *et al.* 1990b
				muscle	2.11±2.00			
				kidney	4.71±3.33			
	f	28	1977-83	liver	64.86±52.90			
				muscle	2.31±2.08			
				kidney	5.50±3.05			
Spotted dolphin, *Stenella attenuata*	m/f	27	1977-83	liver			8.72±8.79	André *et al.* 1990a
				muscle			0.29±0.17	
				kidney			48.69±26.36	

Table 2b

Heavy metal concentrations (mean ± SD) in different tissues of marine mammals from South America.
Provenance of researchers: [1]The Netherlands [2]Argentina.

Area/Species	sex	n	Date	Tissue	Units: ppm wet weight tHG	Cd	Zn	Cu	Reference
SURINAM[1]									
Guiana river dolphins, *Sotalia guianensis*	-	2	1971	liver	5.7±5.32	0.39±0.02	62.5±3.5		Koeman *et al.* 1972
ARGENTINA (MAR DEL PLATA)[2]									
Cuvier's beaked whale, *Ziphius cavirostris*	-	1	< 1990	liver	0.1				Marcovecchio *et al.* 1995
				muscle	0.23				
				kidney	0.17				
Bottlenose dolphin, *Tursiops gephyreus*	male	1	1982	liver	54				Moreno *et al.* 1984
				muscle	3.2				
				kidney	8.3				
Bottlenose dolphin, *Tursiops gephyreus*	-	2	< 1990	liver	86±7.3	0.8±0.2	196.2±34.1	77.7±3.8	Marcovecchio *et al.* 1990
				muscle	5.5±0.8	0	93.3±13.1	6.3±1.1	
				kidney	13.4±2.5	28.4±4.3	93.6±5.9	29.5±3.9	
Franciscana dolphin, *Pontoporia blainvillei*	-	7	< 1990	liver	3.8±1.6	3.3±1.4	83.4±40	16±3.3	Marcovecchio *et al.* 1990
				muscle	3±1.2	0.1±0.1	49.3±4.8	2.5±1.5	
				kidney	1.9±0.7	9.9±3.9	79.4±21.4	14±4.9	
Pygmy sperm whale, *Kogia breviceps*	-	1	< 1990	liver	11.7	7.6	163.2	10.3	Marcovecchio *et al.* 1990
				muscle	1.6	0.6	47.8	2.5	
				kidney	10.5	412.6	169.7	7.4	
Sea Lion, *Otaria flavescens*	both	7	1983-85	liver	47±13	1.1±0.66			Peña *et al.* 1988
				muscle	1.2±0.7				
				kidney	2±0.7	5.65±2.3			
URUGUAY[2]									
Fur seal, *Arctocephalus australis*	male	3	< 1990	liver	25±0.04	54±7.73	68.5±5.8	13.5±2.54	Gerpe *et al.* 1990
				muscle	0.58±0.04	0.41±0.09	46.1±4.51	1.7±0.16	
				kidney	1.02±0.25	86.4±15.1	44.4±11.8	4.47±1.05	
	female	5	< 1990	liver	34.9±8.03	23.2±3.41	49.3±3.43	11.2±0.23	
				muscle	0.46±0.09	0.33±0.08	17.5±3.92	1.74±0.14	
				kidney	0.8±0.14	47.1±5.3	44.1±3.27	3.6±0.48	

wildlife populations. In mammals, mercury accumulates with age and, in females, it crosses the placental membranes and passes to the milk (Aguilar *et al.*, 1999).

Levels of total mercury (irrespective of whether in its organic or inorganic form) have been determined in all the species studied (Table 2). As expected, mean concentrations in liver were consistently higher than those in muscle or kidney. Levels were extremely variable, ranging from 0.1-106$\mu g.g^{-1}$. Liver concentrations reported in marine mammals from northern waters typically ranged from 3-200$\mu g.g^{-1}$ (Wagemann and Muir, 1984), indicating that concentrations of this element in Central and South American cetaceans and pinnipeds are moderate overall. However, marine mammals from the regions where contamination by this metal is likely to be higher, such as the Amazon river, have not been studied in this regard.

Methyl-mercury has only been analysed in five short-finned pilot whales from the Caribbean (Gaskin *et al.*, 1974). The proportion of methyl-mercury in relation to that of total mercury in the liver was very low (3%), in contrast to the situation observed in the muscle (60%) and kidney (18%) of the same individuals. This is a common finding in marine mammals. In most vertebrates, methyl-mercury is the most abundant derivative of all the forms of mercury present in tissues. However, the fraction of organic mercury in the liver of marine mammals heavily contaminated by this element is much lower than in the rest of the body, an anomaly explained by the apparently unique ability of marine mammals to demethylate organic mercury to transform it into its inorganic form and, in this way, reduce its toxic impact (Koeman *et al.*, 1975). The mean levels of total-mercury (tHg) found in the liver of short-finned pilot whales, particularly in males, were high (106$\mu g.g^{-1}$). The authors of the paper explained these high levels by the fact that the Caribbean is a tectonically active region with a higher than average environmental level of mercury. However, spinner dolphins from the same region presented much lower levels (6-13$\mu g.g^{-1}$) of tHg (Gaskin *et al.*, 1974).

The spotted dolphin from the tropical waters of the Pacific is the only species from which sufficient numbers of individuals have been studied to identify variations of mercury levels with age, body weight, sex, geographical origin or date of sampling (André *et al.*, 1990b). Results indicated that the concentration of mercury increases in all organs throughout the dolphin's life, confirming previous observations in other marine mammal species (Aguilar *et al.*, 1999). It was also found that concentrations increased when the capture site was close to the Equator.

The highest mercury concentrations in tissues from marine mammals from South America ($86 \pm 7.3 \mu g.g^{-1}$) were found in two bottlenose dolphins from Argentina (Marcovecchio *et al.*, 1990). The reasons for this were not evident, although the authors of the study attributed the high levels observed to the fish-eating habits of the species.

CADMIUM

The cadmium industry has increased considerably since the first World War and in particular during the last 25 years (Wagemann *et al.*, 1990). However, it is difficult to determine whether anthropological activity has had a major impact on the natural levels of this element in the biosphere (André *et al.*, 1990a). Cadmium accumulates in the kidney and, in long-lived mammals, its levels usually increase with age; tissue concentrations are generally higher in females than in males (Aguilar *et al.*, 1999). In 27 spotted dolphins from the eastern tropical Pacific, André *et al.* (1990a) reported positive relationships of cadmium renal concentrations with both age and body weight. Peculiar to cadmium is a large individual variation in levels (Wagemann and Muir, 1984).

Mean cadmium concentrations in marine mammals from Central and South America were high overall (5.65-402$\mu g.g^{-1}$ in kidney). Of the seven species examined, two had higher

mean levels than those in the range typical for northern marine mammals (Wagemann and Muir, 1984). The lowest renal mean concentration observed was 5.65μg.g^{-1}, detected in sea lions from Argentina, a level that is still considered to be high. In addition, exceedingly high cadmium concentrations (402μg.g^{-1}) were detected in the kidneys of the oceanic pygmy sperm whale (Marcovecchio *et al.*, 1990), a finding that was attributed to its squid-based diet; squid are known to be large accumulators of cadmium (Martin and Flegal, 1975; Hamanaka and Mishima, 1981).

ZINC AND COPPER

Zinc and copper are well known pollutants that originate from a wide range of mining and industrial activities. While copper levels usually increase with age, zinc tissue concentrations seldom show any age-related trend (Aguilar *et al.*, 1999). For both metals, the main body site for accumulation appears to be the liver. No data on concentrations of zinc or copper are available from marine mammals from Central America. From South America, only five species appear to have been analysed for these metals (Table 2).

Table 2 shows that mean zinc concentrations in fur seals from Uruguay (49μg.g^{-1} in females and 68μg.g^{-1} in males) appear to fall towards the upper bound of means (34-81μg.g^{-1}) observed in the liver of pinnipeds from the Northern Hemisphere (Wagemann and Muir, 1984). Levels detected in bottlenose dolphins (196μg.g^{-1}), franciscanas (83μg.g^{-1}), pygmy sperm whales (163μg.g^{-1}) from Argentina and tucuxis (62μg.g^{-1}) from Surinam are more than double those commonly found in cetaceans (26-59μg.g^{-1}) from the Northern Hemisphere.

In contrast, copper levels from most individuals and species analysed appear to be in the usual range of northern marine mammals, with the exception of two bottlenose dolphins from Argentina, which showed very high concentrations (78μg.g^{-1}) of this metal (Marcovecchio *et al.*, 1990).

OTHER METALS

Apart from the low levels of arsenic (0.17μg.g^{-1}) detected in tucuxis by Koeman *et al.* (1972), data for other metals are limited to a survey on concentrations of lead in sea lions from Argentina (Peña *et al.*, 1988). In this survey, several tissues from seven individuals were analysed but only the bone from three of them presented detectable levels (1.6 ± 0.2μg.g^{-1}).

DISCUSSION

The central and southern regions of the American continent support a rich and diverse marine mammal fauna. However, despite the occurrence of extensive agricultural, mining and industrial activities that can be expected to have released vast amounts of pollutants into the marine environment, little attention has been paid to the potential impact of such pollutants on local marine mammal populations. This is reflected in the small number of papers published in the scientific literature on this specific subject, and in the limited scope and heterogeneous sample composition of most of them, as compared to those available from other similarly developed regions of the world.

Only two groups of local researchers, one from Argentina and the other from Chile, have to date published results on the subject. The group from Chile focused its studies on organochlorines in large whales from the Southern Pacific (Pantoja *et al.*, 1984; 1985), while the group from Argentina investigated the incidence of heavy metals in a variety of odontocete and pinniped species (Peña *et al.*, 1988; Gerpe *et al.*, 1990; Marcovecchio *et al.*,

1990; 1994). Although other local groups, mostly from Mexico and Brazil, have also carried out research on the effects of pollutants on marine mammals, their results have not yet been published in the refereed scientific literature.

In addition, a number of researchers from North America and Europe, sometimes in collaboration with local researchers, have undertaken investigations on the effects of pollutants on marine mammals from the region. Although these studies have usually focussed on the northern fringe of the South American continent or the Caribbean region, some have been undertaken along the coasts of Argentina and Uruguay (Koeman *et al.*, 1972; Gaskin *et al.*, 1974; Taruski *et al.*, 1975; O'Shea *et al.*, 1980; André *et al.*, 1990a; b; Borrell *et al.*, 1996).

Most studies were carried out on specimens obtained opportunistically from strandings or fishing interactions and are therefore limited in their sample size and, by extension, in their representativeness of the actual toxicological situation of the populations subject to study. Indeed, only three of them are extensive in terms of sample size, but each deals with only a single species (André *et al.*, 1990a; b; Borrell *et al.*, 1996).

The available data suggest that, overall, organochlorine pollutants, although ubiquitous in the region, do not reach the levels attained in the highly industrialised latitudes of the Northern Hemisphere. In the marine mammals studied, there is a clear predominance of organochlorine compounds of agricultural rather than industrial origin. In some cases (e.g. the DDTs), the pollutant profile found differed little from the original commercial formulations. This indicates that their use was relatively recent, and in some cases virtually contemporary with the time of study.

Information on heavy metals reveals an irregular picture. Although mercury levels were moderate overall, no information was available on concentrations in the tissues of marine mammals found in areas with higher potential risk of pollution, such as the tributaries of the Amazon River affected by mining, or the mouth and adjacent waters of this river. In contrast, cadmium and zinc concentrations were higher overall than those in the range typical for northern marine mammals, while copper and lead levels were comparatively low. However, information on these latter metals was extremely limited.

The lack of comprehensive, long-term studies, renders it impossible to present a reliable evaluation of the impact of pollutants on the marine mammals from the region. This is unfortunate considering that Central and South America harbour a number of endemic cetacean species (e.g. tucuxi, *Sotalia fluviatilis*, black dolphin, *Cephalorhynchus eutropia*, Peale's dolphin, *Lagenorhynchus australis*, boto, *Inia geoffrensis*, franciscana, *Pontoporia blainvillei*, Burmeister's porpoise, *Phocoena spinipinnis*), which are often riverine or coastal, and thus likely to be highly susceptible to the effects of these pollutants. Information on pollutant levels in pinnipeds is equally fragmentary, whilst that on Amazonian manatees or the Central American populations of the West Indian manatee is totally lacking despite their critical conservation status and the comparatively abundant data available from the Florida manatee (O'Shea *et al.*, 1984; Ames and van Vleet, 1996). The impact of pollutants has been identified as a potentially major factor in the conservation of marine mammals (IWC, 1994; Reijnders, 1996) and, given the paucity of data on this subject from central and southern America, further studies are urgently needed to ensure proper management of the local populations and species.

ACKNOWLEDGEMENT

This research was funded by the Comisión Interministerial de Ciencia y Tecnología (CCYT) Project AMB 399/94.

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J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 209–221, 1999

Induction of biotransformation enzymes by polyhalogenated aromatic hydrocarbons (PHAHs): potential impact on animal physiology and health[1]

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ABSTRACT

Biotransformation and its role in the elimination of polyhalogenated aromatic hydrocarbons (PHAHs) has been the subject of many studies from the late seventies onwards. The notion of specific, high affinity interactions of phenolic PHAH metabolites with the plasma transport proteins of thyroid hormone and vitamin A, both *in vitro* and *in vivo*, stimulated further research into the possible role of biotransformation in the toxicity of certain PHAHs such as PCBs. Currently, phenolic metabolites of PCBs and related compounds have been identified as major metabolites in blood plasma of e.g. grey seals (*Halichoerus grypus*) and humans with background environmental exposure to these chemicals. The concentrations of the hydroxy-PCBs were in the same range as the most persistent parent congeners, such as PCB 153, 138 and 180. These phenolic metabolites were found to possess a specific range of biological activities, which differed from the parent compounds. Another potential adverse effect associated with persistent induction of biotransformation enzymes, like UDP-glucuronyl transferases (UGTs) by PHAHs, is a long-term enhanced elimination of several important endogenous ligands such as vitamin A and thyroid hormones. Reduced levels of vitamin A and thyroid hormones have been reported in most experimental animal and wildlife species exposed to PHAHs. The recent observation of the accumulation of high levels of phenolic PCB metabolites in blood and brain of late gestational rat foetuses, in parallel with reductions in both vitamin A and thyroid hormone levels, suggests that these metabolites may play an important role in the observed developmental toxicity of PHAHs.

KEYWORDS: BIOMARKERS; DISEASE; HAZARD ASSESSMENT; PHYSIOLOGY; POLLUTION-HAHs; POLLUTION-ORGANOCHLORINES; REPRODUCTION

INTRODUCTION

Exposure of laboratory animals and wildlife species to various xenobiotic compounds usually causes an enhanced biotransformation response, in most cases resulting in an accelerated catalytic breakdown and elimination of the xenobiotics. Polycyclic aromatic hydrocarbons (PAHs) are an example of this class of xenobiotics. However, due to the highly reactive nature of some of the metabolic PAH intermediates formed, a certain fraction of the PAH metabolites will not be eliminated but rather will give rise to DNA and protein adducts and associated toxicity. On the other hand, there are several classes of very persistent polyhalogenated aromatic hydrocarbon (PHAH) pollutants, such as polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) (Fig. 1), that give rise to a high induction of biotransformation enzymes, but are themselves hardly

[1] This paper was originally submitted to the IWC Scientific Committee as SC/M95/P8.

degraded and eliminated by the enzymes they induce. Consequences of a persistent induction of certain biotransformation enzymes, such as cytochrome P450 isozymes, may involve: increased oxidative stress; increased formation of reactive intermediates from more readily degradable xenobiotic compounds present in the same exposure matrix; and enhanced elimination of physiologically important endogenous ligands (hormones, vitamins). Therefore, these classes of persistent PHAH pollutants may put the exposed laboratory and wildlife species at risk from adverse health effects. In this paper several aspects of the metabolism and physiological/toxicological consequences of exposure to PCBs and related compounds are discussed.

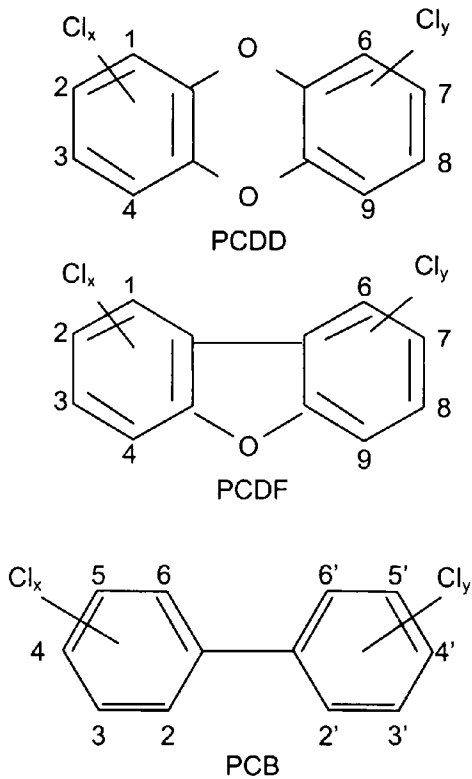

Fig. 1. General structures of PCDD, PCDF and PCB.

POLYCHLORINATED BIPHENYLS, DIBENZO-P-DIOXINS AND RELATED COMPOUNDS

PCBs are a class of ubiquitous and persistent environmental pollutants that accumulate extensively in food chains. All biota and species analysed so far have been found to contain a certain level of PCBs and related contaminants. Relatively high body burdens of PCBs and related compounds are found in species at higher trophic levels, especially those of the aquatic and marine environment. PCBs and related compounds are even detectable in marine species found in the Arctic and Antarctic, far from their source (e.g. harp seals, *Phoca groenlandica*, Antarctic fur seals, *Arctocephalus gazella*, ringed seals, *Phoca hispida* and

white whales, *Delphinapterus leucas*) due to the global transport of these persistent lipophilic compounds from warmer areas to colder areas (Norstrom and Muir, 1994; Oehme *et al.*, 1994a). Body burdens of PCBs and related compounds have been found to be dependent on a variety of factors including species, age, gender, season, diet and location. Therefore, it is difficult to discern temporal trends. However, overall there was a decline in PCB levels in most species in the early eighties, which now seems to have levelled off, at least in some areas (Norstrom *et al.*, 1988; Bignert *et al.*, 1993; De Boer and Hagel, 1994).

PHAH congener patterns

PHAH congener patterns present in biota of various trophic levels differ considerably. These differences are mainly due to variations in exposure and/or biotransformation capacity of the various species. In general, only 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) congeners are found in most biota, indicating a rapid degradation of the non-2,3,7,8-substituted PCDD/Fs by most species (Van den Berg *et al.*, 1994b). The relative concentrations of the various 2,3,7,8-PCDD/Fs increase with the number of chlorine substituents. Therefore, the highest concentrations are observed for octaCDD/F congeners. However, for PCB congeners the picture is more complex. In fact, PCB congeners have been divided into six structurally different groups of congeners that differ in their biostability. Some congeners appear to be very persistent, like the CB congeners 153, 138 and 180. However, the CB congener pattern differs between different species dependent on, for instance their biotransformation capacity. This is well illustrated by the variation of the ratio of the different non-*ortho* substituted, co-planar CBs, 3,3',4,4'-TeCB (CB 77), 3,3',4,4',5-PeCB (CB 126) and 3,3',4,4',5,5'-HxCB (CB 169) in various species (Kannan *et al.*, 1988; 1989a; b). In fish, crabs and blue mussels, CB 77 represents >90% of the non-*ortho* CBs in areas around Japan (Miyata *et al.*, 1994) and Taiwan (Lu *et al.*, 1994) and actually reflects the ratio of non-*ortho* CBs present in river sediments (Ohsaki *et al.*, 1994). In marine mammals, like Antarctic fur seals and harp seals, CB 77 and CB 126 are present in blubber in almost equal amounts, while CB 169 is present at a ratio of 0.1-0.2 towards CB 126 (Oehme *et al.*, 1994a; b). However, in human milk samples CB 77 is only a small proportion (<10%) of the non-*ortho* CB congeners (Koopman-Esseboom *et al.*, 1994).

PHAHs and toxicity

PCBs have been found to cause a wide spectrum of species-specific and congener-specific toxic and biochemical effects in experimental animals, including hepato-, immuno-, reproductive-, and dermal-toxicity, teratogenicity (congenital malformations), developmental neurotoxicity and carcinogenicity (McConnell, 1989; Safe, 1994). In general, early exposure during embryonal and neonatal development is a much more sensitive (more than 100-fold) time period than adult exposure for induction of adverse effects in laboratory animals and probably also in human infants (Brouwer *et al.*, 1995). Moreover, the effects induced early on in life have a much greater chance of being permanent without possibilities for compensation. In addition, PCBs and related compounds have been suggested to be involved in many epizootic diseases in fish, birds and marine mammals, such as 'blue sac like disease' in fish (Peterson *et al.*, 1993), skin lesions and liver tumors in flounder, *Platichthys flesus*, (Vethaak, 1993); reduced immune function in relation to outbreaks of lethal morbillivirus infections in seals in the North Sea and Baltic (Brouwer *et al.*, 1989b; De Swart *et al.*, 1994; Ross *et al.*, 1995, although see Kennedy, 1999); and reduced population size due to reproduction problems in ringed, *pusa hispida*, harbour seals, *Phoca vitulina*, (Helle *et al.*, 1976; Reijnders, 1986) and fish-eating birds, such as cormorants,

Phalacrolorax carbo, (Van den Berg *et al.*, 1994a). Early life stages in wildlife species also appear to be much more sensitive than adults for the adverse health effects of PCBs and related compounds (Colborn and Clement, 1992).

PHAHs and Ah-receptor

Most of the toxic and biochemical effects induced by planar, non-*ortho* substituted PCBs and 2,3,7,8-substituted PCDD and PCDF congeners are mediated through high affinity binding to a cytosolic protein, the aryl hydrocarbon-receptor (AhR; Poland and Knutson, 1982). After ligand binding the AhR is converted into an activated, DNA-binding form, subsequently transformed by the release of two heat shock proteins (HSP90) and translocated to the nucleus and complex formation to a second protein, the Ah-receptor nuclear translocator (ARNT; Whitlock, 1993; Hankinson, 1995). This heterodimer complex is bound to specific sequences, the dioxin responsive enhancer (DRE) sequences located in the promotor-region upstream of dioxin-responsive genes (Denison *et al.*, 1988a; b).

Nowadays a number of genes have been identified that contain one, or several DREs, and therefore their expression may be regulated via the dioxin-AhR pathway. A number of the DRE-containing genes encode cytochrome P450 isozymes, such as CYP1A1, CYP1A2, CYP1B1. In addition, several conjugation enzymes are identified with DREs in their promotor region, such as aldehyde dehydrogenase (Ahd4) gene, NAD(P)H-menadione oxidoreductase (Nmol) gene, UDP-glucuronyl transferase (UGT01*6) gene and the Glutathion-S-transferase (GST-pi) gene (Nebert *et al.*, 1993).

Induction of biotransformation enzymes

In laboratory rodents (mice, rats, guinea pigs) planar, non-*ortho* substituted PCBs cause a 50-fold to several hundred fold persistent induction of CYP1A1/2 activity, similar to 3-methylcholanthrene (3-MC), which therefore is also called 3-MC type of induction. However, in contrast to 3-MC, the CYP1A1/2 induction caused by the planar PCBs is persistent, reflecting the long half-life of PCB congeners in most species. A frequently used model substrate for CYP1A1/2 is ethoxyresorufin, which is deethylated during the enzymatic reaction. This activity is called ethoxyresorufin-O-deethylase (EROD). Another frequently used model substrate is benzo(a)pyrene, and in this case the corresponding enzymatic activity is called arylhydrocarbon hydroxylase (AHH). These activities are extensively used as markers for CYP1A1/2 induction by PHAHs (Safe, 1994). Several of the other AhR-pathway controlled gene products have also been found to be induced, such as increased UGT1 with associated increases in hepatic thyroxine (T_4) and 1-naphtol glucuronidation, following exposure of laboratory animals to PCBs and related compounds (Visser *et al.*, 1993; Van Birgelen *et al.*, 1995).

However, non-planar PCB congeners, such as diortho-substituted PCBs, do not cause induction of CYP1A1/2 genes, but instead induce CYP2B1/2 isozymes, similarly to phenobarbitone (PB). Because of this, such activity is also called PB-type of induction. A model reaction for CYP2B is the depentylation of pentoxyresorufin (PROD). The mechanism of CYP2B gene induction is less clear than that of CYP1A1/2. Both a receptor-mediated enhanced transcription and a post-transcriptional stabilisation of mRNAs have been suggested. In addition to CYP2B isozymes, PB-type inducers also enhance transcription of members of the CYP2C and CYP3A classes. Mono-*ortho* and some di-*ortho* substituted PCB congeners are so-called mixed type inducers' in that, they induce both CYP1A and CYP2B classes of isozymes.

In general, induction of orthologous CYP450 isozymes by PHAHs is also observed in wildlife species, like fish, fish-eating birds and marine mammals. In fish, CYP1A1 and the associated EROD activity is induced by PHAHs via an AhR mediated pathway (Hahn and

Stegeman, 1994). In the case of fish caught in the wild with different levels of environmental exposure to PHAHs, the correlation between exposure and EROD-induction is often less clear. This may be due partly to the problems associated with exposure of species in the wild to complex mixtures of PCBs and related compounds. Several PCB congeners, such as CB 77, appear to be able to competitively inhibit the EROD activity (Hahn and Stegeman, 1994; Morse *et al.*, 1995). In addition, several publications indicate the presence of Ah-receptor antagonists in PCB mixtures that partly abolish the EROD induction by 2,3,7,8-TCDD (Davis and Safe, 1990; Aarts *et al.*, 1993). Fish-eating birds, like the common tern (*Sterna hirundo*) and cormorants, as well as marine mammals, like harp seals and harbour porpoises (*Phocoena phocoena*) also show a good CYP1A1/2 inducibility following exposure to PHAHs (Goksoyr *et al.*, 1992; Van den Berg *et al.*, 1994a; Bosveld *et al.*, 1995). A major distinction between laboratory animals and fish and most fish-eating birds, is the absence of CYP2B isozymes in the latter species. However, there is limited information on the presence and inducibility of CYP2B isozymes in marine mammals (Goksøyr *et al.*, 1992; White *et al.*, 1994; Kannan *et al.*, 1995).

CONGENER PATTERNS IN DIFFERENT SPECIES

Comparison of congener patterns between sediments and fish, fish and marine mammals, fish and fish-eating birds, may provide valuable information on the metabolism of certain PHAH congeners. By comparing ratios of a non-metabolisable PHAH congener, like CB153 with the other CB congeners in different matrices one can deduce the relative impact of metabolism on PHAH congener patterns (Boon *et al.*, 1992). Only 2,3,7,8-substituted PCDD/Fs are found in biota and species at higher trophic levels, such as marine mammals and man, indicating that the non-2,3,7,8-PCDD/Fs are rapidly metabolised in the environment (Van den Berg *et al.*, 1994a). However, 2,3,7,8-TCDF which is present in biota is still rapidly metabolised in laboratory rats with a half-life of about two days. This 2,3.7,8-PCDF congener is probably quite extensively metabolised in marine mammals and human individuals as well. Metabolism of PCBs is more complex. PCB congeners have been divided into different structural groups with regards to cytochrome P450-dependent metabolism, based on experimental and field studies on seals and cetaceans (Boon *et al.*, 1994). Congeners that contribute to dioxin-type toxicity in laboratory animals are given in bold in this list.

I: Congeners without any vicinal hydrogen (H) atoms (e.g. CB153, **CB169**, CB180, CB183, CB187, CB194).

II: Congeners with vicinal H atoms only in the *ortho*- and *meta*-positions in combination with ≥ 2 *ortho*-Cl substituents (e.g. CB99, **CB128, CB138, CB170**).

III: Congeners with vicinal H atoms in the *ortho*- and *meta*-positions in combination with ≤ 1 *ortho* - CL (e.g., CB-28, **CB77, CB105, CB118, CB126, CB156**).

IV: Congeners with vicinal H atoms in the *meta*- and para-positions in combination with ≤ 2 *ortho*-Cl (e.g. CB44, CB49, CB52, CB101, CB110).

V: Congeners with vicinal H atoms in the *meta*- and para-positions in combination with ≥ 3 *ortho*-CL (e.g. CB136, CB149, CB151)

VI: Congeners with vicinal H atoms both in the *ortho*- and *meta*-positions and *meta*- and para-positions in combination with ≤ 1 *ortho*-Cl (e.g. CB31)

In seals, cetaceans and seabirds, congeners belonging to Groups I and II are persistent to biotransformation but, in polar bears, even some of these congeners appear to be metabolisable, especially when at least one unsubstituted para-position is available (Norstrom *et al.*, 1988). Congeners of Group III are metabolisable (by CYP1A) in seals, harbour porpoises and dolphins. This is important because Group III contains most of the dioxin-type congeners. The capacity to metabolise congeners belonging to Groups IV and V seems to be more developed in seals than in cetaceans (Tanabe *et al.*, 1988), although there is evidence for biotransformation of congeners of Group IV (Duinker *et al.*, 1989; Boon *et al.*, 1994) and even Group V congeners (Reijnders, 1994; Bruhn *et al.*, 1995; Reijnders and de Ruiter-Dijkman, 1995) by the harbour porpoise too. The substitution pattern at the four available *ortho*-positions has two possible consequences: it strongly influences the ability of the molecule to reach a planar configuration (Cullen and Kaiser, 1984) which is very important for expressing dioxin-like toxicity, or the bulky chlorine atoms may simply prevent enzymatic attack.

Role of CYP1A1/2 in PHAH metabolism

In vitro incubations of a radiolabelled model compound (^{14}C-CB 77) in hepatic microsomal preparations from rats pretreated with different inducers of P450 isozymes, clearly indicated the importance of CYP1A1/2 in the phenolic CB metabolite formation (Ishida *et al.*, 1991; Morse *et al.*, 1995). The mechanism of CYP1A1/2-dependent formation of phenolic CB metabolites is thought to involve the formation of highly reactive arene oxide intermediates, which may spontaneously rearrange to phenols with a concomittant shift of substituents from the site of hydroxylation to the next neighbour carbon atom in the aromatic ring. Phenolic CB metabolites have been identified as major metabolites of both planar and non-planar CBs in *in vitro* and *in vivo* studies (Klasson-Wehler, 1989; Safe, 1989).

The rate of formation and the nature of the phenolic CB metabolites formed are both species and congener dependent (Murk *et al.*, 1994b). In general, hydroxylation is favoured in the para-position of the least chlorinated ring. However, a phenolic derivative of 2,3,3',4',5-PeCB with the hydroxy-group in the most chlorinated ring was recently identified as the major metabolite present in blood plasma of mice (Bergman *et al.*, 1994). Phenolic products are the major PCB metabolites formed in *in vitro* incubations with liver microsomes and *in vivo* by mammalian and avian species (Murk *et al.*, 1994b). However, in liver microsomes of fish minimal formation of phenolic-PCB metabolites was observed, even when exposed experimentally to cytochrome P450-1A1/2 inducers.

TOXICOLOGICAL CONSEQUENCES OF PERSISTENT CYP1A1/2 INDUCTION BY PHAHs

(a) Phenolic PCB related effects

Metabolism and metabolite formation of most halogenated aromatic compounds, including PCBs have been regarded mainly as an elimination and detoxification route. Recent studies have indicated that phenolic and methylsulphone metabolites of PCBs and related compounds are not readily excreted, but instead may be present in relatively large quantities in organs and body fluids for a prolonged time period. For example, relatively large amounts of phenolic PCB metabolites have been identified in blood plasma of several species (e.g. rats, mice, marine mammals and humans) either experimentally or environmentally exposed to PCBs (Klasson-Wehler *et al.*, 1992; Bergman *et al.*, 1994). Moreover, a considerable accumulation of phenolic PCB metabolites was observed in late gestational foetuses when their mothers were exposed to Aroclor 1254 from days 10 to 16 of gestation (Morse, 1995; Morse *et al.*, 1995).

Effects on thyroid hormone metabolism and transport

Several studies have indicated that phenolic metabolites of PCBs and related compounds do have their own metabolite-specific range of biological activities and may also add to some parent compound-specific biological effects (Table 1). Phenolic PCB metabolites are much more potent than their respective parent compounds in terms of interference with thyroid hormones for their key proteins in transport and metabolism. For example, phenolic PCBs competitively inhibit thyroxine (T_4) binding to transthyretin (TTR; Lans *et al.*, 1993). TTR is the major plasma transport protein of T_4 in most species, except in larger mammals including humans. Phenolic metabolites of PCBs are also much more potent than their parent compounds with respect to competitive inhibition of hepatic type 1-deiodinase (Adams *et al.*, 1990), a pivotal enzyme in the activation/deactivation of thyroid hormone. Moreover, phenolic metabolites, but not their corresponding parent compounds are potent uncouplers of oxidative phosphorylation in rat liver mitochondria (Lans *et al.*, 1990; Narasimhan *et al.*, 1991). Next to these *in vitro* observations there are also *in vivo* observations indicating that both TTR and type 1-deiodinase activities are affected by phenolic metabolites of PCBs (Brouwer and Van den Berg, 1986; Adams *et al.*, 1990).

Table 1

Observed *in vitro* biological effects of phenolic PCB metabolites and relative potency towards parent compounds. TTR: transthyretin; IC: intercellular communication; > or <: order of magnitude.

Biological effects of phenolic PCBs	Potency relative to parent compound
T_4- binding competition on TTR	OH-PCBs >>> PCBs
Inhibition of type 1- deiodinase	OH-PCBs >>> PCBs
Uncoupling mitochondrial respiration	OH-PCBs >> PCBs
Oestrogen receptor binding	OH-PCBs > PCBs
Ah-receptor binding (rat liver cytosol)	OH-PCBs < PCBs
IC inhibition (Hepalc1c7)	OH-PCBs < PCBs
Chick embryo mortality	OH-PCBs << PCBs
EROD induction (Hepalc1c7)	OH-PCBs <<< PCBs

Effects on the oestrogen system

Another interesting finding is that phenolic PCB metabolites, especially lower chlorinated ones, such as 4-hydroxy-2',4',6'-trichlorobiphenyl, show a substantial oestrogen receptor binding affinity, with a relative potency of 0.05 towards oestradiol (Korach *et al.*, 1987). Interestingly, Aroclor 1221 has been shown to increase uterine weight (an oestrogenic activity) *in vivo* in female rats (Gellert, 1978). Increases in uterine weight have also been observed in immature rats, following exposure to 2,2',5,5'-tetrachlorobiphenyl, the Aroclor 1242 mixture and the phenolic metabolite 4'-OH-2,4,6-trichlorobiphenyl (Jansen *et al.*, 1993). It has been suggested that phenolic PCB metabolites may be more potent with respect to oestrogenic activity than their respective parent compounds, but the experimental evidence is scarce.

Effects on the Ah-receptor pathway

Ah-receptor binding has also been observed for some phenolic metabolites of CB77 (Klasson-Wehler *et al.*, 1990). The Ah-receptor binding affinities of 2-OH, 4-OH and 5-OH metabolites of CB77 were quite remarkable, only about two to three times lower than those for the parent compound CB77 itself, using rat liver cytosols. However, the potency to induce the Ah-receptor mediated EROD activity was three orders of magnitude lower for the

phenolic metabolites than for the parent CB77 compound itself, when using mouse Hepa1c1c7 cells (De Haan *et al.*, 1994). This striking difference may indicate that substantial metabolism of phenolic PCBs to more polar derivatives may take place in Hepa1c1c7 cells.

Effects on intercellular communication

Intercellular communication (IC) is an important regulatory mechanism for cell proliferation and differentiation. Recently, we have obtained evidence that phenolic metabolites of CB77 are also able to inhibit gap junctional IC in mouse Hepa1c1c7 cells (De Haan *et al.*, 1994). IC inhibition is believed to be an *in vitro* indicator of the tumor promotion potential of chemicals. The potency to inhibit IC for the phenolic CB77 metabolites was equal or higher than for the parent CB77. At the present time it is unclear as to whether the intrinsic potency of phenolic PCBs to inhibit IC may be even higher, due to the possibility of substantial secondary metabolism of these metabolites in mouse Hepa1c1c7 cells.

In vivo *toxicity of phenolic metabolites*

The *in vivo* toxic potency of phenolic metabolites relative to the parent compound CB77 is at least two orders of magnitude lower when tested in the chick embryo assay (Klasson-Wehler *et al.*, 1990). In addition, acute exposure of rats to phenolic metabolites of PCBs resulted in much less toxicity compared to their corresponding parent compounds (Yoshimura *et al.*, 1987; Koga *et al.*, 1990). However, it may not be possible to delineate the intrinsic toxic potency of phenolic PCBs from these *in vivo* studies. A large proportion of the phenolic PCBs, which were given as a bolus *ip* injection, may never have reached their target sites, due to secondary metabolism. Phenolic metabolites formed *in vivo* are extensively packaged into specific binding proteins, such as TTR. This may affect both the rate of secondary metabolism and the ligand concentration at the target sites, due to a TTR-specific delivery route, such as to the foetal compartment (Morse, 1995). Therefore, exposure studies with phenolic PCBs bound to their specific binding proteins, such as TTR, will be performed in our laboratory to investigate their toxic potency.

(b) Cyp1a1/2-induction and co-carcinogenesis

Induction of CYP1A1/2 and other isozymes also increases the metabolic capacity of the liver towards other xenobiotic compounds that may be present in the same exposure matrix, such as the polycyclic aromatic hydrocarbons (PAHs). Numerous studies have shown the cytochrome P450-dependent accelerated rate of formation of mutagenic, reactive intermediates of PAHs (Dipple *et al.*, 1984; Yang *et al.*, 1984). The well investigated bay-region diol-epoxides, such as benzo(a)pyrene-7,8-diol,9,10-epoxide, are highly mutagenic and readily form DNA-adducts. The formation of carcinogenic metabolites from PAHs by P450 isozymes is highly regio- and stereoselective. For example, the isozyme CYP1A2 is highly selective in the formation of 3,4-dihydrodiol metabolites from 7-methylbenz(c)acridine, while CYP3A4 is mainly involved in the proximate carcinogenic metabolite formation from dibenz(a,j)acridine (Roberts-Thomson *et al.*, 1995). There appears to be a good relationship between cytochrome P450 isozyme induction potency and the mutagenicity of the PAH-metabolites formed. Therefore, it is anticipated that a persistent exposure to PCBs and related P450 isozyme inducers may enhance the risk for co-carcinogenic events considerably when individuals are exposed to complex mixtures of PHAHs and PAHs present in the environment.

(c) Enhanced metabolism of endogenous substrates

Another observed consequence of long-term induction of biotransformation enzymes by PCB exposure is an accelerated metabolism and elimination of endogenously important components, such as vitamins and hormones. In this case, the enhanced elimination of endogenous compounds may not be due to CYP1A1/2, but to concommitantly induced enzymes such as UDP-glucuronyl transferases. Many studies have found an enhanced hepatic glucuronidation of thyroxine (T_4) and a concommittantly reduced level of T_4 in plasma of experimental animals exposed to PCBs and related compounds (Bastomsky, 1977; Collins and Capen, 1980; Visser *et al.*, 1993; Van Birgelen *et al.*, 1995). Both 3-MC and PB-type inducers can cause an accelerated hepatic glucuronidation of T_4, though through different isozymes, UGT1 and UGT2 (Visser *et al.*, 1993). Complex PCB mixtures may therefore possess a high potential to induce T_4-glucuronidation, since both planar and non-planar PCBs may contribute to this effect. Enhanced glucuronidation of certain vitamin A (retinoid) derivatives, such as retinoic acid, was also observed following incubation with hepatic microsomes of TCDD-pretreated rats (Bank *et al.*, 1989; Zile *et al.*, 1989). This enhanced conjugation of retinoid derivatives may also be involved in the dramatic depletion of hepatic and extrahepatic retinoid concentrations in laboratory animals exposed to PHAHs (Brouwer *et al.*, 1988; 1989a; Håkansson *et al.*, 1991; Chen *et al.*, 1992).

Reductions in both vitamin A and thyroid hormone levels in relation to PHAH exposure have been observed in several fish-eating bird species and in marine mammals (Brouwer *et al.*, 1989b; De Swart *et al.*, 1994; Murk *et al.*, 1994a; c; Van den Berg *et al.*, 1994a). In addition, Murk *et al.* (1994a; c) recently found a significant correlation between T_4-glucuronidation and PCB body burdens in common terns.

CONCLUSION

In conclusion, persistent induction of P450 isozymes by environmental pollutants, such as CYP1A1/2 by PHAHs, may put individuals (laboratory animals, wildlife species and man) at risk from adverse health effects, such as developmental toxicity and carcinogenesis. Therefore, given the long-lasting and relatively high levels of exposure of marine mammals to a variety of persistent chemical pollutants, it is of utmost importance to develop and use mechanistically-based biomarkers for PHAHs and other pollutants to monitor exposure levels and subtle indications of effects.

ACKNOWLEDGEMENTS

The author is grateful to D.C. Morse, Department of Toxicology, Agricultural University Wageningen and J.P. Boon, Netherlands Institute for Sea Research (NIOZ), Texel, The Netherlands for their critical reading of the manuscript and valuable suggestions.

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J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 223–248, 1999

Environmental pollutants and marine mammal health: The potential impact of hydrocarbons and halogenated hydrocarbons on immune system dysfunction

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ABSTRACT

This paper provides a detailed review of the immunotoxicological effects of environmental pollutants on the health of marine mammals, particularly in relation to their impact on the immune system and mechanisms of toxicity. Environmental pollutants are increasingly implicated (both directly and indirectly) with the onset of infectious disease and related mortality incidents in marine mammals,. The release of chemicals into the marine environment and the subsequent bioaccumulation up the food chain may pose a serious threat to marine mammals inhabiting contaminated areas; this has been documented in various studies of pollutant concentrations in tissue samples and large scale mass mortalities. Data correlating pollutant residues with altered reproductive/developmental states, and immune system dysfunction in particular, are reported for terrestrial mammals and suggest a similar association in marine mammals. Immunology is emphasised as a tool for assessing marine mammal health using quantitative and qualitative techniques to establish the effects of chemical pollutants. This has become increasingly important in relation to the subsequent dangers that may be posed to humans through any indirect exposure via the food chain.

KEYWORDS: POLLUTANTS; ORGANOCHLORINES; DISEASE; REVIEW; IMMUNOSUPPRESSION

INTRODUCTORY CONCEPTS OF IMMUNOTOXICITY IN MARINE MAMMALS

During the last twenty years there has been increasing attention directed toward the role of chemical pollutants as causative factors in the onset of disease. This has been generated, at least in part, by intense media coverage of accidental and deliberate chemical releases into the environment, and has been focused primarily on the initiation of disease in humans exposed to environmental pollutants and in seals and sea lions dying from viral diseases. Critical incidents have led to the association between chemical exposure and diseases in humans. However, this attitude is less prevalent with respect to consideration of the widespread pollution of the oceans, or the bioaccumulation of lipophilic chemicals up the food chain resulting in detectable levels of chemical pollutants in marine animals. However, with the increased availability of information on the effects of eating pollutant-contaminated

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seafood, it is becoming generally recognised that marine animals may also suffer both directly and indirectly from diseases caused by chemical pollutants, for example as a result of the loss of immune system protection against micro-organisms in the environment. Few direct data exist which correlate pollutant residues in marine mammal tissues with altered reproductive or developmental states, or with depressed immune function and increased levels of disease (Reijnders *et al.*, 1999). However, a number of physical entities and chemical agents are known to initiate immunosuppression in other animals, including radiation, chemotherapeutic agents, immunointeractive viruses and some of the chemicals found as environmental pollutants. Many chemicals have been clearly established to have immunotoxic properties in some laboratory animals. The following sections will emphasise those chemicals known to be immunosuppressive in terrestrial mammals, including man. Their distribution is reviewed from the perspective of what types of chemicals exist in the environment, whether they are found in marine mammals and how they might interact either directly with cells of the immune system or with physiological mechanisms regulating the immune system. This review also summarises some of the basic concepts in immunology and discusses the types of immunologically-based investigative tools and data that will be needed to accurately determine the effects of chemical pollutants on immune function in marine mammals.

ENVIRONMENTAL POLLUTANTS: IMPACT ON MAMMALS

Background

The hydrocarbons, including aromatic hydrocarbons, polycyclic aromatic hydrocarbons and halogenated hydrocarbons, include several broad groups of chemicals extensively used in agriculture and industry and widely disseminated as pollutants (Kimbrough and Jensen, 1989). A large number of these compounds, including members of the polychlorinated biphenyl (PCB), polybrominated biphenyl (PBB), naphthalene (PCN), benzene, phenol, and terphenyl (PCT), polynuclear aromatic hydrocarbon (PAH), halogenated phenols, anilines, benzenes, dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and chlorinated pesticides, insecticides and herbicides have been found as pollutants at terrestrial and aquatic sites. The stability and lipophilicity of such chemicals result in their tendency to be concentrated in fatty tissues, leading to their bioaccumulation up the food chain. Detectable residue levels of a variety of organochlorines and aromatic hydrocarbons, including the PCBs, have been identified in the liver, milk and adipose tissues of both terrestrial and aquatic animals, and in human adipose, milk and serum (Safe *et al.*, 1985).

The PCBs comprise a large group of 209 isomers and congeners which have two to ten chlorine atoms substituted in the phenol rings and differ only in the number of chlorine residues and their positions on the biphenyl structure (Mullin *et al.*, 1984). PCBs were originally synthesised in 1881 for commercial use where a stable oil with a high flash point was required. They were used for cutting oils and heat transfer oils, in generators and transformers and in a variety of industrial formulations and processing procedures. Their use was restricted in the USA in the early 1970s and banned throughout much of the industrial world by the late 1970s. However, considerable quantities of the total global PCB production is still in use in closed systems (Van der Gaag and Marquenie, 1991). Although the accuracy of the proposal has been questioned (S. Safe, pers. comm.), Peterle (1991) suggested that a number of industrialised countries might still have synthesised PCBs into the 1990s. Public awareness of and concern for the ecosystem damage and potentially adverse health effects resulting from exposure to the variety of PCBs and related organochlorine pollutants was minimal until the last two decades. That awareness has increased significantly with reports of human agricultural and occupational exposures (e.g. Cook *et al.*, 1980; Brown and Jones,

1981; Hardell, 1981; Suskind, 1983; 1985; Gustavsson *et al.*, 1986), a series of PCB poisonings in Asia resulting in immunosuppression (Kashimoto *et al.*, 1981; Chen and Hsu, 1987), the broad media discussion of the dioxin contaminant controversy (Patterson and Hoffman, 1976; Kimbrough and Carter, 1977), the widely reported health problems in Vietnamese populations and American military personnel resulting from Agent Orange contamination (Lawrence *et al.*, 1985; Lathrop *et al.*, 1987) and the toxic effects of a PBB contamination in Michigan (Reich, 1983).

The PCBs were initially identified by Jensen (1966) as a tissue residue in seals three decades ago, with significant bioaccumulation occurring from ingestion of PCB-contaminated fish. The ocean serves as a major reservoir for pollutant PCBs. While the time of residence in any one oceanic compartment may be relatively short, the persistence of PCBs allows for recycling between different geographical areas and system components before being removed through metabolism or photodecomposition (Manchester-Neesvig and Andren, 1989). It has been estimated that 61% of the total environmental load of PCBs is found in the marine ecosystem, but the overall marine concentration of PCBs tends to be low, on the order of parts per trillion. These calculations are complicated by the fact that up to 80% of pollutant PCBs in the total marine environment are concentrated in the North Atlantic (Tanabe, 1988). Virtually all of the PCBs are considered to be toxic to one degree or another, but only 20 of the 209 known isomers and congeners found in PCB mixtures have planar, non-*ortho*, chlorine substitutions in the biphenyl ring. Of these, only three, 3,3',4,4'-tetrachlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl and 3,3',4,4',5,5'-hexachlorobiphenyl (IUPAC No. 77, 126 and 169 respectively; Fig. 1) are proximate isostereomers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The planar non-*ortho* PCBs are thought to be responsible for most of the toxic effects of PCBs on biological organisms (Hansen, 1987; Safe, 1990; McKinney *et al.*, 1992), with the determination of toxicity between PCBs being, at least in part, associated with the fact that non-*ortho* congeners interact with the *Ah* receptor, while congeners with *ortho*-chloro substituents are inactive as *Ah* receptor agonists.

While animals taken directly from contaminated sites may have high tissue concentrations of lipophilic chemicals, as evidenced by PCB concentrations as high as 600ppm in the fat of white whales in the St. Lawrence Estuary (e.g. Massé *et al.*, 1986), marine mammals sampled in virtually all oceans, including some pristine waters generally considered to be uncontaminated, have also been found to have high, although not acutely toxic, organochlorine residues in their tissues (Aguilar *et al.*, 1999). The physiological consequences of chronic cellular exposure to potentially toxic organochlorines and hydrocarbons are unknown; however, elevated PCB tissue residues are reported to have occurred concurrently with the recent mass mortalities of dolphins in the United States (Geraci, 1989; Kuehl *et al.*, 1991; 1994) and with the 1990 western Mediterranean epizootic dolphin deaths, where PCB levels as high as 3,000ppm were detected (Aguilar and Borrell, 1994). An initial report suggests the possible existence of a cause-effect relationship between tissue residues of organochlorines and decreased mitogen-initiated lymphocyte blastogenesis in cetaceans (Lahvis *et al.*, 1993). The relationship certainly has been shown in humans (Chen *et al.*, 1985; Chen and Hsu, 1987; Kashimoto and Miyata, 1987), but cannot be assumed for marine mammals from the very limited experimental data. Considerable data/sets from most ocean areas reveal residues of persistent organochlorines in tissues of marine mammals, but with few exceptions, studies do not address the physiological consequences of chemical residues, and neither support nor refute the proposed association between chemical exposure and immune system dysfunction. Data do, however, show that organochlorine and hydrocarbon pollutants act as inducers of cytochrome P450s in marine vertebrates, including dolphins (Geraci and St. Aubin, 1982; Geraci, 1990), that cytochrome

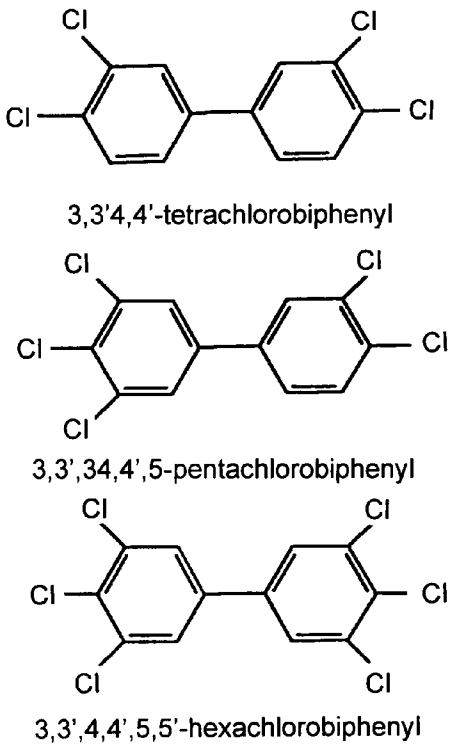

Fig. 1. Three polychlorinated biphenyls, 3,3',4,4'-tetrochlorobiphenyl, 3,3',4,4',5-pentachorobiphenyl, and 3,3',4,4',5,5'-hexachorobiphenyl, IUPAC No. 77, 126 and 169 respectively, which are proximate isostereomers of 2,3,7,8-tetrachorodibenzo-*p*-dioxin.

P450s metabolise aromatic hydrocarbons to form electrophilic metabolites capable of causing DNA adducts (Poland *et al.*, 1979; Poland and Knutson, 1982; Safe, 1984; 1990; Whitlock, 1986; 1987) and that DNA-hydrocarbon adducts have been reported for cetaceans from both pristine and polluted waters (e.g. Ray *et al.*, 1991). These data tend to support the proposal by Payne *et al.* (1987) that hydrocarbon induction of cytochrome P450 enzyme systems may serve as an early warning indicator qualifying as a 'most sensitive biological response' for evaluating the presence of organic pollutants in marine ecosystems.

This brief review does not address the impact of oil and oil spills on marine mammals. Geraci (1990) compiled an extensive review of the effects of oil on marine mammals. The studies considered the potential for oil as an agent leading to increased mortality in marine mammals due to physical and physiological alterations, but did not address the potential effects of oil and oil-derived chemicals on immune system function or the overall health of the animals.

Environmental impact of hydrocarbon pollutants

Most organochlorine pollutants exhibit similar chemical and environmental properties. They are stable lipophilic compounds that are only slowly degraded by acids, bases, heat and oxidative processes, resulting in their being persistent and in their tendency to bioaccumulate up the food chain. For organochlorines, the degree and position of halogen substitution is directly related to stability and lipophilicity, as well as to toxicity (Goldstein and Safe, 1989)

and immunotoxicity (Saboori and Newcombe, 1992). Mechanisms by which the organochlorines initiate toxic phenomena are diverse; however, for a number of organochlorines, interactions with the *Ah* receptor are required to elicit toxic phenomena (see below). Specific classes of polycyclic aromatic hydrocarbon and halogenated hydrocarbon pollutants, including the PCBs (with 209 possible congeners), PCDFs (with 135 possible congeners), PCDDs (with 75 possible congeners) and a wide variety of PAH, have been identified as significant environmental contaminants. Of these, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; Fig. 2) is the most toxic. TCDD has a reported ED_{50} (the concentration at which a 50% determination of response occurs) that may be as low as 10^{-13} M dependent on the test species, and has been described as a cytochrome P450 inducing agent and a suspected carcinogen (Schwetz *et al.*, 1973; Whitlock, 1987; Safe, 1990). Different species vary greatly in their responses to TCDD, with guinea pigs showing

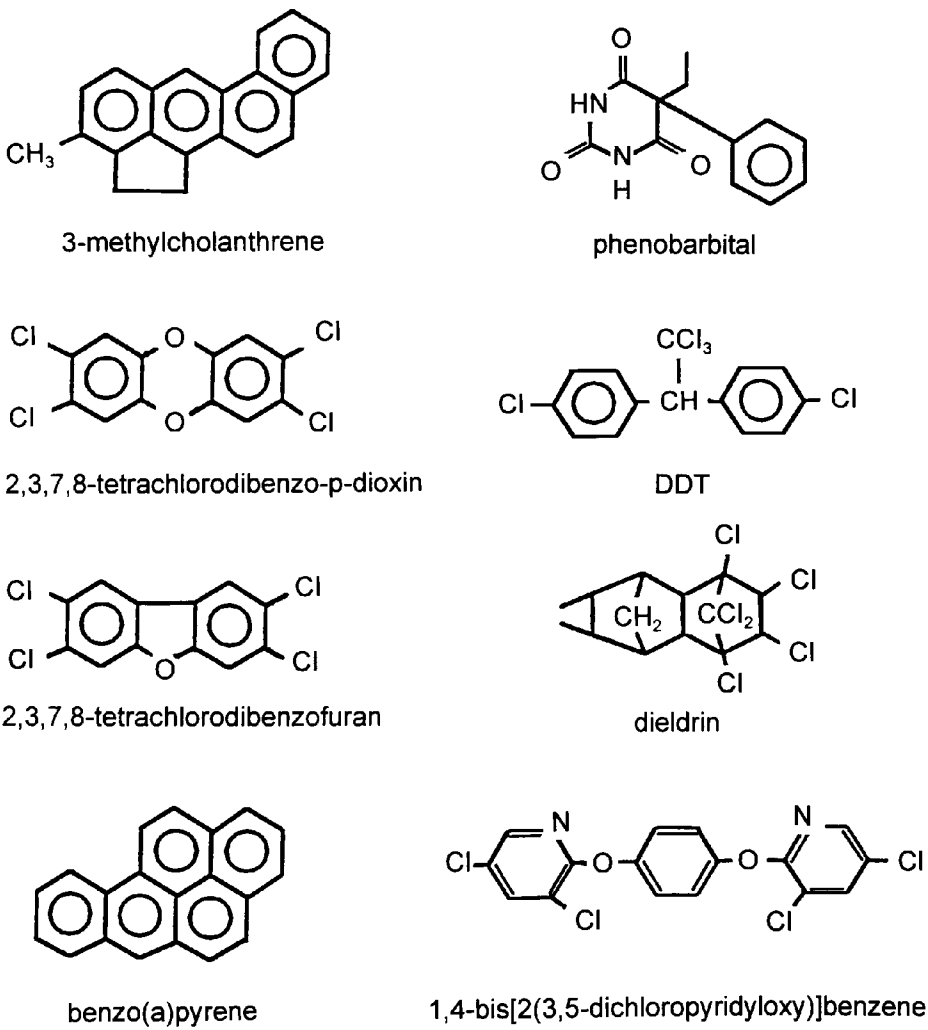

Fig. 2. A partial list of polycyclic aromatic hydrocarbons (PAH) and halogenated hydrocarbons (organochlorines) that are carcinogens, or are cytochrome P450 inducing chemicals, or interact with basic helix-loop-helix interactive cellular receptors.

an LD_{50} two orders of magnitude lower than some mouse strains (Esposito *et al.*, 1980). These differences have contributed to significant disagreement about the actual concentration of TCDD required to produce toxic effects. Nevertheless, it is clear that TCDD (and related compounds) is toxic, ubiquitous, and, with a reported biological half-life in humans of approximately ten years (Poellinger, 1995), persistent. Environmentally dispersed organochlorines such as TCDD and related compounds are clearly of concern and represent a long-term threat to aquatic animals in waters bordering both industrialised countries and densely populated emerging countries.

When animals come in contact with TCDD, the sequence of physiological effects can be complex and differ dramatically between species. In 1971, TCDD contaminated oil was used to spray multiple sites in Missouri for dust control, including a show ring where a number of animal deaths subsequently occurred. Autopsies of seven horses which died within weeks after the application of oil to the ring revealed oral ulcers, gastric ulcers, ascites, hyperkeratosis, nephritis and cystitis, with the onset of illness days to weeks after initial exposure, and deaths progressing over a period of several weeks (Kimbrough and Carter, 1977). Numerous other animals, including dogs, cats, mice and birds died in or around the ring within that time period, and a number of humans became ill with persistent headaches and skin lesions. Analyses of soil collected from the show ring revealed TCDD concentrations of 30ppm, with TCDD concentrations at 1ppb in up to 42 other affected sites. An extensive study did not indicate the existence of depressed B or T cell blastogenesis in TCDD-exposed human populations (e.g. Patterson and Hoffman, 1976); however, thymosin alpha-1 levels completed on serum frozen for three years suggested that the TCDD exposed group, which were depressed from 1,148.7±482 pg/ml in unexposed controls to a low of 9.73±304 pg/ml in the exposed group, apparently demonstrated decreased thymic function associated with TCDD exposure (Stehr-Green *et al.*, 1989; Hoffman, 1992).

Such clinical data are consistent with experimental findings (Morris *et al.*, 1993; Wood *et al.*, 1993) showing that TCDD significantly suppresses IgM secretion and background proliferation in both human and marine animal B-cells. The proposal that TCDD in some way interferes with cell maturation is further supported by data showing that the *Ah* receptor-dependent depression of immune function may be due to alteration in the maturation rate of thymocytes associated with organochlorine-initiated changes in thymocyte interactions with epithelial cells (Esser and Welzel, 1993). Investigations of marine thymocyte maturation further suggest that 3,3',4,4'-tetrachlorobiphenyl (one of the non-*ortho* isostereomers of TCDD) inhibits proliferation of thymocytes, perhaps by enhancing premature differentiation of thymocytes into cytotoxic T lymphocytes (Lai *et al.*, 1994). TCDD treatment and determinations of function in T-cells from *Ah* receptor positive and *Ah* receptor negative mice suggested that inhibition of splenic plaque-forming-cell responses was primarily *Ah* receptor-independent, while antigen-mediated immune responses were apparently sensitive to *Ah* receptor-dependent immunotoxicity of halogenated hydrocarbons (Harper *et al.*, 1993). Thus, the organochlorines appear to have at least two generalised groups of mechanisms for interfering with physiological functions including function of the immune system. One family of mechanisms is apparently dependent on xenobiotic binding to the *Ah* receptor. At least one more family is independent of *Ah* receptor binding, but is associated with binding to one or more other classes of cellular receptors.

Xenobiotics that do not bind (or bind with low affinity) to the *Ah* receptor may elicit altered physiological states by binding other cellular receptors. Exogenous chemicals that interact with cellular steroid receptors to initiate gene expression typically regulated by endogenous hormones have been broadly characterised as xenoestrogens. Oestrogen mimetic activity has been demonstrated for a variety of environmentally encountered

chemicals, including chlorinated pesticides, insecticides and herbicides, endosulfans, the polychlorinated biphenyls, dibenzofurans and dioxins, octylphenol and nonylphenol, detergents, phytoestrogens and metabolic products of this diverse group of chemicals. Other than the apparent requirement for a phenolic ring, the array of compounds found to have estrogenic activity may differ widely, pointing out the difficulty in using structure/function analyses to predict the oestrogenicity of chemicals (Duax and Griffin, 1985; Müller *et al.*, 1995).

Endogenous steroids may either maintain or alter states of physiological function by interaction with a variety of nuclear receptors. They bind receptors with high affinity and specificity, forming ligand-dependent complexes that initiate gene transcription in cells that express the appropriate ligand-binding receptor. Xenobiotic chemicals which bind steroidal receptors typically stimulate the same responses in cells as natural ligands, but, in pure form, usually have significantly less (up to 10,000-fold) affinity of binding to steroid receptors (Arnold *et al.*, 1996). Pollutant chemicals, however, almost never exist in pure form, and synergistic effects of complex pollutant mixtures result in oestrogen receptor binding affinities which may be multiple log units higher than those of individual chemical components (Arnold *et al.*, 1996).

Xenoestrogenic pollutants, either individually or as components of complex mixtures, have endocrine disruptive activities known to adversely affect physiological homeostasis, including reproduction, foetal development, sex determination and immune system function, and have been proposed to be etiologic factors in hormonally associated cancer initiation. Xenobiotic-altered reproductive capacities can potentially be related to sex determination abnormalities during foetal development or to physiological effects on adult organisms. As an example of altered physiology, the quality of human semen is reported to have declined over the last twenty years along with a decline in the sperm counts of males (Carlsen *et al.*, 1992; Sharp and Skakkebaek, 1993). Xenobiotic-associated feminisation has also been reported as a mechanism altering normal sex determination in birds, fish, shellfish, non-human mammals and reptiles (Colborn *et al.*, 1993; Crews *et al.*, 1994; 1995).

Foetal development is apparently altered in both subtle and overt ways by exposure to endocrine-disrupting xenobiotic chemicals. This may result in altered structure and function of reproductive tract tissues (Iguchi *et al.*, 1995; Jones and Hajek, 1995) and both skeletal and soft-tissue morphological abnormalities (Migliaccio *et al.*, 1995). These abnormalities appear related to the agonistic or antagonistic regulatory effects of xenobiotics on growth factors, and point out that interactions of xenobiotics with a variety of cellular receptors in addition to the *Ah* and steroid receptors may adversely impact foetal and neonatal development (Birnbaum, 1995).

The pleiomorphic expression of immune system dysfunction in response to xenobiotic exposure has puzzled investigators for years. The variety of immune system responses to xenobiotics is apparently due to the degree to which the immune and endocrine systems are intimately interactive, with many steroids being exquisite regulators of immune system function (Ralston *et al.*, 1990; Laird *et al.*, 1993). Different isomers and congeners of xenobiotics such as dioxin and PCBs can have strikingly different actions to either elicit or inhibit expression of different cytokine and growth factor genes (Safe, 1994). The end result of immune system dysregulation from xenobiotic altered expression of different cytokines or growth factors remains, however, much the same. Animals exhibit increased susceptibility to viral, bacterial, fungal and parasitic infections, and may present a wide variety of individual and species responses.

Chronic TCDD toxicity in rodents is evidenced primarily as hepatic dysfunction, whereas chronic administration of low TCDD concentrations to monkeys leads to skin abnormalities,

gastritis and gastric ulcers, and to immune dysfunction as an apparent consequence of hypocellular bone marrow resulting in decreased levels of circulating myeloid and lymphoid cells. Acute and subacute TCDD toxicity in the same animals leads to thymic involution and a variety of immunological defects resulting in failure of the immune system. Thus, the effects of chronic low-level exposure to TCDD as a model organochlorine may differ from those observed in acutely exposed animals, and species-specific effects of chronic exposure to low levels of organochlorines may occur.

Determination of hydrocarbon pollutants in marine mammals

Halogenated hydrocarbons, particularly the PCBs, have been reported as residues in cetaceans from virtually all of the world's oceans. PCBs and related chemicals are found in essentially all high triglyceride deposition tissues of cetaceans, including blubber, mammary glands and melon fat as well as in serum, brain, spleen, liver, muscle and kidney of marine mammals (Aguilar, 1985).

PCBs are adsorbed onto high lipid content membranes of phytoplankton, and are subsequently internalised (Broman *et al.*, 1992) and sequestered in intracellular lipids until the organism is consumed by a higher order species. Tanabe (1988) reported that organochlorine bioaccumulation factors between ten thousand and one million times the source concentration may occur in specific species, and that these values are dependent on body fat content and the trophic level occupied by the organism. While the total oceanic content of PCBs has been estimated at 1 part per trillion, specific areas such as the North Atlantic have significantly higher PCB concentrations. A million-fold bioaccumulation of 1 part per trillion would yield only a few parts per million for high lipid tissues, whereas higher organochlorine concentrations have been reported for many cetacean species. For example, harbour porpoises and bottlenose dolphins stranded on the coast of Scotland were reported to have about 23ppm PCBs, with 10.2ppm of DDT (Wells *et al.*, 1994). Striped dolphins that died in the western Mediterranean epizootic exhibited a high of 3,000ppm PCBs, with the majority of animals evaluated showing PCB levels between 500 and 2,000ppm in blubber (Aguilar and Borrell, 1994). Elevated levels of PCBs would be expected for near-shore cetaceans that exhibit high fat per total body weight ratios, are long lived and are upper trophic level carnivores (Honda *et al.*, 1992). However, the levels reported by Wells *et al.* (1994), Aguilar and Borrell (1994) and de Kock *et al.* (1994) are clearly excessive and indicative of both high pollutant concentrations in resident waters and efficient bioaccumulation. Pelagic cetaceans inhabit relatively pristine waters and feed lower on the food chain tend to exhibit lower tissue residue levels of organochlorines (O'Shea and Brownell, 1994).

The consistent findings of organochlorine residues in tissues from cetaceans in polluted and pristine waters have resulted in the need to correlate data on residue levels with the health of the animals. This is particularly true in those instances where the highly toxic non-*ortho* coplanar congeners, such as IUPAC 77, 126 and 169, have been found (Falandysz *et al.*, 1994). Kuiken *et al.* (1994) were unable to correlate PCB levels in tissues of harbour porpoises (*Phocoena phocoena*) with healthy animals that died of trauma as opposed to animals that died of infectious disease of some sort. Kannan *et al.* (1993) completed isomer-specific analyses of PCBs from striped dolphins in the western Mediterranean epizootic, reporting concentrations ranging from 94-670μg/g (wet weight). They reported that TCDD toxic equivalents for non-, mono- and di-*ortho* congeners were several times higher than is typical for humans or other marine mammals, and that, even though non-*ortho* congeners are more toxic, mono-*ortho* congeners contributed higher levels of TCDD toxic equivalents in these animals than did non-*ortho* congeners. Aguilar and Borrell (1994) proposed that the high levels of PCBs in the liver, as opposed to the blubber, of animals in

the striped dolphin epizootic suggest that blubber lipid reserves may have been mobilised resulting in elevated serum levels of PCBs with deposition of mobilised PCBs in the liver. However, they were unable to determine whether this led to any pathological effect in the animals that died during the epizootic. Kannan *et al* (1993), however, proposed that the ratio of IUPAC 169/126 in the epizootic animals suggested the likelihood of a significant induction of mixed function oxidase enzymes, a factor that might be indicative of physiological stress.

While induced cytochrome P450 levels and elevated metabolism of hydrocarbons had been previously shown in dolphin tissues, the presence of a TCDD-binding *Ah* receptor protein was first demonstrated in bottlenose dolphin epithelial cells (CDK cell line) established from foetal dolphin kidney tissue (Carvan *et al.*, 1994). TCDD-induced CDK cells metabolised benzo (a) pyrene (BP) *in vitro* to form BP-DNA adducts capable of initiating DNA excision repair (Carvan *et al.*, 1995). Cellular proliferation induced with 10^{-8} M TCDD and treated with 10^{-7}M BP was reduced to 50% of control levels. Proliferation in non-TCDD-induced cells treated with 10^{-5}M BP as both an inducing agent and carcinogen source was approximately 10-fold lower than the control. The BP-decreased proliferative capacity was effectively eliminated by the inhibition of cytochrome P450 induction with α-naphthoflavone. These data suggest the possibility that *Ah* receptor-mediated induction of gene expression in dolphin cells could be associated with cytotoxic phenomena *in vitro*.

Pinnipeds

Seals, sea lions, fur seals and walruses have been found to contain variable tissue concentrations of a number of persistent organochlorines, including PCBs, the halogenated hydrocarbon pesticides and their variety of metabolites (e.g., DDT, dichlorophenyldichloroethane, hexachlorocyclohexane, chlordane and its metabolites, and certain of the cyclodienes such as dieldrin and aldrin), along with complex mixtures of other lipid soluble hydrocarbons (e.g. Hutchinson and Simmonds, 1994).

The variety of contaminant analyses reported from animals differing in species, age, gender, location, times of year, time since death occurred, states of lactation and states of nutrition, coupled with difficulties in identification of specific chemicals and different congeners of the same class of chemicals, has made it very difficult to compile data useful for a determination of the correlation between chemicals and the onset of contaminant associated diseases. For instance, Duinker *et al.* (1988) reported the number of chlorine atoms of significant PCB congeners to range from 3-7, stating that the composition of PCB mixtures in tissue residues cannot easily be accurately determined in terms of a single formulation (e.g. Aroclor vs Clophen). Determination of specific congeners of PCBs is extremely important since they may differ widely in toxicity dependent on the number and location of chlorine groups (Safe, 1984; 1994; Harper *et al.*, 1995), and since complex mixtures of the chemicals may or may not be additive in their immunosuppressive characteristics (Harper *et al.*, 1995) and distribution patterns (Duinker *et al.*, 1988). In addition, they may exhibit interspecies differences in uptake, transformation and excretion patterns that will continue to make it difficult to evaluate mixtures of compounds as to their effects on animals.

Many of the early studies of organochlorines in pinniped tissues expressed chemical concentrations in terms of wet tissue weights, with the greatest concentrations of organics found in the blubber. The reported chemical concentrations differ dramatically when expressed in terms of extractable lipids. This is not surprising, considering that blubber organochlorine residues account for approximately 98% of total body residues. Hutchinson and Simmonds (1994) point out that a major problem in determining whether

organochlorines are exhibiting significant increases in tissue residues lies in the historical absence of standardised sampling and analysis methods. The recent adoption of more standardised analytical methods and the availability of more highly purified chemical standards has resolved many of the past problems inherent in residue analysis. However, complex mixtures of organics and their metabolites found in pinniped tissues continue to be difficult to determine, and to be dependent on environmental persistence and mobility, the variety of congeners and congener toxicity of the chemicals, the age, gender, state of nutrition, health of the animal and species capacity to be induced for enzymes that conjugate or metabolise residue chemicals.

The relationship between exposure to specific chemicals or mixtures of chemicals in the environment and health has been difficult to establish. Developmental abnormalities, evidenced as skeletal defects, in ringed seals, harbour seals and grey seals from areas of the Baltic Sea were reported to have increased after 1955, correlating with the increase in tissue organohalogen levels (Zakharov and Yablokov, 1990; Bergman *et al.*, 1992; Mortensen *et al.*, 1992). These defects increased in occurrence during a time when environmental organochlorine concentrations were increasing and the seal population was decreasing, and were proposed to be associated with altered endocrine function in pregnant females. Abnormalities in foetal development have also been reported to be potentially associated with blockage of the uterus in seals (Helle, 1980; Reijnders, 1994), with the suggestion by Reijnders (1994) that perturbations in the endocrine system caused by PCBs could be a cause of foetal abnormalities, foetal resorption or uterine pathologies. All of these could potentially be indicative of a broader state of reduced reproductive efficiency in animals exposed to specific endocrine interactive organochlorines; however, alternative causes of these abnormalities cannot be excluded. Potential organochlorine-associated reproductive dysfunction has also been reported for sea lions in California, where tissue concentrations of PCBs in prematurely born pups were from 200% to 800% higher than in pups born full-term (De Long *et al.*, 1973). Although complicating factors, such as differences in metal concentrations and the potential presence of micro-organisms capable of causing spontaneous abortion, clouded the initial conclusions of the study, the presence of elevated PCB concentrations could not be ruled out as being causative to the reproductive dysfunction (Addison, 1989).

Endocrine alterations induce reproductive and developmental disorders. They have long been associated with known states of immune suppression in humans and experimental laboratory animals (Margolick, 1992; Newcombe, 1992) leading to increased indices of infectious disease. The epizootic-associated deaths of large numbers of marine mammals, along with recently developed data providing potential understanding of the mechanisms by which organochlorines might impact endocrine function, have generated considerable interest in the possible association between pollutant-initiated immune suppression and infectious diseases in marine mammals.

Large scale mortalities of marine mammals, the vast majority of which have occurred in modern times, include: harbour seals in Iceland in 1918, and in 1979 and 1980 in the northeastern United States; Bering Strait walruses in 1978; Baikal seals in 1987; bottlenose dolphins on the east coast of the United States in 1987 and 1988; bottlenose dolphins in the Gulf of Mexico in 1990, 1992, 1993 and 1994; and striped dolphins in the Mediterranean and Aegean Seas in 1990, 1991 and 1992. In most of these, useful data were obtained from some of the animals pertaining to age, gender, body condition, overt pathologies and tissue burdens of organohalogens. However, the variable states of decomposition resulted in some difficulties in evaluation of chemical residues, viral and immune function, leaving open the question(s) regarding underlying causes of death in virtually all of the animals that died in even the most recent of epizootics. Nevertheless, immune system dysfunction resulting from

organochlorine exposure has been widely demonstrated in laboratory animals (Safe, 1984; 1990; Harper *et al.*, 1995), and there is evidence suggesting that impaired immune function in pinnipeds with elevated tissue residues of organochlorines may be associated with increased indices of infectious disease (Brouwer *et al.*, 1989).

Mechanisms of organohalogen and hydrocarbon toxicity

Environmental exposure of animals to TCDD and related chemicals, including a variety of PCB congeners, is associated with a number of toxic physiological responses, including immunosuppression, thymic involution, weight loss, hepatotoxicity, porphyria, severe dermal lesions, foetotoxicity and severe birth defects, and a dramatic wasting syndrome ultimately leading to death (Safe, 1986; 1990; 1994). These responses differ dramatically between inbred strains of animals, with LD_{50} values between 0.6-2.0 μg/kg for guinea pigs and 1,157-5,000 μg/kg for hamsters (88), and have not been defined for any of the marine mammals. Toxic equivalency factors (TEF) are available for many of the more active isomers and congeners of TCDD, PCDD and related compounds, and are expressed as potential measures of toxicity in laboratory animals relative to the effects of TCDD (Goldstein and Safe, 1989; Safe, 1990; Harper *et al.*, 1995).

The variety of animal responses to toxic hydrocarbons, including some of the organochlorines, is related to the fact that these chemicals may induce expression of a variety of enzyme systems (Poland and Knutson, 1982; Safe, 1984; Whitlock, 1986; 1987), including the phase I cytochrome P450 enzymes such as ethoxyresorufin O-deethylase (EROD), and the phase II enzymes such as glutathione S-transferase (Tables 1 and 2). The mechanisms by which polycyclic aromatic hydrocarbons induce P450 expression, and the subsequent metabolism of PAHs by P450-associated enzymes to produce reactive compounds capable of causing macromolecular adducts and cell damage has been extensively investigated. In contrast, the precise mechanisms by which organochlorines exert cytotoxic effects are not well understood. Some of the organochlorines also act by binding cellular receptors with subsequent altered expression of both cytochrome P450 and non-P450 genes. Altered gene expression may, in turn, alter critical cellular processes

Table 1

Selected Inducible Phase I Enzyme Systems.

*Cytochrome P450 (multiple families and enzymes within families)
*Nicotinamide adenine dinucleotide-b5 reductase
*Nicotinamide adenine dinucleotide-P450 reductase
*Epoxide hydrolase
*Esterases
*Aldehyde/ketone reductase
*Monoamine oxidase
*Diamine oxidase (histaminase)
Amidohydrolases (amidases)
Amine oxidase (flavin-containing monooxygenase)
Alcohol oxidoreductase (alcohol dehydrogenase)
Aldehyde oxidoreductase (aldehyde dehydrogenase)
Xanthine oxidase
Aldehyde oxidoreductase (aldehyde oxidase)

*Enzyme activity has been measured in lymphocytes.

Table 2

Selected Phase II Detoxification Enzymes.

*N-acetyl transferase
*Thiopurine methyltransferase
*Glutathione-S-transferase
*Catechol-O-methyltransferase
*Uridine diphosphate glucuronyl transferase
Cytoplasmic sulfotransferases
Amino acid conjugases (glutamine, cysteine)
Thio methyltransferase

*Enzyme activity has been measured in lymphocytes.

resulting in the onset of toxic phenomena. Not all organochlorines bind the cellular receptors, and exert cytotoxic effects by other mechanisms.

Recent data show that the characteristic interaction of some organochlorines with normal cells is dependent on the initial binding to cellular receptors. These may include members of the steroid receptor superfamily (Dolwick *et al.*, 1993b) and at least one member of the basic helix-loop-helix (bHLH) superfamily of DNA binding proteins, the *Ah* receptor (Poellinger, 1995). The protein product specified by the *Ah* receptor gene, AhR, is one of the ligand-activated transcription factors that belongs to the bHLH family of DNA interactive proteins. These are activated by binding to either exogenous anthropogenic ligands such as dioxin (saturable high affinity binding, K_d of about 1 nM), exogenous dietary ligands such as indolo[3,2-*b*]carbazole, or to endogenous ligands, which have not been identified. Ligand binding is followed by loss of the chaperone heat shock protein, hsp90, dimerisation of the AhR-ligand complex with the nuclear transport protein, Arnt (Li *et al.*, 1994; Whitelaw *et al.*,

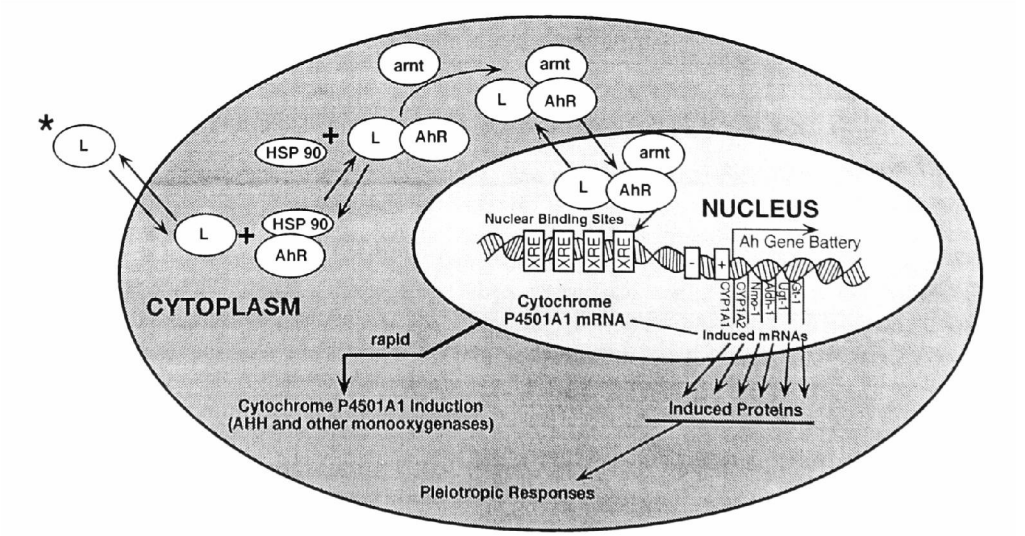

Fig. 3. A depiction of the sequential mechanisms by which AhR-binding ligands interact with the cell to initiate expression of a variety of different genes. The ligand, TCDD, PCBs, benzo(a)pyrene, 3-methylcholanthrene, or any number of other AhR-interactive agents, binds AhR, the chaperone protein HSP-90 dissociated from the AhR-ligand complex, the aryl hydro-carbon receptor nuclear transport protein, arnt, binds the complex as it is transported into the nucleus, and the AhR-ligand complex binds to XRE (DRE) sites, allowing transcription of downstream genes to give variety of physiological responses.

1994), and transport of the receptor-ligand complex into the nucleus (Fig. 4). The intranuclear AhR-ligand/Arnt complex interacts with xenobiotic response elements of DNA, XRE (also called dioxin response elements, DRE), resulting in the initiation of mRNA transcription and the synthesis of a variety of gene products, including phase I enzymes such as the CYP isoforms. In addition, AhR-ligand binding to DNA appears to regulate expression of a number of growth modulatory genes, such as plasminogen activator inhibitor-2 and interleukin 1-β (Sutter *et al.*, 1991). Dioxin binding to AhR in mice has been linked to expression of growth modulatory genes that may be important in the development of AhR-mediated toxic phenomena, including thymic involution and cleft palate formation (Poland and Knutson, 1982; Safe, 1990). AhR is the only ligand activated bHLH transcription factor known at this time; however, other bHLH type receptor proteins are known to regulate cell type-specific transcription, the initiation of mitosis and cell proliferation, and cell transformation (Prendergast and Ziff, 1992). These include the myc oncoproteins, and drosophila genes regulating neurogenesis, mesoderm formation, sex determination, formation of the peripheral nervous system (Jan and Jan, 1994) and early development of olfactory and autonomic neurons (Guillemot *et al.*, 1993).

The *Ah* receptor gene is constitutively expressed in the cells of all normal animals and in a variety of tissues within each animal (Dolwick *et al.*, 1993a), and expression of the *Ah* receptor gene is essential for the health of the animal. Genetically engineered mice (knockout mice) which lack production of AhR ($Ah^{-/-}$) either die at birth or live a short life marred by liver damage and immune dysfunction resulting in repeated infections (Fernandez-Salguero *et al.*, 1995). $Ah^{-/-}$ mice that live past birth never develop normal liver function and are subject to progressive liver damage leading to fibrosis and an early death. Birnbaum and co-workers (Abbott *et al.*, 1991) speculate that a yet unknown endogenous ligand for AhR plays a critical role in foetal development and homeostasis in mice. This is seen in the fact that $Ah^{-/-}$ mice lacking the AhR protein also lack the capacity to initiate normal immune system function when faced with foreign antigens against which an immune response would be initiated in normal animals, apparently due to the inability to initiate T cell proliferative responses. The interaction of dioxins and PCBs to produce the sequel of toxic effects seen in animals (Hebert *et al.*, 1990; De Vito *et al.*, 1994), and the inability of $Ah^{-/-}$ mice to develop normally clearly shows a role for AhR in the regulation of a variety of normal physiological functions in animals. Birnbaum has speculated that the orchestrated interaction of the AhR with its normal endogenous ligand(s) may be significantly perturbed by the presence of exogenous ligands capable of binding the AhR protein with high specificity, and that exogenous ligand binding to AhR may induce the inappropriate expression of a variety of genes, the products of which interfere with normal cell development and function. While the AhR-binding xenobiotics are toxic to adult animals, leading to the loss of immune function associated with thymic atrophy and hypocellularity of bone marrow, they can be devastating in prenatal and neonatal animals, leading to a broad spectrum of developmental defects in addition to the inability to develop normal immune system function. Again, the cellular effects initiated by organochlorine binding to AhR are species specific and toxic effects are not initiated by organochlorines in all species.

These very recent data provide a foundation for understanding the possible mechanisms by which environmental pollutants interact with marine mammals to decrease their immune function and increase their susceptibility to infectious diseases. This initial understanding of the potential mechanisms of immunotoxicity was developed in laboratory animals and must be evaluated to demonstrate applicability of the mechanism to marine mammals. Expression of the *Ah* receptor, binding of the AhR protein to dioxin, and increased cytochrome P450-associated metabolism of hydrocarbons in dioxin-induced cells has been demonstrated in bottlenose dolphin cells *in vitro*, and has been indirectly shown in white whales and other

marine animals. These data provide a starting point for investigations into the cellular mechanisms by which organochlorines and related compounds initiate immune dysfunction in marine mammals.

BASIC IMMUNOLOGY: AN UPDATE FOR THE NON-SPECIALIST

Introduction

If an animal is to survive, it must be able to exclude and/or defend against invading pathogens. There are two major components of the immunological defensive response to micro-organism invaders, each effecting a defence against one of the two relatively distinct categories of infectious agents. The antibody response is directed primarily against exogenous antigens, such as bacterial proteins and polysaccharides, while the cell-mediated response is directed primarily against antigens from transformed cells or endogenous viral antigens that originate in virus infected cells.

When a foreign antigen enters an animal, it is initially engulfed, hydrolytically processed and presented so that it can be recognised as non-self. This information is transmitted either to the antibody-forming system or to the cell-mediated system. These respond by producing specific antibodies and/or activated cells that function to eliminate or neutralise the foreign antigen. The immune system also has a memory component so that when it encounters the same antigen again, its response will be faster and more efficient.

The immune system thus has four basic components. It needs (i) a method of trapping and processing antigens; (ii) a mechanism for recognising and stimulating a response to specific antigens; (iii) cells that either produce antibodies specific for reactive domains on the antigen or that participate in cell-mediated immune attack on the antigen; and (iv) memory cells capable of reacting rapidly to the same antigen if it is encountered again. Each component is associated with specific cell types. Antigens are engulfed, processed and eventually eliminated by combinations of cells that may include macrophages, dendritic cells and lymphocytes. Lymphocytes have specific receptors that interact with and respond to foreign proteins presented as processed antigenic peptides. The memory function of the immune system is resident in specific lymphocytes capable of initiating a secondary immune response. Cell-mediated responses are initiated by T lymphocytes, while antibody synthesis and secretion is a function of B lymphocytes.

Antigen processing

There are several steps in exogenous antigen capture and processing by macrophages. The antigen must initially be phagocytised and incorporated into phagosomes (Gerlier and Rabourdin-Combe, 1989). The phagosomes are fused with cytoplasmic lysosomes to form endosomes containing acidic proteases, which degrade exogenous proteins into fragments about 10-20 amino acids long. Endosomes containing these antigenic peptide fragments then fuse with other endosomes carrying specialised type II major histocompatibility complex proteins (MHC type II) which function on the cell membrane as a type of receptor. As the antigenic peptide fragments and MHC II proteins move to the cell surface, the non-self peptide fragments become aligned with a groove on the MHC II molecule and bind there. The endosome vesicle fuses with the cytoplasmic membrane and the MHC II/peptide complex is oriented on the cell surface in a form that can be recognised by receptors on T cells. T cells which bind the MHC II complex and have a unique receptor capable of interacting with the antigenic peptide are triggered into responding by binding of their receptor with the antigen. Macrophages regulate the dose of antigen presented on their plasma membrane along with MHC II proteins, and so prevent the inappropriate

development of tolerance to antigens. If an antigen is presented to T cells without being linked to an MHC II molecule, the cells may be turned off and tolerance to the antigen may result.

Receptors of the immunoglobulin superfamily

The key proteins having domains which serve as receptors that bind antigenic peptide fragments belong to the immunoglobulin superfamily, which includes many functionally different proteins (Burton, 1990; Cambier and Campbell, 1992). Some have multiple immunoglobulin domains, others have only a single domain. The globulins with multiple domains include antibody molecules (immunoglobulins), T-cell antigen receptors (TCR) and MHC class I and II molecules. Common features of the immunoglobulin superfamily are that all members are involved in binding to other molecules, most are found on cell surfaces and none have enzymatic activity. In many cases, cell interactions are mediated by two different members of the immunoglobulin superfamily, for example, TCR and MHC molecules mediate interaction between antigen presenting cells and T cells.

Immunoglobulins may serve as B cell receptors or as antibodies

The macrophage also secretes a variety of cytokines, including interleukin 1, or IL-1, which binds to receptors on CD4+T cells, called T helper or Th cells. Activated CD4+T cells then secrete a milieu of lymphokines, including IL-2, which interacts with receptors on a number of different cells, including CD8+T cells, B cells, and, in an autocrine or self-activating mode, CD4+T cells. B cells are capable of synthesising immunoglobulins that are unique to each B cell type. The globulin genes have gone through a splicing process during maturation of the cell that results in all B cells having a different variable region on their membrane bound globulins, and in each cell producing only one type of globulin variable region. When that variable globulin domain is capable of interacting with the peptide presented by MHC II, the B cells bind the MHC II-peptide complex and are stimulated via one or more signal transduction mechanisms to enter mitosis and begin formation of B cell clones. Cells of the clone, a group of cells derived from a common progenitor B cell, further mature and begin secreting immunoglobulins, each of which has an identical variable domain capable of interacting with the antigenic peptide presented to the progenitor B cell by the macrophage.

Processed antigens bind special receptors called MHC molecules

MHC groups I and II are complex proteins that are uniquely polymorphic. That is, they differ in their unique combination of different proteins between almost every individual, and their complex structure determines which antigenic peptides can bind to the MHC. MHC molecules determine whether an individual can respond to a specific antigen, and MHC binding is essential for initiating an immune response against a specific peptide region, called an epitope. If an antigenic peptide does not bind to an MHC molecule, no immune response can be initiated against that peptide. For this reason, the genes determining MHC molecules are called 'immune response genes'. These genes dictate resistance or susceptibility to many diseases in animals.

MHC Class II molecules are glycoproteins consisting of two polypeptide chains called α and β. Two domains of each peptide chain ($\alpha 1$ and $\beta 1$) fold together to form an open-ended groove which functions as an antigen binding site. The overall shape of the MHC II groove is determined by conserved peptides of the α and β domains while the polymorphic residues determine the precise shape of the groove. The MHC groove can interact with a peptide of

12-24 amino acids as a straight chain that projects out of both ends. The individual shape of specific MHC determines the ability of each class II molecule to bind antigen fragments, and thus dictates the ability of an animal to respond to a specific antigenic peptide. MHC II are found only on antigen presenting cells, whereas all nucleated cells of an organism contain MHC I proteins.

A second type of MHC molecule involved in binding endogenous antigens, tumour cell or viral antigens generated within the body, is called MHC class I. MHC class I molecules bind peptides typically originating within the cell. The processing of these peptides is very different from those associated with class II molecules. Normal cells continually break down within the body and recycle proteins. During this process abnormal proteins are removed, regulatory peptides are not allowed to accumulate and amino acids are made available for other purposes. Newly formed proteins in virus-infected cells are hydrolysed by large proteolytic complexes called proteasomes (Goldberg and Rock, 1992). As a protein is degraded, peptides are rescued from further breakdown by attachment to transporter proteins that carry protein fragments from the cytoplasm to the endoplasmic reticulum, where MHC class I molecules are synthesised and assembled around the peptides which lie within the MHC groove. MHC class I molecules carry peptides to the cell surface for recognition. When the MHC I/peptide complex reaches the cell surface, the bound peptides are displayed and are available for interaction with specific cells having receptors for which the MHC-associated peptides have interactive specificity.

T cells recognise antigens through a special receptor

T cells will only respond to antigens attached to an MHC molecule if those antigens are recognised by the T cell antigen receptor, or TCR (Hodgkin and Kehry, 1992). Each T cell has between 10,000 and 20,000 identical TCRs on its surface. These TCRs are protein complexes closely associated with an additional protein designated as either CD4 or CD8. A CD4 or CD8 molecule is required in order to link the T cell to the MHC molecule on the antigen-presenting cell. CD4 binds to MHC class II molecules while CD8 binds to MHC class I molecules. CD4+ T cells bind and respond to processed exogenous bacterial and parasitic antigens, while CD8+ T cells bind and respond to processed endogenous viral and tumour antigens.

The TCR is a complex structure, as one would expect for a critical antigen-binding receptor. It consists of two major parts, one that confers binding specificity to antigens and MHC, and a second that is involved in signal transduction mechanisms that initiate signals from the receptor protein to the T cell, triggering intracellular responses. TCR are always associated with a set of invariant glycoproteins collectively called CD3. These CD3 glycoproteins are the signal-transducing parts of the TCR receptor. They do not function simply as an on/off switch for mitosis, but are linked to at least three different signal transduction pathways (Weaver and Unanue, 1990). Depending on which combination of pathways is triggered, the T cell may divide and/or secrete proteins called cytokines. If the receptor is only partially triggered then the T cell may be turned off. This is a protective device to ensure that T cells do not respond inappropriately to self antigens.

Two types of mammalian TCR have been identified. One has two component peptide chains called gamma and delta. The other consists of two different peptide chains, alpha and beta. In humans, mice and (probably) most non-ruminants, between one and ten percent of T cells carry gamma and delta receptors. The remaining 90-99% of T cells in these species carry alpha and beta receptors. In artiodactyls, in contrast, T cells with gamma and delta TCR can account for as much as 60% of the total T cell population. The four T cell receptor peptide chains are similar in structure although their molecular weights differ. Each TCR

chain is divided into four well defined domains. The C-terminal end of each chain is attached to the T cell membrane and has a constant amino acid sequence, thus, it is called the constant region (C-region). The TCR domain exposed at the cell surface has a highly variable sequence and is therefore called the variable region (V-region). The TCR protein functions essentially as an immunoglobulin with a constant domain imbedded within the cytoplasmic membrane as an effector oriented towards the signal transduction target. When two cytotoxic cells compete for binding to the same MHC I and antigenic peptide only one is triggered, perhaps the one with highest binding affinity for the antigen.

Summary

In a model immune response to bacterial invasion, macrophages engulf a bacterium and hydrolytically degrade the bacterial proteins, presenting them on the cytoplasmic membrane in association with the MHC II complex. The macrophage also secretes a variety of cytokines, including interleukin 1, or IL-1, which binds to receptors on CD4+ T cells, called T helper or Th cells. Activated CD4+ T cells then secrete IL-2, which interacts with receptors on a variety of cells, including CD8+ T cells, B cells, and, in an autocrine or self-activating mode, CD4+ T cells. Activated B cells that produce a variable globulin domain capable of interacting with the peptide presented by MHC II bind the MHC II-peptide complex and are stimulated via one or more signal transduction mechanisms to enter mitosis and begin formation of a B cell clone. Cells of the clone, a group of cells derived from a common progenitor B cell, mature and begin secreting immunoglobulins, each of which has an identical variable domain capable of interacting with the antigenic peptide presented to the progenitor B cell by the macrophage.

If the immune response is triggered by endogenous antigens such as those on a virally infected cell or a transformed (neoplastic) cell, the macrophage engulfs the infected cell, digests the proteins to peptides and presents the antigenic non-self peptides in concert with an MHC I complex. Again, the macrophage secretes IL-1, which stimulates CD4+ T cells to secrete IL-2. When IL-2 binds receptors on CD8+ T cells (cytotoxic T lymphocytes) the CD8+ T cells recognise the MHC I complex and bind to it. Those CD8+ cells that have a globulin receptor, TCR, capable of responding to the antigenic peptide are stimulated to initiate mitosis and the cytotoxic T cells form a clone, each cell of which is capable, by virtue of its globulin receptor, of binding to cells in the body having virally encoded or transformed specific membrane proteins like the one the macrophage presented. When cytotoxic T cells bind to cells expressing the viral or transformed cell proteins they mount an enzymatic attack on the cell that kills it (O'Rourke and Mescher, 1993).

Lymphoid organs and cells of the immune system are strategically located to monitor physiological portals of entry and may be among the first cells to participate in the absorption, distribution and biotransformation of xenobiotic compounds entering an animal. In mammals, ingested fat-soluble chemicals are taken up into the lacteal absorption from the gut and transit the mesenteric lymphatic drainage to the vascular circulation via the thoracic duct, bypassing the liver (Yoo *et al.*, 1984; Busbee *et al.*, 1985; Wilson *et al.*, 1985). Therefore, fat soluble chemicals in the diet incorporated into chylomicrons are peripherally distributed prior to encountering hepatic detoxification mechanisms, with potential exposure of essentially all peripheral organs and cells to unmetabolised fat-soluble hydrocarbons. Immune function cells in the lymphatic organ-rich thoracic duct are directly exposed to extremely high levels of fat-soluble chemicals. Cells of myeloid and lymphoid origin constitute the first line of defence against microbial invaders, and conduct constant immunosurveillance against cells showing antigens indicative of virus infections or of neoplastically transformed cells (Newcombe, 1992). Anything capable of interfering with

the complex physiological processes required for myeloid cell function and activation of either the antibody-associated or cell-mediated lymphoid responses could ultimately lead to the loss of protection against microbial pathogens and neoplastic cells.

IMMUNOLOGY AS A TOOL FOR ASSESSING MARINE MAMMAL HEALTH

Practical limitations are imposed upon establishment of unequivocal associations between environmental contaminants and marine mammal health. Marine mammals are protected species and thus fulfilling Koch's postulates relative to infectious agents and potential toxic compounds is not realistic (e.g. see Mims and White, 1984). Furthermore, the ability to sample individual animals over time in their natural environment is not an easy task and the use of invasive techniques to obtain samples is probably not appropriate. Development of quantitative and qualitative techniques to measure immune system function represents the most viable and sensitive monitor of marine mammal health. The characterisation of a given animals' immune system components will provide a sensitive indicator of perturbations to the system, provided sufficient age, sex and reproductive status baseline data are available for the species. Blood of such species may be relatively easily obtained in sufficient quantities by non-invasive means (venipuncture via the fluke in cetacean species or accessing a venous sinus in pinnipeds) with the resulting serum, plasma and leukocytes providing a window for assessing immune system status.

Knowledge about the immune system of marine mammals is limited. Normal ranges of total white blood cell (WBC) counts, and associated differentials, serum immunoglobulin (Ig) levels and limited blood chemistry values have been reported for some species as summarised by Bossart and Dierauf (1990) and Kennedy-Stoskopf (1990). However, the number of animals sampled is often quite small and minimal data available relate to the influence of animal age and reproductive status on the reported values. Such measurements of immune system components are valuable to assess an animal's health and provide gross insight into existing potential disease (Bossart and Dierauf, 1990). The measurement of antigen-specific antibody, as a measure of current and past exposure to infectious agents, can be realised using plasma, or preferably serum, in standard serologic assays. Serologic assays such as enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA) require secondary reagents for identifying the presence of specific antibody. For the large part, anti-Ig reagents readily available for human, marine and domestic animal species have not been adequately characterised as to their specificity for equivalent Ig molecules in marine mammals. While some cross-species reactivity has been documented (Nash and Mach, 1971; Cavagnolo and Vedros, 1978; Cavagnolo, 1979), it is desirable to establish reagents specifically for use with each marine mammal species. The use of protein A (Ross *et al.*, 1993) or G (conjugated to a detection system) for detection of marine mammal Ig can serve as a secondary reagent; however, efficacy of such binding may not be uniform between animal species and antibody isotypes. The preferable reagent for routine serologic analysis would be species-specific anti-heavy and –light chain Ig such that all antibody isotypes would be identified. Such reagents, anti-harbour seal Ig (Carter *et al.*, 1990) and anti-dolphin Ig (Romano *et al.*, 1992), have undoubtedly been prepared by multiple investigators (our laboratory has developed such reagents for dolphins, harbour seals and sea otters) but are not available on a commercial basis. Furthermore, polyclonal (Kennedy-Stoskopf, 1990) or monoclonal antibodies with Ig heavy chain specificities are also of value; identification of high titer antigen-specific IgM and low titer IgG would be indicative of an active infection and preclude the necessity for paired serum samples (samples obtained over a period of time) for demonstrating a rise in antibody titer to a given antigen.

Identification of immune system dysfunction as a mechanism of assessing marine mammal health using serologic data, gross haematology and clinical chemistries are probably of limited value. Acquired immunodeficiency, as opposed to malfunction at the inherited or genetic level, has classically been identified by measurement of lymphocyte function employing a lymphocyte blastogenesis assay on peripheral blood mononuclear leukocytes. Such assays measure the ability of lymphocytes to proliferate following polyclonal stimulation with mitogens; proliferation is typically detected by incorporation of ^{3}H-thymidine. Lymphocyte blastogenesis data have been reported for bottlenose dolphins (Colgrove, 1978; Lahvis *et al.*, 1993) and harbour seals (De Swart *et al.*, 1993; Ross *et al.*, 1993), using a variety of mitogens including phytohemagglutinin (PHA), concanavalin A (ConA), pokeweed mitogen (PWM) and bacterial lipopolysaccharide (LPS). Antigen-specific induction of lymphocyte proliferation (blastogenesis) and delayed-type hypersensitivity (DTH skin test) have been reported in harbour seals following immunisation (De Swart *et al.*, 1993). However, such antigen-specific analysis is not realistic when assessing the immunocompetence of free-ranging animals. While blastogenesis assays provide gross insight into lymphocyte function and can identify animals with substantial immunosuppression, they have inherent disadvantages including: (i) poor reproducibility (especially between laboratories); (ii) the lymphocyte subset(s) induced to proliferate are unknown; and (iii) the counts/minute (CPM) or stimulation index (SI) should probably not be used in a quantitative sense but rather divided into low, moderate or high responders. The latter point becomes obvious as larger numbers of apparently healthy animals are analysed for lymphocyte blastogenesis; while those animals with negligible or very high stimulations tend to cluster, the majority of animals present as a continuum of responses ranging from low (10,000-20,000 CPM) to high (80,000-100,000 CPM) stimulations. The significance between animals on the low versus high end is unknown.

Based upon the above-mentioned problems associated with interpretation of blastogenesis relative to identification of immune system function/dysfunction, additional reagents and assays will be required. As described above, the adaptive immune system has been classically described as having two arms, cellular and humoral. Generation of a balanced immune response ensues following processing and presentation of the antigen by MHC class II-bearing cells of myeloid and lymphoid origin to a subpopulation of T lymphocytes that express the differentiation antigen, CD4. These CD4+T lymphocytes, T helper cells, play a pivotal role in facilitating the differentiation and expansion of antigen-specific B lymphocytes and CD8+ cytotoxic T lymphocytes. When considering establishment of techniques for measurement of marine mammal immune system function/dysfunction it is most logical to follow the advancements in human medicine. The extensive development of reagents and techniques that are currently providing advanced capabilities in assessing immune system status in humans, and many domestic and laboratory animal species, is lacking in marine mammals. Phenotypic and functional characterisation of an animals immune system has been dramatically enhanced in many species using panels of reagents including monoclonal antibodies specific for leukocyte differentiation and activation antigens and cytokine probes (monoclonal antibodies and/or cDNA probes to detect intracellular cytokine mRNA). Unfortunately, the majority of monoclonal antibodies, and many of the cDNA probes, are species-specific, requiring reagent development for individual marine mammal species. Development of monoclonal antibodies specific for cell-surface differentiation antigens, adhesion molecules and activation antigens is critical to understanding the immune systems of marine mammals and identifying acute to chronic perturbations in the system. To date, there is a complete absence of published reports describing such reagents for any marine mammal species other than monoclonal antibodies specific for harbour seal immunoglobulin (King *et al.*, 1993a) and antibodies specific for

human MHC class II proteins that cross-react with bottlenose dolphin MHC proteins (Romano *et al.*, 1992). While polyclonal antibody preparations have been developed that recognise surface Ig on B lymphocytes from a number of marine mammal species, antibodies specific for T cells and subsets thereof are lacking. From the authors' perspective, antibodies specific for CD3, a complex of invariant proteins associated with the T lymphocyte antigen receptor (TCR), could be considered a reagent in critical need relative to identification of immunocompromised marine mammals. Many of the CD3-specific monoclonal antibodies available for other species are capable of inducing T cell activation and proliferation in the absence of exogenous mitogens or interleukin(s). Not only can these reagents be used to enumerate circulating T lymphocytes by analytical flow cytometry, but can also activate T lymphocytes in a mechanism similar to that induced by antigen via the TCR. Such *in vitro* T cell activation is far more physiologically relevant than the polyclonal activation of lymphocytes via use of mitogens, LPS or phorbol esters. Thus, when attempting to assess T lymphocyte dysfunction, results will probably differ substantially between TCR- and mitogen-induced signal transduction and activation; we would speculate that activation induced via engagement of the TCR will identify immune suppression that would not be obvious following other non-physiologic activation mechanisms.

While lymphocyte blastogenesis, or lymphocyte proliferation, has classically been considered a measure of immune system function, advances in immunology have established that activation of lymphocyte subsets can result in production of cytokines (see below) with potent immunoregulatory capabilities; this attribute does not necessarily require cell proliferation. Thus, an additional assay gaining popularity for identifying immune system function measures the ability of lymphocytes to activate by identification of the expression of cell-surface activation antigens such as the IL-2 receptor (IL-2R). Such assays typically utilise analytical flow cytometry for enumeration of in vitro-activated lymphocytes (predominantly T cells) with fluorochrome-conjugated recombinant IL-2 or monoclonal antibodies specific for IL-2R (CD25). Not only can such analysis provide the absolute number of lymphocytes that were activated by a given stimulus but can also be used in two-colour analysis for identification of the lymphocyte subsets (e.g., CD4+ or CD8+) that have been activated. The species cross-reactivity exhibited by human recombinant IL-2 has permitted application of this technique to assessment of T cell function in a variety of marine mammals (dolphins, killer whales, sea otters and harbour seals). The commercial availability of fluorochrome-conjugated human IL-2 has made this flow cytometry technique possible. Upon development of monoclonal antibodies specific for lymphocyte differentiation antigens of marine mammal species, ability of multiple subsets to activate following an appropriate stimulus will provide an invaluable tool for assessing subtle levels of immune system dysfunction.

In addition to the physical interaction between antigen-presenting cells, T lymphocytes and B lymphocytes, an array of small proteins (cell-surface and/or secreted), cytokines, are pivotal in development and control of the immune response. Cytokines may exhibit autocrine (act upon the cell producing the cytokine), paracrine (act upon a nearby cell) and/or hormonal (act upon cells at a distant site) types of effects on receptor-bearing cells; the ability of a cell to express specific receptors plays a large role in controlling cytokine effects. A single cytokine can be produced by multiple cell types and induce different effects on different cell types. Furthermore, different cytokines can act in synergism or antagonism on a given cell. The development of reagents (monoclonal antibodies; generally species-specific) for identifying cytokines, transcription of cytokine genes and cytokine receptors has been extensive in human and marine species. Such analysis has provided a new dimension to understanding immune system function and dysfunction. Cytokine-dependent or responsive cell lines have been classically used to measure levels of multiple cytokines

with some being cross-species responsive. Harbour seal interleukin 6 (IL-6) has been identified in the plasma of animals with indications of systemic infection using such a cross-species bio-assay (King *et al.*, 1993b). IL-6 is a pleiotropic interleukin and becomes detectable in peripheral blood during an inflammatory response. Antigen-trapping enzyme-linked immunosorbent assays (ELISA) are beginning to find routine use in human medicine for identification of circulating levels of cytokines with hormonal type effects that are produced during an inflammatory response (tumour necrosis factor, IL-1 and IL-6) and thus have diagnostic value. An alternative approach currently being used for research purposes is identification of cytokines produced by individual cells via identification of their respective cytoplasmic mRNA. Many of the cytokine genes have sufficient cross-species homology to develop oligonucleotide primers for use in establishing polymerase chain reaction (PCR)-based identification of mRNA; this attribute is facilitating development of such probes in marine mammals. Such capabilities should prove valuable in the functional subdivision of CD4+T (T helper, TH) lymphocytes into those that facilitate inflammatory responses (TH1) versus those that facilitate Ig production (TH2); an evaluation of perturbations in such T cell subpopulations should prove very useful in the identification of subtle immune system perturbations.

In summary, advances are being made in improving our understanding of the immune systems of marine mammal species and such progress has the potential to accelerate in a manner analogous to that currently being experienced for human and marine systems. Establishment of the immune system as a sensitive monitor of the health of marine mammals will ultimately hinge upon acquisition of the above-mentioned reagents, techniques and application to normal healthy animals to establish baseline values.

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J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 249–265, 1999

Cancer in beluga whales from the St Lawrence Estuary, Quebec, Canada: A potential biomarker of environmental contamination[1]

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ABSTRACT

A population of approximately 500 white whales (*Delphinapterus leucas*) inhabits a short stretch of the St Lawrence Estuary which drains one of the most industrialised areas of the world. Over a 12-year period (1983-1994), 73 carcasses out of 175 beluga[2] whales reported stranded on the St Lawrence Estuary shoreline have been examined. Of these 73 carcasses, 14 (19%) were affected by 15 different malignant tumours (cancers), one animal being affected by two different types of cancer. Overall, 23% of necropsied sexually mature animals were affected by cancer. Forty percent of the 35 cancer cases reported world-wide in cetaceans occurred in this population. The estimated annual incidence rate (AIR) of cancer in St Lawrence beluga whales, a minimum figure of 233/100,000 animals, is much higher than that reported for any other population of cetaceans, and is similar to that of man, and of hospitalised cats and cattle. More specifically, the AIR of small intestinal cancers in the studied population, a minimum figure of 83/100,000 animals, is much higher than that observed in man and all animals, except in sheep in certain parts of the world, where an environmental carcinogen is believed to be etiologically involved.

KEYWORDS: POLLUTION-PAHS; WHITE WHALES; BIOMARKERS; DISEASE

INTRODUCTION

Despite the significant number of post-mortem examinations performed on cetaceans over the last 50 years, a relatively small number of cancers (malignant tumours) have been reported in these animals (e.g. Landy, 1980; Howard *et al.*, 1983; Geraci *et al.*, 1987). In a review of cetacean tumours, Geraci *et al.* (1987) examined the accuracy of diagnosis of reported neoplasms and concluded that no causal association could be identified between the reviewed tumours and environmental pollutants. Since that publication, most of the additional cases of cetacean cancers have been reported from the small (*ca* 500-1,000 animals; Kingsley, 1996) beluga whale population inhabiting the St Lawrence Estuary (Martineau *et al.*, 1988; 1995; Girard *et al.*, 1991; De Guise *et al.*, 1994). It has been

[1] A version of this paper was submitted to the IWC workshop on Chemical Pollution and Cetacean, March 1995, Bergen as SC/M95/P10.

[2] The official IWC common name for *Delphinapterus leucas* is the white whale. However, as the common name used in previous papers relating to this population is the alternative 'beluga whale', this has been retained for this paper.

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suggested that this high prevalence might be etiologically related to environmental contaminants (Martineau *et al.*, 1988; 1994; 1995; De Guise *et al.*, 1994). The purpose of this paper is to review reported cases of cancers in free-ranging cetaceans, and to compare the cancer prevalence found in St Lawrence beluga whales with that prevailing in other cetaceans, domestic animals and man. Two new cases of gastrointestinal cancer for cetaceans, a gastric adenocarcinoma and a small intestinal adenocarcinoma are also described.

MATERIALS AND METHODS

Previously reported cases

Published cases of cancers reported in free-ranging cetaceans and those listed in the Marine Mammal Database of the Registry of Comparative Pathology (Armed Forces Institute of Pathology (AFIP), Washington DC, 20306) were reviewed. The database includes tissue glands, glass slides, paraffin blocks and photographs from captive and free-ranging marine mammals affected by neoplasia, congenital conditions and diseases caused by parasites, bacteria and viruses. The samples are supplied from the USA and other countries on a voluntary basis by professionals (mostly biologists and veterinarians) involved with marine mammals. At the end of 1995, the database included reference to 834 diseased cetaceans (including 13 of the 14 St Lawrence white whales affected with cancer). Over 90% ($n = 774$) of the database examples originated from necropsies with the remainder ($n = 60$) from surgical submissions from live animals. In addition to the 13 white whales from St Lawrence a further 14 white whales affected by various diseases were included, all of which were captive. Of these, six were adults (10, 15, 19 and 26 years and two of unknown age but considered 'adults'), one was a new-born (4 days), a second was a juvenile (3 months) and the last was immature (3 years); the age of five animals was not reported. Samples from all of these whales were obtained from necropsies whilst three came from live diseased animals.

Biological sampling

Since January 1983, 175 beluga whale carcasses have been found drifting or stranded along the shoreline of the St Lawrence Estuary, Quebec, Canada. Some 42% ($n = 73$) were examined at the Faculté de Médecine Vétérinaire of Université de Montréal, of which 85% ($n = 62$) were sexually mature.

Cancer epidemiology in St Lawrence beluga whales

Assuming that the St Lawrence animals are a distinct population, the total incidence of cancers in the studied population during the study period can be expressed as:

$$\hat{N}_C = \frac{N_c}{M_A} \times M \text{ where:}$$

$\hat{N}_C$ = the estimated number of cancers in the population;
N_c = the number of autopsied animals with cancer;
M_A = the number of autopsied animals; and
M = the estimated minimum total mortality.

This assumes that the animals that are autopsied are representative of the total stranded 'population'. If it is assumed that all dead animals strand, then $M = M_s$, where M_s = the number of stranded animals. However, weather conditions in the region mean that strandings can only be detected for 9 months of the year. If it is assumed that mortality is uniform throughout the year then;

$$M = M_s \times \frac{12}{9}$$

For other species, the parameter usually estimated is the annual incidence rate of cancers (AIR) expressed as the number of cancers per 100,000 individuals. Following Dorn and Priester (1987), for the study population this would be:

$$AIR = \frac{N_c}{T} \times \frac{100{,}000}{N_t}$$

where T is the study period in years and N_t is the estimated total population size. This assumes that N_t has been constant over the duration of the study.

The validity of the assumptions involved is discussed later in the paper. A crude sensitivity test is carried out by assuming different values for population size, N_t, and the correction factor for poor weather (see Tables 4 and 5). The scenarios tested have been termed: 'best' i.e. reflecting our view of the most likely values; 'optimistic' i.e. assumed values that lead to a lower AIR in the population and 'pessimistic'; i.e. assumed values that lead to a higher AIR.

RESULTS

Cancers reported in cetaceans

Malignant neoplasms that have been reported in free-ranging cetaceans are listed in Tables 1, 2 and 3. A total of only 35 cancers have been reported in two species of Mysticeti and nine species of Odontoceti.

High percentages of those cancers were found in the digestive (12/35 or 35%) and reproductive (9/35 or 26%) systems. The high number of ovarian cancers is probably explained by the emphasis put on the examination of the reproductive system during evaluation of the productivity of commercially exploited species (Geraci *et al.*, 1987).

Cancer incidence in the St Lawrence population

From equations (1) and (2) above and the parameter values listed in Table 5, the total incidence of animals with cancer in the studied population is about 45, translating to an AIR of about 750 animals per 100,000 for the 'best' scenario. The results of the sensitivity analysis are considered in the 'Discussion'.

Nature of the cancers in St Lawrence beluga whales

Two new gastrointestinal cancers

The carcass of an adult male beluga whale (*Dl-1-94*) was found drifting offshore near Tadoussac (48°7'N, 69°43'W) on 24 May 1994. The major finding of the necropsy was a large (9×7×2cm deep) ulcer present on the mucosa of the second gastric compartment. The ulcer was connected to the abdominal cavity by a 7mm-diameter perforation. Microscopic examination (performed in May 1995) revealed that the *tunica submucosa* and the *tunica muscularis* of the gastric wall adjacent to the ulcer were heavily infiltrated by large numbers

Table 1
Cancers reported in beluga whales from the St Lawrence Estuary (1983-1994).

Organ	Cancer type	Age (yrs)	Sex	Date of stranding	AFIP accession no.[1]	Reference
Urinary bladder	Transitional cell carcinoma	17	M	1983 (Dl-18-83)[2]		Martineau *et al.*, 1985
Intestine	Adenocarcinoma	29+	M	1989 (Dl-7-89)	2462295-3	De Guise *et al.*, 1994
Intestine	Adenocarcinoma	20+	M	1989 (Dl-8-89)	2462247-4	De Guise *et al.*, 1994
Intestine	Adenocarcinoma	25+	M	1993 (Dl-2-93)	2461200	Martineau *et al.*, 1995
Intestine	Adenocarcinoma	27+	M	1994 (Dl-2-94)	2464226-6	Martineau *et al.*, 1995
Intestine	Adenocarcinoma	27+	F	1994 (Dl-7-94)	2508083-900	This report
Stomach	Adenocarcinoma	21+	F	1988 (Dl-4-88)	2456949-3	De Guise *et al.*, 1994
Stomach	Adenocarcinoma	27+	M	1994 (Dl-1-94)	2508095-300	This report
Salivary gland	Adenocarcinoma	24	M	1986 (Dl-6-86)	2457053-3	Girard *et al.*, 1991
Liver	Adenocarcinoma	22+	F	1988 (Dl-9-88)[3]	2456952-7	De Guise *et al.*, 1994
Mammary gland	Adenocarcinoma					
Ovary	Granulosa cell tumour	24.5	F	1985 (Dl-2-85)	2519612	Martineau *et al.*, 1988
Ovary	Granulosa cell tumour	21+	F	1988 (Dl-13-88)	2462292-0	De Guise *et al.*, 1994
Ovary	Dysgerminoma[4]	25+	F	1989 (Dl-6-89)	2462229-2	De Guise *et al.*, 1994
Mediastinum	Poorly differentiated malignant neoplasm (cancer)[5]	18+	M	1990 (Dl-1-90)	2519747	De Guise *et al.*, 1994

[1]All cases have been submitted to the AFIP except Dl-18-83 which has been confirmed by Daniel Cowan, Department of Pathology, University of Texas Medical Branch, Galveston, Texas 77555-0588, USA. This case has also been confirmed in Geraci *et al.*, 1987. [2]Dl-18-83 is the 18th beluga examined in 1983. The same system was used for the classification of all carcasses. [3]Dl-9-88 was affected by two cancers. [4]Classified as Granulosa Cell Tumor in De Guise *et al.*, 1994. Reclassified as Dysgerminoma after consultation with AFIP. [5]Classified as metastatic poorly differentiated carcinoma in De Guise *et al.*, 1994. Reclassified as poorly differentiated neoplasm after consultation with AFIP.

of irregularly-shaped tubular glands separated by moderate amounts of connective tissue (Fig. 1). The tubular structures were lined by closely packed cuboidal cells that generally formed a single layer but occasionally piled up in a disorderly manner (Fig. 2). The tumour cells had varying amounts of eosinophilic cytoplasm and the generally round nuclei were hyperchromatic, often crowded, varied moderately in size and occupied a central position. Chromatin was finely to coarsely clumped and nucleoli could not be seen. The nucleus:cytoplasmic ratio was higher than one. Mitoses were rare. Multifocal aggregates of predominant macrophages were observed in the stroma with small numbers of plasmocytes and neutrophils. Accordingly, a gastric adenocarcinoma with a secondary perforating ulcer was diagnosed.

The carcass of a female beluga whale (*Dl-7-94*) was found at Saint-Paul du Nord (48°34'N, 69°15'W), on 25 December 1995. During the necropsy, it was found that the duodenal segment wall was diffusely thickened (3cm) over a length of 20cm at the junction with the stomach. The demarcation between the distal normal intestinal wall (1cm thick) and the thickened segment was abrupt. Microscopically, the intestinal wall was thickened by cellular nodules present mostly in the *tunica submucosa*. These nodules, separated by an abundant fibrous stroma, were composed of irregularly-sized and poorly-formed tubular glandular structures and nests of tumour cells that were separated by smaller amounts of stroma where single tumour cells were often present. Infiltrating tumour cells were also present in large numbers in the subjacent *tunica muscularis* where they formed small

Table 2

Cancers reported in free-ranging cetaceans, other than St Lawrence beluga whales.

Species	Organ	Cancer	Age	Sex	Sources	Reference
Bottlenose dolphin	Liver, lungs	Reticuloendotheliosis		F		Pers. comm. in Landy, 1980
Bottlenose dolphin	Pancreas	Carcinoma	Adult	M		Pers. comm. in Landy, 1980
Bottlenose dolphin	Spleen, lymph nodes	Lymphosarcoma	Adult	F		Pers. comm. in Landy, 1980
Pacific white-sided dolphin	Spleen, lymph nodes	Lymphosarcoma	Adult	M		Howard *et al.*, 1983
Pacific white-sided dolphin	Spleen, lymph nodes, liver, kidney	Eosinophilic leukemia	Adult	M		Howard *et al.*, 1983
Pilot whale	Ovary	Granulosa cell tumour	Adult	F		Bernischke and Marsh, 1984
Harbour porpoise	Unknown	Adenocarcinoma		F	British waters	Baker and Martin, 1992
Harbour porpoise	Stomach	Adenocarcinoma	Adult	F	Northern Wadden Sea	Breuer *et al.*, 1989
Amazon river dolphin	Lung	Squamous cell carcinoma	Adult	F		Geraci *et al.*, 1987
Blue whale	Ovary	Granulosa cell tumour	Adult	F	Antarctic	
Fin whale	Ovary	Granulosa cell tumour	Adult	F	Antarctic	Rewell and Willis, 1949
Fin whale	Ovary	Granulosa cell tumour	Adult	F	Antarctic	Rewell and Willis, 1949
Fin whale	Ovary	Carcinoma[1]	Adult	F	Antarctic	Stolk, 1952

[1]Reclassified as Granulosa cell tumour by Geraci *et al.*, 1987.

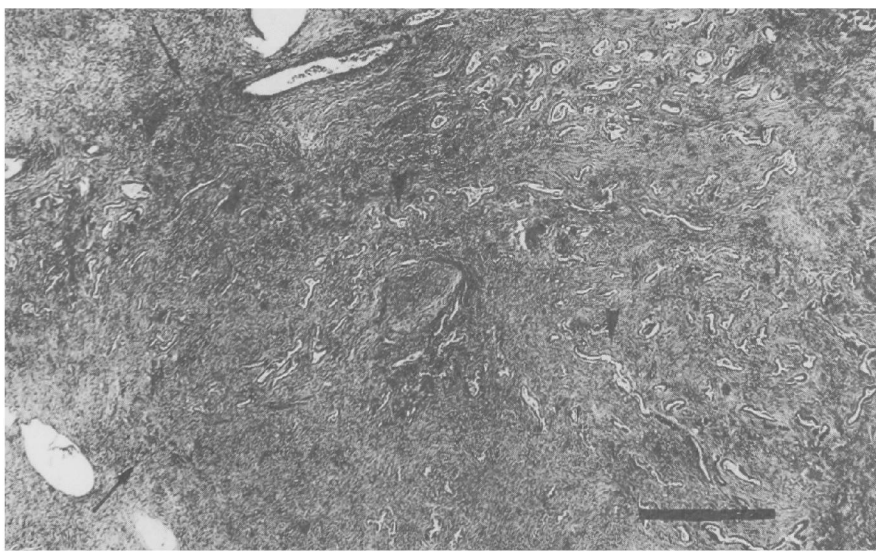

Fig. 1. Gastric adenocarcinoma in a beluga whale (DL-1-94). Numerous irregularly-shaped, tubular, gland-like structures (arrowheads) infiltrate the *tunica submucosa* of the second gastric compartment and are separated by a moderately abundant collagenous stroma. Arrows show the boundary between the normal *tunica submucosa* (left and right corners) and the infiltrating tumour cells (right). Bar: 0.5mm.

Table 3

Cetaceans affected by cancer listed in the Marine Mammal Database, AFIP (updated, December 1995).

Species	Status	Organ	Cancer type	Age	Sex	Sources	%[1]	Accession no.
Beluga	Captive	Brain	Carcinoma	19yrs	M	Arctic	7.1% (14)	2034441
Bottlenose dolphin	Wild	U[2]	Tubulopapillary adenocarcinoma	Adult	M	Gulf of Mexico	0.3% (384)	2304654
Bottlenose dolphin	Wild	Kidney	Renal cell carcinoma	Adult	F	Atlantic Ocean (S. Carolina)	0.3% (384)	2445679
Fin whale	Wild	Kidney	Lymphosarcoma	Adult	F	U	20% (5)	1470245
Killer whale	Captive	Liver, lymph node, spleen	Reticuloendotheliosis	Adult	F	U	5.3% (19)	162636
Killer whale	Captive	Lymph node	Hodgkin's disease-like[3]	Adult	M	Iceland	5.3% (19)	2337420
Pygmy sperm whale	U	Liver	Cholangiocarcinoma	U	U	U	1.7% (57)	1777514
Spotted dolphin	Wild	Testis, lymph nodes, adrenal glands	Malignant seminoma, Pheochromocytoma	Adult	M	Gulf of Mexico	16.7% (6)	2428264

[1](Number of animals affected by cancers of a given species) over (the total number of animals of this species) × 100; ( ): total number of animals that are listed in the Marine Mammal Database of the AFIP. [2]Unknown. [3]Yonezawa *et al.*, 1989.

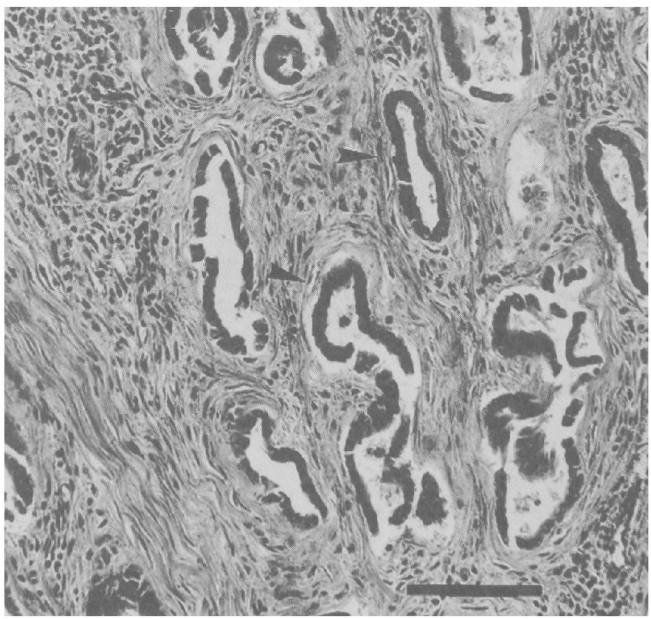

Fig. 2. Gastric adenocarcinoma in a beluga whale (DL-1-94). The glandular structures (arrowhead) are lined by a single layer of well differentiated cuboidal cells. The collagenous stroma contains several interspersed aggregates of mononuclear cells. The apparent detachment of tumour cells into the tubular lumen is due to autolysis. Bar: 100μm.

irregularly-shaped nests or poorly-formed acini with indistinct lumen (Fig. 3). In addition, single tumour cells were generally large, tended to be columnar, were irregularly-sized and shaped and showed loss of polarity. The cytoplasm was scarce, the nucleus:cytoplasm ratio was higher than one and the nuclei were large, variably-shaped and sized. A highly infiltrative small intestinal adenocarcinoma was diagnosed.

Summary of cancer types found

Fifteen cancers were diagnosed in 14 (19%) of the animals necropsied (Table 1). If only sexually mature adults are considered this rises to 23% (Martineau *et al.*, 1988; 1994; De Guise *et al.*, 1994). Some 60% of the malignant tumours originated from the epithelium lining the digestive system (adenocarcinomas of the intestine, stomach, liver and salivary gland) (Girard *et al.*, 1991; De Guise *et al.*, 1994; Martineau *et al.*, 1995). Four intestinal adenocarcinomas were close to the stomachs and one was closer to the anus (Martineau *et al.*, 1995).

DISCUSSION

Cancers previously reported in cetaceans

Limitations of the source material

The published material prior to this study does not comprise a systematic survey of cancer incidence in cetacean populations. Rather it reflects the interest of a relatively small number of scientists, particularly in earlier years. For example, only a tiny proportion of the baleen whales killed for commercial purposes this century, even those flensed with biologists present, were examined for cancer (or indeed any other diseases). This reflected the prevailing interest at that time, which was the estimation of population parameters, particularly with respect to reproduction. The same is true for catches of odontocetes. Thus, whilst the low number of reports might be taken as indicative of the relative rarity of cancer, it is impossible to quantify this in any way.

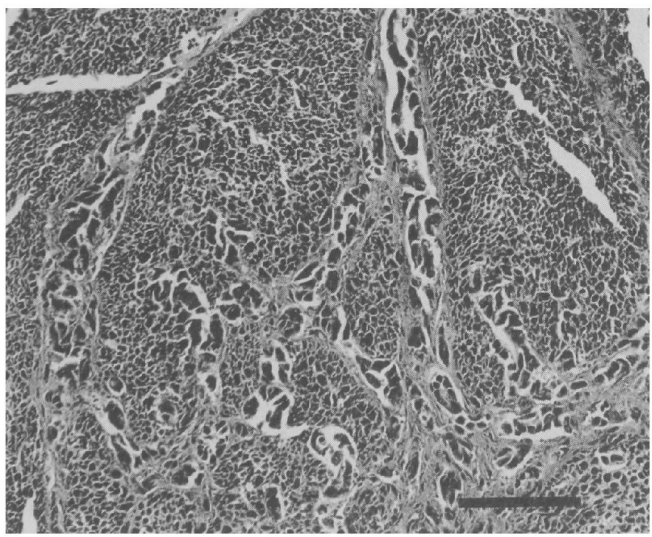

Fig. 3. Small intestinal (duodenal) adenocarcinoma in a beluga whale (DL-7-94). Multiple poorly-formed glandular structures and nests of epithelial cells infiltrate the duodenal *tunica muscularis* along fibrous septa. The empty spaces result from autolysis. Bar: 200μm.

However, there have been some relatively systematic studies. For example, a single cancer was found during the post-mortem examination of 301 cetaceans from British waters (J.R. Baker, University of Liverpool, pers. comm.), only one cancer was found by Geraci *et al.* (1987) in over 1,800 cetaceans examined and no tumours were found in approximately 50 beluga whales examined in the Arctic (D.J. St. Aubin, pers. comm. *in* De Guise *et al.*, 1994). In two other studies, neither Stroud and Roffe (1979) nor Howard *et al.* (1983) reported neoplasms from 21 stranded cetaceans and 65 stranded common dolphins (*Delphinus delphis*), respectively. Philo *et al.* (1993) reported that only one (apparently non-malignant) tumour had been found in 130 bowhead whales (*Balaena mysticetus*) examined between 1980 and 1989, but cautioned that the quality of the examinations were not consistent, depending on the personnel present and the prevailing conditions.

However, there are problems in quantitatively interpreting data from each of the above studies. For most, there is insufficient information available on the animals examined, in terms of how they were selected, associated biological data and their species and population identity. Interpretation of the Arctic beluga data is confounded by the fact that the ages of the animals are not known and, as discussed later, age may be a significant factor in cancer incidence.

The present review reveals that the St Lawrence population is the best studied cetacean population (as a proportion of the total population size) with respect to pathological studies.

The other major source of information was the AFIP database. This again clearly cannot be taken as providing a representative sample. It is by its nature confined to diseased animals submitted on a voluntary basis. Reasons for submission will be variable and unpredictable.

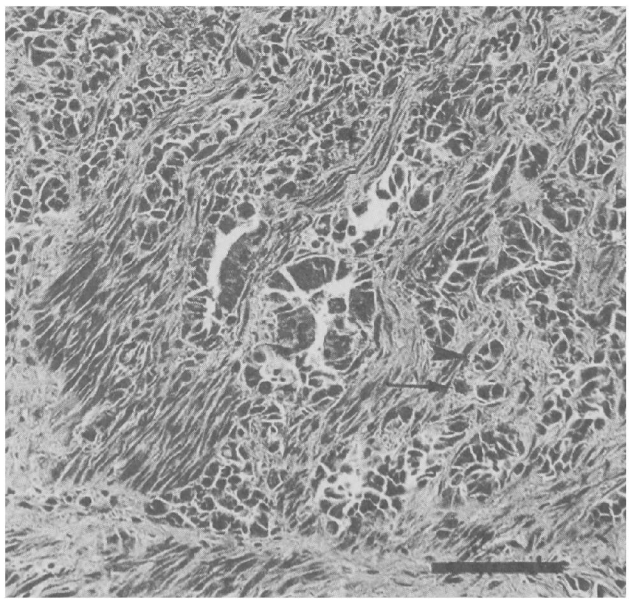

Fig. 4. Small intestinal (duodenal) adenocarcinoma in a beluga whale (DL-7-94). Tumour cells infiltrating the intestinal tunica muscularis tend to form glandular structures. Cellular nests (arrowhead) and individual tumour cells (arrow) are also observed. Note the loss of polarity of tumour cells and their markedly variable shape and size. Bar: 100μm.

Cases are usually sent to the AFIP in order to obtain the opinion of the AFIP's pathologists and/or because the cases are unusual or otherwise of interest. Samples will be biased towards captive animals (of which the number of species is limited and which may also include animals kept for most, if not all of their life in an artificial environment) and stranded animals, and probably the North American region. There are a number of well-known problems in the interpretation of such data. Age data will be of varying sophistication (as witnessed in Table 3 where only one animal is of known age). This is not to suggest that the data are of no value but rather to insert a note of caution into their interpretation.

Cancer incidence in the St Lawrence population

In the 'Materials and Methods' section, a number of assumptions were identified in the calculation of AIR. The validity of these is discussed below.

Can the St Lawrence animals be considered a separate population?

In veterinary, as in human epidemiology, the number of individuals at risk must be known precisely in order to determine the prevalence of disease. This requirement explains why there have been few epidemiological studies of cancer in wild mammals and especially in marine mammal populations, which are notoriously ill-defined and/or widespread.

However, the geographical isolation of the St Lawrence animals in a restricted, relatively small area at the southernmost range of the species means that they can be treated as a separate population (e.g. Bjørge *et al.*, 1994, pp.83-4). This population is believed to have declined from an estimated 5,000 to about 500-1,000 animals (*ibid.*, Table 9; Reeves and Mitchell, 1984), and is confined to a short stretch of the St Lawrence Estuary located downstream of a basin heavily contaminated by industrial pollutants. These whales have been the objects of numerous censuses, using a variety of techniques (Michaud *et al.*, 1990; Kingsley and Hammill, 1991; Michaud, 1993). All recent censuses have provided results within approximately the same range (see Table 4).

Are autopsied animals representative of the total population?

The only criterion used to determine whether a given carcass was autopsied was its state of preservation. Thus, it does not seem unreasonable to assume that the autopsied animals represent an unbiased sample of the total stranded animals.

It is assumed that all carcasses have equal chances of being recovered and are examined whatever the cause of death for the following reasons. Given the restricted range of this population and the fact that all carcasses have been found within that range or downstream as a result of drifting (Michaud *et al.*, 1990; Kingsley and Hammill, 1991; Michaud, 1993), apart from the winter period discussed above, it is probable that almost all deaths will result in detectable strandings. Thus, while some deaths may occasionally escape our attention, our

Table 4

Recent population estimates for the St Lawrence beluga population.

Year	Method	Estimate	SE	95%CI	Source
1988	Photo	491	69		Kingsley and Hammill, 1991
1990	Photo	606	308		Kingsley and Hammill, 1991
1992	Visual	490	n/a		Michaud, 1993
1992	Photo	525	71	410-725	Kingsley, 1996
1995	Photo	705	108	540-1035	Kingsley, 1996
	Average	563			

Table 5

Calculation of AIR for the St Lawrence beluga population, including a crude sensitivity test to some of the assumptions made (see text).

	Pessimistic	'Best'	Optimistic
No. of autopsied animals with cancer	14.00	14.00	14.00
No. of autopsied animals	73.00	73.00	73.00
No. of stranded animals	175.00	175.00	175.00
Correction for poor weather	1.50	1.33	1.00
Estimated total mortality	262.50	233.33	175.00
Estimated no. of cancers in the population	50.34	44.75	33.56
Study period in years	12.00	12.00	12.00
Annual estimated no. cancers	4.20	3.73	2.80
Estimated total population size	500.00	500.00	1,000.00
AIR	839.04	745.81	279.68

sampling is most likely representative of the population in terms of causes and extent of mortality (it is in practice a minimum total mortality). However, the correction used for poor weather may be biased downwards to an unknown degree in that it assumes equal mortality throughout the year, when in fact the poor weather may result in higher mortality during those months, as has been seen in some other odontocete species. The extreme ice and weather conditions found at that time of year effectively break down any carcasses into small pieces. Thus, the possibility that carcasses from animals that died during the winter period may still be available for detection, and thus allowed for twice, can be ruled out.

Sensitivity of the results to the assumptions made

Whilst the estimate of an AIR of about 750 per 100,000 individuals represents our best estimate, it is clear that this is assumption dependant. In order to examine the sensitivity of the estimate to the assumptions we have varied some of the parameter values used as shown in Table 4. The table shows that the AIR is heavily dependent on the assumptions made. The crude sensitivity analysis shown in the table (which only examines questions of weather correction and population size) gives a range of AIR values from about 280-840.

Comparison of St Lawrence Estuary beluga whales with other species/populations

Cetaceans

The present review has shown that 40% of the 35 cancers reported in cetaceans have been found in the St Lawrence beluga whale population. This dramatically high figure clearly needs to be interpreted with caution in view of the limitations of the source material discussed above. However, the cancer prevalence in this population is much higher than that observed in the albeit limited studies of other cetacean populations, including Arctic populations of the same species (D.J. St. Aubin, pers. comm. *in* De Guise *et al.*, 1994).

Cancer is generally a disease of old age and thus a longer life expectancy for St Lawrence beluga whales might explain their high rate of cancer. All animals with cancer in our study were over 16 years. A comparison of the age composition of the 135 stranded St Lawrence whales of known age with that of 412 harvested belugas from the Alaskan population (Burns and Seaman, 1985) shows that there are older animals in the Alaskan population (Fig. 5). However, as one might expect, the stranded population is bimodal, with a peak of newborns and then one of older animals in the 18-26 age group, in contrast to the harvested population which is skewed towards younger animals with a single peak at around 6-8 years followed

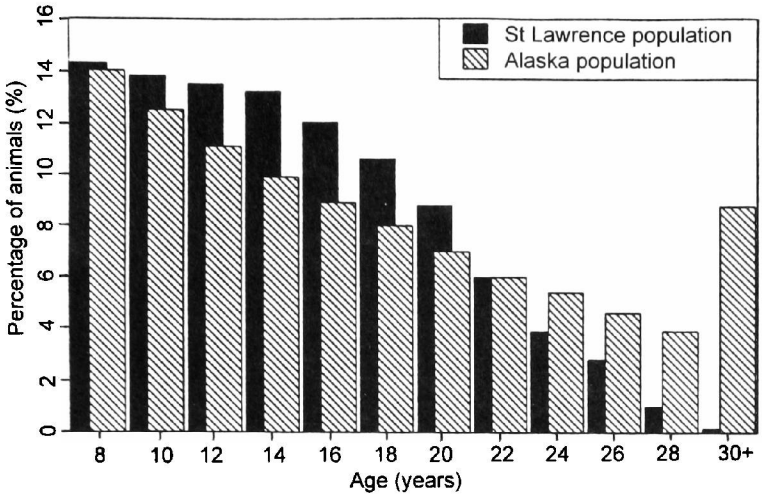

Fig. 5. Comparison of the age structure of the St Lawrence beluga whales (Béland *et al.*, 1988) with the Alaskan population (Burns and Seaman, 1985) according to published life tables.

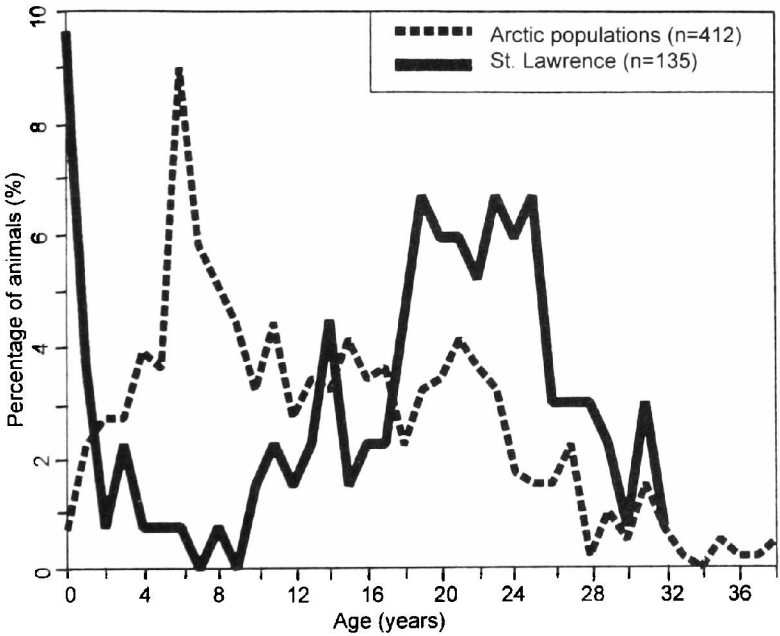

Fig. 6. Comparison of the age distribution of the St. Lawrence Estuary beluga whales found stranded (1983-1994) and the beluga whales sampled by aboriginal hunting in the Arctic (adapted from Burns and Seaman, 1985).

by a general decline (Fig.6). Without knowledge of the age structure of the Arctic population referred to (D.J. St Aubin pers. comm. in De Guise *et al.*, 1994) it is difficult to make a strict comparison of those data with the St Lawrence data that is not potentially confounded by age.

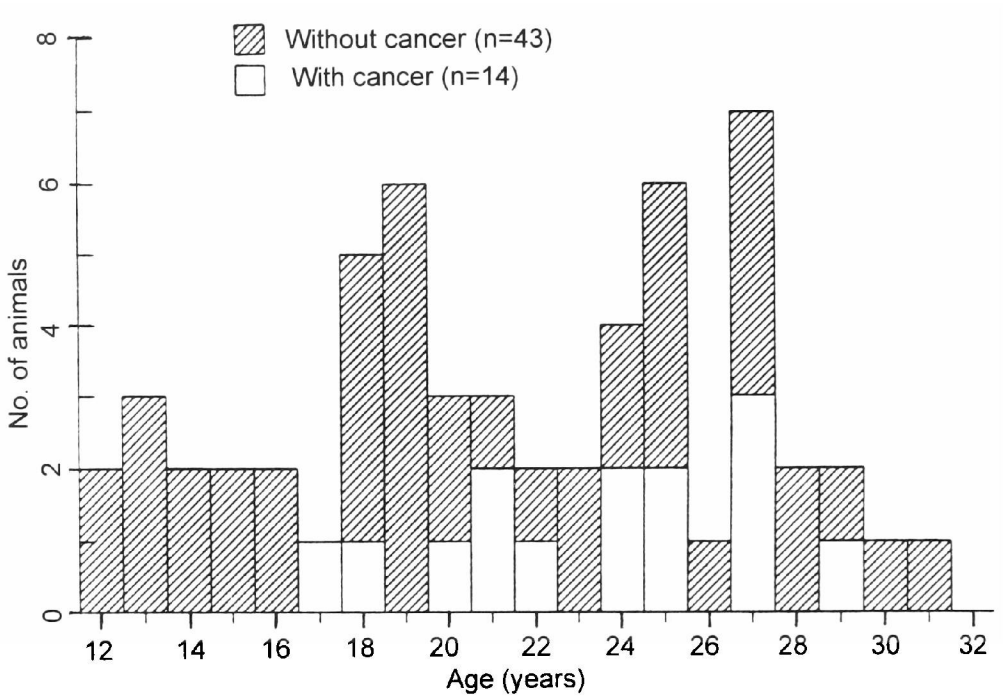

Fig. 7. Age distribution of stranded St Lawrence Estuary beluga whales with and without cancers and older than 12 years (1983-1994).

In mature whales (age 12 and older) with cancer and mature animals without cancers, the mean age of animals with cancer is 23.36 whereas that without is 20.84 (Fig.7). The difference is not significant at the $p > 0.10$ level.

Eight cases of cancers are reported among 834 cetaceans listed in the AFIP's Marine Mammal Database (1%) (Table 3). Whilst this is significantly lower (chi-square = 86.81:1df, $p < 0.01$) than the incidence in the St Lawrence population, the limitations of this database referred to earlier render such statistical tests difficult to interpret in anything more than a general way. It might seem more appropriate to compare the St Lawrence population with only the 14 beluga whales (11 necropsy cases with 1 cancer and 3 surgical biopsy cases with no cancers) included in the database. However, all of the animals were captive and from a variety of populations, and given the small sample size, the fact that there is no significant difference (chi-squared test) between the frequency of cancer (1/11) among the necropsied APIF beluga whales and those from the St Lawrence again does not allow one to reach any firm conclusions.

Other taxa

The populations of domestic species used in epidemiologic studies are animals that were examined at veterinary colleges (Priester and Mantel, 1971; Priester and McKay, 1980). These are probably under better medical care and include more sick animals than the general animal population. In addition, the older animals are more numerous in the pet animal population owing to curative and preventative improvement in veterinary medicine (Dorn and Priester, 1987).

Free-ranging animals generally have a shorter life span than captives because of predation, harsh environmental conditions and malnutrition. Since the risk of developing cancer increases with age, it is reasonable that cancer rates in pet, zoo and aquarium animals would be higher than in free-ranging mammals (Fowler, 1987). Yet, for all cancer types, our 'best' AIR estimate for St Lawrence belugas is much higher than that observed in horses and cats, is slightly lower than the rate observed in dogs and is twice that of man. Even if the 'optimistic' scenario is chosen, the value is comparable with that of cats and horses and close to that of man.

In addition, the rate of gastrointestinal cancers affecting St Lawrence belugas appears much higher than the rate observed in man and domestic species, with the exception of sheep in certain parts of the world. Thus, not only does this whale population appear to have a high prevalence of cancer, but this is particularly marked with regard to adenocarcinomas of the gastrointestinal tract (intestine, stomach and salivary glands); such tumours accounted for half of the malignant tumours found (Table 6).

Table 6

Frequency of cancer in St Lawrence estuary beluga whales over 12 years (1983-1994) compared to that of man and domestic animals. Estimated annual incidence rate of cancer per 100,000 animals (AIR).

	Total cancer	Epithelial cancer of the small intestine	Epithelial cancer of the gastrointestinal tract[1]
Beluga	233 (750)[2]	83 (266)[7] 67 (212)[8]	117 (327)
Man[3]	363.4	0.8	55.6
Cattle[4]	177.2	2.78	2.78
Dog[5]	828.3	6.87[9]	24.3
Cat[5]	257.4	26	29.2
Horse[5]	256.3	0	7.7
Sheep[6]	0.03	up to 2,000	ND

[1]Gastrointestinal tract: glandular stomach, small and large intestine combined. [2]( ): AAIR: adjusted AIR accounting for mortality in winter months and for total mortality. [3]Man (Anonymous, 1991; Anonymous, 1992). [4]Cattle (Priester and Mantel, 1971; Priester and McKay, 1980; Bristol *et al.*, 1994). [5]Dog, cat, horse (Priester and Mantel, 1971; Priester and McKay, 1980). [6]Sheep (Georgsson and Vigfusson, 1973). [7]Five cancers of the small intestine (if the most distal intestinal adenocarcinoma is considered as affecting the small intestine). [8]Four cancers of the small intestine (if the most distal intestinal adenocarcinoma is considered as affecting the large, instead of the small intestine, Martineau *et al.*, 1995). [9]Sum of epithelial cancers listed under 'small intestine' and 'intestine not otherwise specified' in Priester and McKay, 1980.

Several etiologies for the high prevalence of a specific cancer cell type in such a small population can be envisaged.

In cattle, small intestinal cancer results from an interaction between exogenous carcinogens and viruses. Bovine papillomavirus type-4 causes papillomas in the bovine upper digestive tract. In cattle infected with that virus and fed with bracken fern, a plant that contains powerful carcinogens, these benign tumours become malignant and are accompanied by intestinal adenomas and adenocarcinomas that do not contain viral DNA (Campo *et al.*, 1994). thus, viral infection alone does not cause cancer. A similar interplay might be at work in St Lawrence belugas since gastric papillomatosis has been observed in a significant number of carcasses and particles consistent with papillomaviruses have been visualised in papillomas (Martineau *et al.*, 1988; De Guise *et al.*, 1994).

In contrast to their rarity in other animals, high prevalences (0.2-1.58%) of intestinal adenocarcinomas are observed in sheep in some regions of Australia and New Zealand.

Ingestion of environmental carcinogens, particularly phenoxy and picolinic acid herbicides, are thought to be major etiological factors of these endemic cancers (e.g. Dodd, 1960; Webster, 1966; Cordes and Shortridge, 1971; Georgsson and Vigfusson, 1973). Carcinogens are also present in the environment of St Lawrence belugas and are probably ingested by these animals. The Saguenay River is part of the St Lawrence belugas' habitat. Its sediments contain 500-4,500ppb of total polycyclic aromatic hydrocarbons (PAH) (dry weight) (Martel *et al.*, 1986). Benzopyrene DNA adducts were detected in St Lawrence beluga tissues, and were absent from the brain and liver of four Arctic whales (Mackenzie Estuary) (Shugart *et al.*, 1990). Beluga whales, unique among odontoceti in that regard, are known to feed in significant amounts on bottom invertebrates (Vladykov, 1944; 1946). They dive comfortably down to 400 meters (Ridgway *et al.*, 1984). In addition, field observations suggest that these whales dig into sediments (Dalcourt *et al.*, 1992). Considered together, these observations suggest that St Lawrence belugas ingest carcinogenic compounds, which might explain the high rate of digestive tract cancers seen in this population.

This situation would not be without precedent. In bottom-dwelling fish, labial papilloma and liver cancer are strongly associated with chemical contamination of sediments (Harshbarger and Clark, 1990). Interestingly, in humans, gastric cancers and the rare epithelial cancers of small intestines have been associated with the ingestion of smoked food, which are contaminated with benzopyrene (Chow *et al.*, 1993).

That small intestinal cancer might be a feature of beluga whales as a species appears unlikely; the presence of only a single case of cancer (not intestinal) among the 17 beluga whales listed in the Marine Mammal Database and the absence of this condition in the literature do not support this hypothesis.

Inbreeding has led to some degree of genetic homogeneity in St Lawrence belugas (Patenaude *et al.*, 1994). Cancers have not been reported in genetically highly homogenous wild animals such as free-ranging cheetahs and Florida panthers (O'Brien, 1994). In man, rare forms of cancers are known to be inherited (Cotran *et al.*, 1994). However, in free-ranging animal populations there are no reports which document genetic susceptibility to certain types of cancers, although some reports do suggest that such susceptibility concerns free-ranging animals that have been kept in captivity for months or years (Carpenter *et al.*, 1981).

CONCLUSION

This paper has highlighted the limited amount of data available on cancer in cetaceans that can be used in a rigorous quantitative manner. Despite this, it is clear from examining the available data from cetaceans and other mammals, that there appears to be an unusually high incidence of cancer in the St Lawrence beluga population. There is evidence to suggest that this may be related to chemical pollutants but the difficulties of establishing such a cause-effect relationship are well-known and will require a major dedicated research programme (see Reijnders *et al.*, 1999).

Convincing statistics would require much larger numbers of whales and/or the follow-up of St Lawrence belugas for many more decades. These are drawbacks that have long been recognised by cancer epidemiologists and that have been partly solved by studying large numbers of bottom-dwelling fish (Harshbarger and Clark, 1990) or small numbers of dogs in well designed epidemiological studies using age, breed and sex-matched controls. In the latter studies, cancer specific types have been associated with exposure to asbestos and insecticides (e.g. Glickman *et al.*, 1983; 1989; Hayes *et al.*, 1991).

The observation of high cancer prevalences in other populations of marine mammals similarly exposed to carcinogens would strengthen an etiological relation with chemical

carcinogenesis (Fox, 1991). Such observations have recently been made; 65 cases of metastatic carcinomas have been reported in 370 California sea lions stranded alive from 1979 to 1994 along the Californian coast (7.6% of necropsied animals) (Gulland *et al.*, 1996) and in eight adult sea lions out of 82 (9.7% of necropsied animals) in 1993-94 (Holshuh *et al.*, 1995). In both instances, exogenous carcinogens have been considered as possible etiological agents.

The etiological role of contaminants in cancer of marine mammals would be supported by the absence of tumours in stranded animals from non-exposed populations and by the detection of specific, identical mutations in genes such as *ras* or p53 in tumours. The possible contribution of other factors to the etiology of cancer in St Lawrence belugas such as genetic homogeneity and viruses should also be examined.

Acknowledgments

We wish to thank Bruce Williams from the Department of Veterinary Pathology, AFIP, for his help, B. Clausen from the Environmental Investigation Agency, the Ministry of Environment, Government of Denmark and Greg Donovan of the International Whaling Commission for reviewing the manuscript and providing useful comments.

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J. CETACEAN RES. MANAGE. (SPECIAL ISSUE 1), 267–273, 1999

Morbilliviral infections in marine mammals[1]

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ABSTRACT

Epizootics of infectious disease were unknown in cetaceans prior to 1987. However, since then there have been at least three epizootics in dolphins and two in pinniped species. Many of the clinical, pathological and epidemiological features of these events were similar to those of morbilliviral infections in terrestrial mammals. There has been speculation that contaminants may have predisposed marine mammals to these and this is discussed. Morbilliviruses are highly pathogenic viruses and caused epizootics in terrestrial mammals long before the advent of anthropogenic contaminants.

KEYWORDS: POLLUTION; DISEASE; EPIZOOTICS; IMMUNOSUPPRESSION; PATHOLOGY; PINNIPEDS; STRIPED DOLPHIN; HARBOUR PORPOISE; BOTTLENOSE DOLPHIN; WHITE WHALE; REVIEW

REPORTED INFECTIONS

Recognised morbillivirus infections had not been reported in aquatic mammals prior to 1987, but since then there have been a number of epizootics of morbilliviral disease in cetacean and pinniped populations in several regions of the world (e.g. Simmonds, 1992).

Pinnipeds

An epizootic of morbillivirus infection killed approximately 18,000 harbour seals (*Phoca vitulina*) and several hundred grey seals (*Halichoerus grypus*) in Europe in 1988 (Kennedy *et al.*, 1988b; Osterhaus and Vedder, 1988; Dietz *et al.*, 1989; Bergman *et al.*, 1990; Kennedy, 1990). This die-off apparently began along the Baltic coast in April of that year and subsequently spread to seal colonies along the North Sea coasts of Norway, Sweden, Denmark, Germany, The Netherlands, the United Kingdom and Ireland. It appears to have terminated in late 1988 although a small localised outbreak occurred in northern Norway in late 1989 (Krogsrud *et al.*, 1990). In addition, a morbilliviral epizootic killed several thousand Baikal seals (*Phoca sibirica*) in Lake Baikal from late 1987 to late 1988 (Grachev *et al.*, 1989).

Laboratory studies (Cosby *et al.*, 1988; Curran *et al.*, 1990; Blixenkrone-Moller *et al.*, 1992; Rima *et al.*, 1992) indicated that the morbillivirus which infected European seals was a newly recognised virus (the phocine distemper virus, PDV). Similar studies of a virus isolated from Lake Baikal seals (Osterhaus *et al.*, 1989; Visser *et al.*, 1990; Barrett *et al.*, 1992) indicated that it was a strain of canine distemper virus (CDV). No epidemiological link could therefore be established between the Siberian and European epizootics.

[1] This paper was originally submitted to the IWC Scientific Committee as SC/M95/P15.

Cetaceans

The first evidence of morbillivirus infection in cetaceans emerged from the coast of Ireland during the 1988 European seal epizootic. At that time a morbillivirus disease was diagnosed in six harbour porpoises (*Phocoena phocoena*) found stranded on the coast of Northern Ireland (Kennedy *et al.*, 1988a; 1991). Morbilliviral disease was subsequently found in a few harbour porpoises found stranded on the coasts of England, Scotland and The Netherlands in 1990 (Kennedy *et al.*, 1992; Visser *et al.*, 1993). As the porpoises from the coast of Northern Ireland were found in a region inhabited by morbillivirus-infected harbour seals, it was originally believed that interspecific transmission of PDV had occurred. However, the porpoise morbillivirus was subsequently isolated and characterised as another newly discovered morbillivirus distinct from PDV (McCullough *et al.*, 1991; Welsh *et al.*, 1992; Barrett *et al.*, 1993; Visser *et al.*, 1993; Blixenkrone-Moller *et al.*, 1994).

A die-off of striped dolphins (*Stenella coeruleolba*) began along the Mediterranean coast of Spain in July 1990 (Domingo *et al.*, 1990; 1992; Duignan *et al.*, 1992). This epizootic rapidly spread to other areas of the Spanish and French Mediterranean coasts and probably also to the coasts of Morocco and Algeria. It subsided in late 1990 but re-emerged along the southern coast of Spain between June and September 1991 and eventually reached the southern Adriatic Sea, Ionian Sea, Sicilian Channel and southern Tyrrhenian Sea. A third outbreak occurred in the region of the Greek Islands in early 1992. At least several thousand animals are believed to have died during these Mediterranean outbreaks (Di Guardo *et al.*, 1992; 1995; Aguilar and Raga, 1993). Laboratory characterisation of a morbillivirus isolated from affected dolphins in the western, central and eastern regions of the Mediterranean Sea indicated that all three outbreaks were phases of a single epizootic (Van Bressem *et al.*, 1993).

It has been suggested that the northwestern European harbour porpoise and Mediterranean striped dolphin viruses may represent two distinct strains of the same morbillivirus (Bolt and Blixenkrone-Moller, 1994). Antigenic and genomic analyses indicate that the viruses isolated from these cetaceans are distinct from other morbilliviruses including the newly recognised PDV (Curran *et al.*, 1990; 1992; McCullough *et al.*, 1991; Welsh *et al.*, 1992; Barrett *et al.*, 1993; Van Bressem *et al.*, 1993; Visser *et al.*, 1993; Blixenkrone-Moller *et al.*, 1994; Bolt and Blixenkrone-Moller, 1994). Although they cause a distemper-like disease in harbour porpoises and striped dolphins (Kennedy *et al.*, 1988a; 1991; 1992; Domingo *et al.*, 1992; Duignan *et al.*, 1992), the cetacean morbilliviruses appear to be more closely related to rinderpest, peste-des-petits-ruminants and measles viruses than to CDV and PDV, which comprise the distemper subgroup of morbilliviruses (Visser *et al.*, 1993).

From June 1987 until May 1988, hundreds of Atlantic bottlenose dolphins (*Tursiops truncatus*) died along the eastern coast of the United States. Strandings commenced along the coast of New Jersey and eventually spread to the Atlantic coast of Florida. It has been estimated that more than 50% of the inshore population of bottlenose dolphins in this region died (Federal Register, 1993).

An initial investigation concluded that brevetoxin produced by the 'red tide' marine dinoflagellate *Ptychodiscus brevis* was the main cause of the die-off (Geraci, 1989). However, a recent study of tissues from affected animals revealed the presence of morbillivirus infection and associated lesions in more than 50% of animals examined (Lipscomb *et al.*, 1994b).

The most recent known epizootic of morbillivirus disease in cetaceans occurred among bottlenose dolphins in the Gulf of Mexico from June 1993 to mid-1994. This mortality event evolved slowly but eventually affected dolphins from Florida to Texas. The full extent of dolphin mortality in this incident is unknown (Lipscomb *et al.*, 1994a).

Virus isolation or genomic analysis of the morbillivirus present in tissues of bottlenose dolphins from the western Atlantic and Gulf of Mexico epizootics has not been achieved. It is therefore not yet possible to determine the relationships of the morbillivirus or morbilliviruses in these populations to those affecting the harbour porpoise and striped dolphin populations referred to above.

EPIDEMIOLOGY OF MARINE MAMMAL MORBILLIVIRUSES

Epizootics of morbillivirus infection are believed to have occurred in terrestrial species from antiquity. Measles epidemics in humans date back to the early years of this millenium and waves of rinderpest have raged in large ruminant populations in Africa, Asia and Europe for centuries (Norrby and Oxman, 1990; Scott, 1990). Mortality rates have frequently approached 100%. The epidemiology of these events and our knowledge of experimental morbilliviral infections in animals indicate that these viruses are highly pathogenic agents capable of causing very high mortality in susceptible populations. Since morbilliviral epidemics in terrestrial mammals obviously pre-date the manufacture of organochlorine compounds, it is apparent that these viruses can cause disease outbreaks of epidemic proportions in the absence of contaminants. The pathology of morbilliviral infection in aquatic mammals has been well documented and is very similar to that in terrestrial mammals (Kennedy *et al.*, 1989; Norrby and Oxman, 1990; Domingo *et al.*, 1992; Lipscomb *et al.*, 1994b).

Extrapolating from our knowledge of the epidemiology of morbillivirus infections in terrestrial mammals, morbillivirus epizootics in marine mammals are likely to have resulted from the introduction of morbilliviruses to previously unexposed and therefore immunologically naive populations. Direct contact with an infected animal appears to be the probable method of introduction to a new population. Morbilliviruses frequently cause epizootics in susceptible host species (Norrby and Oxman, 1990; Scott, 1990). They are highly infectious and are excreted in large numbers by many routes including respiratory aerosol, and via a range of body secretions and excretions. Although they are relatively unstable in the environment, their low minimum infectious dose results in a high transmission rate provided the population density exceeds a minimum threshold value. It is therefore not surprising that such viruses can cause major epizootics in marine mammal species.

In non-exposed populations of terrestrial mammals, morbillivirus outbreaks usually affect individuals of all ages, while in those with previous exposure to the virus they predominantly affect young individuals with less developed immune systems (Hoffman, 1983). In the North Sea harbour seal epizootic and the Mediterranean striped dolphin epizootic, mortality centred on adult individuals as well as juveniles. The cause for apparent discrepancy in age-specific mortality in marine mammals between some areas remains unknown, although behavioural factors that would result in a lower exposure of juveniles to the virus have been suggested (Härkönen and Heide-Jørgensen, 1990; Calzada *et al.*, 1994).

IMMUNOSUPPRESSION IN MORBILLIVIRUS-INFECTED ANIMALS

Lymphoid, epithelial and central nervous system tissues are the major host targets for morbilliviruses. Marked damage to lymphoid tissues has been demonstrated in a wide range of morbillivirus-infected terrestrial and marine mammals including cetaceans (Norrby and Oxman, 1990; Kennedy *et al.*, 1991; Domingo *et al.*, 1992; Duignan *et al.*, 1992; Lipscomb *et al.*, 1994b). The effects of this damage in natural infections are difficult to quantify but clinically significant immunosuppression in morbillivirus-infected marine mammals is

evidenced by the destruction of lymphoid tissues and an increased incidence of secondary fungal, bacterial, protozoal and parasitic infections (Kennedy *et al.*, 1989; 1991; Baker and Raga, 1992; Domingo *et al.*, 1992; Duignan *et al.*, 1992; Lipscomb *et al.*, 1994a; b).

Our understanding of the epidemiology of the aquatic mammal epidemics is incomplete, but the rapid spread of infection and high mortality rates mirror morbilliviral infections in terrestrial animals. These similarities suggest that it is likely that the cause of the recent epizootics in marine mammals was the introduction of a virus into previously unexposed and therefore susceptible populations.

Although there is no evidence that contaminants facilitated recent morbillivirus epizootics in marine mammals, it is well known that organochlorines, including polychlorinated biphenyl compounds (PCBs), accumulate to high concentrations in tissues of marine mammals. These compounds have been demonstrated to reduce immune function in several terrestrial mammalian and avian species (Busbee *et al.*, 1999). Furthermore, altered *in vitro* indices of immune function have been demonstrated experimentally in harbour seals fed fish from environmentally-contaminated waters (De Swart *et al.*, 1994; 1995; Ross *et al.*, 1995) and *in vitro* tests on bottlenose dolphin line cells have shown the capability of organochlorines to induce immune dysfunction in cetacean cells (Busbee *et al.*, 1999). It is therefore likely that such substances could produce immunosuppression in wild marine mammals.

Several studies have attempted to identify a relationship between tissue levels of PCBs and mortality due to morbillivirus infection in marine mammals. For example, higher levels of PCBs were reported in tissues of seals that died during the 1988 European morbillivirus epizootic than in survivors (Hall *et al.*, 1992), whilst exceptionally high concentrations of PCBs were found in tissues of striped dolphins that died during the die-off in the Mediterranean Sea in 1990 compared to concentrations in tissues of this species in years prior to and after this epizootic (Aguilar and Borrell, 1994). Tissue concentrations in bottlenose dolphins that died during the morbillivirus epizootic along the eastern coast of the USA in 1987 and 1988 were also considered to be high although comparisons could not be made with levels in tissues of surviving dolphins (Kuehl *et al.*, 1991). However, under experimental conditions, exposure to PCBs did not increase the susceptibility of seals to morbilliviral disease (Harder *et al.*, 1992).

In essence, these studies provide quantitative data on tissue pollutant concentrations and an indication that some pollutants cause alterations in *in vitro* indices of immune function. However, there is no evidence that they have affected mortality or morbidity due to morbilliviral infection. It should be recognised though, that discovery of a cause and effect relationship between tissue concentrations of contaminants and morbillivirus mortality in these epizootics would be difficult. As morbilliviruses cause direct damage to the immune system and are therefore likely to be a major cause of mortality in infected animals, it is clear that such immunosuppression and any resulting from contaminants will be difficult to quantify separately.

Detection of changes in the prevalence of such diseases can best be achieved by postmortem examination of affected individuals and subsequent correlation of lesions with tissue contaminant concentrations or biomarkers of the toxic effects of these substances. It is currently impossible to separate any possible immunosuppressive effects of contaminants from those due to morbillivirus infection in aquatic mammals that have died as a result of natural morbillivirus infections.

Morbilliviral epidemics are therefore unlikely to be the ideal scenario for investigating possible immunosuppressive effects of contaminants in marine mammals. Such effects are more likely to be insidious and manifest as an increase in susceptibility to neoplasms and diseases caused by organisms normally less pathogenic than morbilliviruses. As in human

immunodeficiency virus infection and in morbilliviral infections in mammals, immunosuppression is associated with an increased incidence of opportunistic fungal, protozoal, bacterial and viral infections. An increase in the rate of neoplasms in a contaminated population, as reported in white whales (*Delphinapterus leucas*) in the St Lawrence Estuary (Martineau *et al.*, 1988), may also be an indication of immunosuppression.

Many morbilliviral-infected bottlenose dolphins and striped dolphins were found in poor body condition (Geraci, 1989; Domingo *et al.*, 1992). Mobilisation of blubber reserves in these animals probably resulted in increased plasma concentrations of organochlorines and a consequently increased risk of toxicity from these compounds. Mortality and morbidity during morbilliviral epizootics may therefore have been higher in dolphins with high body burdens of lipophilic contaminants than in those with lower tissue levels. However, given the lethal effects of morbilliviruses, it is this author's view that it is unlikely that organochlorine toxicity had anything other than a marginal effect on mortality.

In conclusion, the recent epizootics of morbilliviral infection in marine mammals are likely to have resulted from introduction of morbilliviral infection into susceptible populations. The high mortality reported in many of these die-offs is consistent with our knowledge of morbilliviral infections in terrestrial mammals. Although epizootics of infectious disease had not been reported in aquatic mammals prior to 1987 and many of the animals involved in recent die-offs had relatively high tissue concentrations of organochlorines, there is no evidence that contaminants contributed to increased mortality in these populations. However, the increasing data on potentially significant deleterious effects of contaminants on marine mammal health, i.e. immunosuppression, warrants further investigation.

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ISSN 1561-073X