Life History Parameters and Polychlorinated Biphenyls (PCBs) in Long-finned Pilot Whales *(Globicephala melas)* from New Zealand Strandings

A report to WWF-New Zealand, June 1998

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Key Points

- A first-time study of PCB levels and life-history parameters in long-finned pilot whales (*Globicephala melas*) from strandings around New Zealand revealed that all individuals sampled carried a mean PCB burden of 311 ng/g (pts per billion) and a range of 33-931 ng/g in the blubber. This is 2-3 orders of magnitude lower than in Northern Hemisphere pilot whales.

- The high variability in the PCB levels was seemingly related to differences in sex, age and sexual maturity.

- The middle order feeding level of this toothed whale (odontocete), for the most part in the open ocean, is suggested as an explanation for the low level of PCB burden, derived from inferred relatively low environmental concentrations in the Southern Ocean.

- Life history parameters of age, growth and reproductive status, described for the first time for this species in the Southern Hemisphere, closely match those of the Northern Hemisphere population i.e. 14.3 yr and 494 cm age and body length at sexual maturity for males and 8.7 yr and 379 cm for females.

- Males accumulate PCBs throughout whole of life; females show an increase prior to maturity, then a decrease throughout active parturition due to unloading of PCBs to their calves, and then increase again post-parturition.

- The male PCB age trend indicated that in those that are first-born progeny, PCB levels and congeners were exceptionally high and distinctive respectively.

- These whales appear to exhibit a limited capacity to metabolise particular PCB congeners.

- Though actual PCB levels were highly variable, the actual congener profiles were remarkably consistent in all individuals sampled, as in other NZ open ocean cetaceans, pointing to exposure to a common PCB contaminant source.

- Continuity of the PCB contamination profile over time, space and species suggests a distant source for these compounds, rather than from local emissions. Atmospheric transport is likely to be the mode of dissemination of some of the more volatile PCB congeners.

- The relatively low levels of PCB contaminants appear to be below those that result in adverse health effects of a dioxin-like nature. However, a PCB dose-effect relationship and sensitivity to these compounds has yet to be quantified.

- When coupled with biological information such as age, sex and reproductive status, PCB levels and profiles can usefully contribute to assessing the well-being of long-finned pilot whale populations. However, though this finding is novel for this species, a longer-term study, with a larger data base and including other similar species is required.

- This study exemplifies the fact that knowledge of the biology and human impact on cetaceans, though good for some species, is fragmentary for others, such as the rarer oceanic toothed and baleen whales.

- Long-finned pilot whales and possibly other oceanic species clearly have value as pollution bioindicators in the Southern Ocean, provided that the fullest use practicable is made of data available at mass strandings of these animals.

- The conservation of such species should therefore be given more attention for that reason, and not only for the reason of protection of these species for their own sake as widespread great whales. This cause would benefit from greater public awareness of the ubiquitous nature of PCBs, their biological significance, especially for marine mammals, and that New Zealand is not isolated from distant as well as immediate sources of these and like contaminants.

CONTENTS

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INTRODUCTION

Polychlorinated biphenyls (PCBs), are one type of the many persistent organic pollutants (POPs) of anthropogenic origin that contaminate the environment worldwide (Kimbrough, 1980). They are toxic, resistant to biodegradation and readily accumulate in fatty tissues. In doing so, they are known to cause significant health threats to many animals, particularly marine mammals. These threats include reproductive, carcinogenic and immunosuppressive effects.

In the ocean, which is a major repository for these contaminants, PCBs accumulate to the highest levels in top predators, such as toothed whales, dolphins and seals and sealions (Boon *et al.* 1994). As such, these species can serve as bioindicators of marine pollution. They have been used in that way in the Northern Hemisphere, through extensive studies of cetaceans in particular, but little in the Southern Hemisphere (Tanabe *et al.* 1994, Muir *et al.* 1996a). PCBs have, however, been detected in low amounts in the New Zealand marine environment (Day, 1996, Jones *et al.* in press), but further study to establish the actual scale of the contamination is clearly warranted.

The choice of marine mammal species as suitable and reliable bioindicators for study purposes is largely a matter of access to, and adequacy of samples (Muir and Norstrom 1991). Some accessible species, namely those that live on or near shore (pinnepeds, dolphins) have proven to show relatively high levels of PCBs (Jones *et al.* 1996). This could be expected, since the sources of these contaminants are likely to be close at hand and the species feed on organisms at higher trophic levels at which there is bioaccummulation of contaminants. The levels and distribution of pollutants in the open ocean is not so readily determined, since those mammals whose lives are spent far offshore are generally less accessible for study, especially since the establishment of moratoria on cetacean capture of many species of cetaceans.

The usefulness of mass strandings of oceanic whale species as a source of information on oceanic contamination by PCBs is therefore clear. The long-finned pilot whale species, *Globicephala melas*, a toothed whale or odontocete, is one of the few whale species that strands in numbers, as many as 200 on occasion, and typically in pods of mixed ages and sexes (Evans 1987). This fact, coupled with knowledge that PCB contaminant levels in the Southern Ocean generally, and in oceanic marine mammals specifically, have been poorly known and understood, largely precipitated the study presented here. Of immediate relevance in this context was that pilot whales and their PCB levels had been extensively studied and reported on in the Northern Hemisphere (Taruski *et al.* 1975, Alzien and Duguy

1979, Martin *et al.* 1987, Muir *et al.* 1988, Simmonds *et al.* 1994, Borrell *et al.* 1995) and comparative information was thereby available.

The determination of actual PCB and other contaminant levels is of initial importance for its own sake and for comparative purposes in general. However, the implications of this for the health of marine mammals that carry a contaminant load are ultimately of the greater concern, and of significance also in life history studies. It has been shown, for example, that a portion of the PCBs accumulated by the female parent are unloaded to the offspring by way of the milk (Borrell *et al.* 1995). That being so, fluctuations in PCB levels can be an index to female reproductive status and thus contribute overall to an understanding of life history. PCBs progressively accumulate in body tissues with age and appear also to accumulate differentially in the sexes and with the degree of maturity. PCB contaminant profiles can provide a lead to the mechanism(s) by which long-finned pilot whales metabolise specific PCB congeners and in this way offset to some degree the effects of bioaccumulation and biomagnification.

In general terms, this study reports on aspects of PCB contamination in long-finned pilot whales from the Southern Hemisphere for the first time. Additionally, through accompanying observations from the available study material, life-history parameters of this species in the Southern Hemisphere are given and compared with those from the Northern Hemisphere.

In greater detail the scope of this study encompasses the following:

1) PCBs, their nature, usage, toxicity, distribution and fate in the environment; the long-finned pilot whale as a study species, its ecology in New Zealand, and as a source for data on PCBs in an oceanic species; study protocols and logistics for collection and analysis of PCBs and life history information; and problems of analysis;

2) The determination of age, growth and reproductive parameters and associated methodology;

3) The degree of PCB accumulation and the relation of these contaminants to age and sex, including trends and significance;

4) The profile of the various PCB congener accumulation and its toxicology and implications;

5) The suggested conservation management applications of the study and their significance in NZ and worldwide.

PCBS AND THEIR ENVIRONMENTAL SIGNIFICANCE

The Nature of PCBs

The term "PCBs" is a generic one referring to a group of synthetic organic compounds with the general formula $C_{12}H_{10-n}Cl_n$ where n is the number of chlorine atoms within the range 1-10 (Kimbrough 1980). Chlorine atoms are substituted in a variety of positions in a biphenyl- ring to give up to 209 possible congeners, each with its own properties. They were first synthesised late last century, then produced commercially, and subsequently marketed worldwide under a variety of trade names (Kimbrough 1980). Production peaked between the late 1950s and early 1970s.

The toxicity of PCBs varies according to their particular chemical structure, especially the number and position of the chlorine atoms (Safe 1990). The so-called 'coplanar' congeners are the most toxic, resembling in structure the highly toxic 2, 3, 7, 8 - tetrachlorodibenzo - *p* - dioxin (TCDD). Of the 20 possible coplanar PCBs, numbers 77, 126 and 169 (IUPAC numbers) are the most toxic and along with 2, 3, 7, 8 - TCDD have been shown to produce toxic effects in experimental animals (Ahlborg *et al.* 1994).

PCBs are both chemically and physically highly stable and degrade very slowly in the environment (Erickson 1986). They are virtually non-flammable, highly soluble in fats and organic solvents and have a low solubility in water. They have a high boiling point and a high dielectric constant, making them good insulators. These unique properties endow PCB mixtures with versatility in commercial terms, having found widespread application in transformers, hydraulic fluids, lubricating and cutting oils, fire retardants and pesticides, and as plasticisers in plastics, adhesives, textiles, surface coating, sealants, varnishes, paints, printing inks and copy papers.

PCBs in the Physical and Biological Environment

These compounds are also significant contributors to environmental contamination and have precipitated widening public concern since the late 1960s (Jensen 1966, Kuratsune *et al.* 1972), resulting in their restricted use and eventual prohibition on manufacture. Although most production stopped during the 1970s, some continued to at least 1983 (Erickson 1986). World estimated total production to date has been 1.2 million tonnes (WHO 1993). Despite the above restrictions, significant quantities of PCBs are still in use worldwide, posing a continuing risk of environmental pollution. Highest contamination, gross in places, has been observed in the highly populated and industrialised regions of the Northern Hemisphere, often those closest to the ocean, estuaries, rivers and lakes (Tanabe *et al.* 1988). Contamination generally decreases with increasing distance from the source, though paradoxically, relatively high PCB levels have been detected in the Arctic and Antarctic.

The ocean acts as the final repository for PCBs liberated elsewhere, with highest contamination in the mid-latitudes of the Northern Hemisphere, the major source area of production and use (Tanabe and Tatsukawa 1986). Contamination is apparently less in the Southern Ocean. This trend has also been observed in other oceanic and atmospheric components of air, plankton, fish and marine mammals. Further to this, there has been a southward shift of PCB usage to developing countries of the tropics, which are increasingly acting as major emission sources (Tanabe *et al.* 1994).

The persistent and lipophilic ('fat loving') nature of PCBs allows them to accumulate in the fatty tissues of organisms. PCBs in aquatic organisms lower in the food chain such as plankton, crustaceans, shellfish and some fish can, by exchange across the gills and body surfaces, generally balance PCB levels between their body lipids and the surrounding water (Tanabe *et al.* 1988). Those of higher trophic levels such as larger fish and marine mammals do not have this capacity. As a result, PCBs are progressively accumulated by the ingestion of food already contaminated with PCBs (Kimbrough 1980, Boon *et al.* 1992). This is the process of bioaccumulation. An accompanying process is biomagnification by which contaminant concentrations are increased with each successive step in the food chain (Boon *et al.* 1992).

The amounts and nature of PCBs in these higher organisms further depends on their ability to metabolise these contaminants. Mammals, birds and some fish species can metabolise and eliminate the more water soluble, less lipophilic PCBs and hence they tend not to accumulate (Kimbrough 1980, Boon *et al.* 1992). In contrast, the less water soluble and more lipophilic PCBs are less able to be metabolised and more readily accumulate. This is especially so for organisms having a longer life span.

Much attention has been focussed on pollutant contamination in marine mammals. They can accumulate PCBs to high levels, principally because they occupy upper trophic levels, possess much lipid materials, have a relatively long life expectancy, but also a comparatively low capacity to metabolise and eliminate these compounds (Boon *et al.* 1994). Moreover, the PCB contaminated lipids in the mother's milk can be passed to offspring during the characteristic lengthy lactation (Borrell *et al.* 1995).

Elevated levels of PCBs in various marine mammals have been associated with reproductive failure and sterility (Helle *et al.* 1976a, Helle *et al.* 1976b), premature births (De Long *et al.* 1973), uterine stenosis and occlusions (Helle *et al.* 1976b), reduced testosterone levels (Subramanian *et al.* 1987), impaired immune function (Ross *et al.* 1995), skull bone lesions (Bergman *et al.* 1992) and tumours (De Guise *et al.* 1994). The exposure of the animal to a wide variety of marine pollutants complicates efforts to establish a proven cause-effect relationship between observed PCB levels and apparent toxic effects. However, epidemiological evidence is building up.

Though quantitative information on PCBs in New Zealand is lacking, it is clear that significant quantities of PCBs have been used in the electrical supply industry here and to a lesser extent as heat transfer and hydraulic fluids, plasticisers and as lubricants in vacuum pumps (Ministry of Health 1994). Quantities imported or still held may have been as high as 680 tonnes (the least conservative figure - OECD 1987) or much lower at about 225 tonnes (PCBs Core Group, Hazardous Wastes Task Group 1988). Leakages of, or incidents concerning PCB containing materials have been reported, and considerable quantities of PCBs have been regularly disposed of in landfills (PCBs Core Group, Hazardous Wastes Task Group 1988).

Environmental contamination by these compounds in New Zealand has been known of as early as 1976 and have since been detected in fish, birds and mammals, including humans, as well as in soil and marine sediments (Solly and Shanks 1974, Solly and Shanks 1976, Fox *et al.* 1988). The more recent reports by Hannah *et al.* (1993), Jones *et al.* (1996) and Jones *et al.* (in press) describe PCB levels in New Zealand cetaceans and have thus been the most relevant to the present study.

The tendency for marine mammals to accumulate PCBs and carry high burdens of these environmental contaminants make these animals suitable bioindicators or sentinel species with which to monitor marine pollution (Muir and Norstrom 1991). They have, in fact, been used in this capacity in the Northern Hemisphere and figure prominently in pollution studies.

THE RELEVANCE OF PILOT WHALES TO THIS STUDY

There are two species of the odontocete (toothed whale) genus *Globicephala: G. melas* (the long-finned pilot whale) and *G. macrorhynchus* (the short-finned pilot whale). The two are different in distributional range, size, external markings, length of flippers, size of tail flukes, tooth count and skull characteristics (Evans 1987). However, for all practical purposes it is the flipper size and body markings that are most useful for identification. For the long-finned pilot whale there are some differences in body markings between individuals of the Northern and Southern Hemispheres, which perhaps merits their recognition as subspecies, but this distinction is not currently recognised (Evans 1987).

Long-finned pilot whales occur widely in the cold-temperate waters of the North Atlantic Ocean from Greenland, Iceland and the Barents Sea in the north, Cape Hatteras in the west and northwest Africa (including the Mediterranean) in the east (Evans 1987). In the Southern Hemisphere they occur mainly north of the Antarctic Convergence in the cold currents (Falkland, Humboldt and Benguela) associated with the West Wind Drift. These whales migrate seasonally, apparently in response to changes in prey distribution and water temperature (Zachariassen 1993). Inshore movements have been observed in the Northern Hemisphere over summer and outside the continental shelf in winter. There is a north-south seasonal movement of Southern Hemisphere whales into the Antarctic (Evans 1987).

Pilot whales are extremely social and show strong herding behaviour, swimming in large pods of between 40-200 animals (Sergeant 1962, Zachariassen 1993). The pods usually contain animals of various sizes, ages and both sexes. Though essentially oceanic, pilot whales enter coastal waters from time to time in pursuit of prey, squid being the preferred item (Desportes and Mountsen 1993). However, fish and other organisms may prevail in the diet when squid are scarce.

Pilot whales are sexually dimorphic. At maturity, which is at ca. 12 yr, males are 4-5 m in body length, though they may reach 6.2 m; females mature at 6-13 years and at length of 3-4 m (Sergeant 1962, Kasuya *et al.* 1988, Bloch *et al.* 1993). Males live up to 50 yr, while females may live more than 60 yr.

The long-finned pilot whale is one of the few cetaceans that tend to mass strand. Their tight social cohesion is a likely important factor for pods to strand and to re-strand after being refloated by human-initiated efforts (Sergeant 1982). Animals tend to strand on gently sloping beaches and in large numbers of mixed ages and sexes. Strandings are one of the sources of data for life-history and pollution studies.

Historically, mass strandings of long-finned pilot whales occur frequently on the New Zealand coast though the reason(s) why they do so remain a matter of conjecture (Brabyn 1991). From the 1840s to 1991, 148 strandings involving 6331 individuals were recorded and between 1989 and 1993 there were 43 strandings of 1099 individuals (National Whale Stranding Database 1989-1993). Strandings occur year round but more often over the summer period. Whangarei Harbour, Mahia Peninsula, Golden Bay and the Chatham Islands appear to be particularly prone to strandings and could be referred to as stranding ‘hot-spots’.

Long-finned pilot whales have been exploited by communities of the North Atlantic, who for centuries have herded them into bays where large numbers could be beached and killed (so-called drive-fisheries) (Evans 1987). These activities ceased in eastern Canada in 1972 but have been continued in the Faroe Islands as a traditional year round subsistence hunt taking around 800 whales annually (Zachariassen 1993).

The drive-fishery catches, together with mass strandings, have provided extensive information on the biology of long-finned pilot whales. Data from the international research programme on these whales, conducted from July 1986 to July 1988 and based on Faroe Islands catches, included those on external characteristics, age and growth parameters, genetics, and social organisation, reproductive biology, food and feeding, energetics, pollution and parasitology (Donovan *et al.* 1993).

Mass stranded and harvested animals have also provided apparently reliable information on PCB contamination, though the studies involved only the Northern Hemisphere populations. The observed PCB levels ranged from very low (0.5 ng/g, Muir *et al.* 1988) to very high (189 ng/g, Alzieu and Duguy, 1979). Some data are available for age and sex accumulation trends, indicating that females transfer substantial amounts of PCBs to their offspring at birth, and during subsequent lactation which is relatively long (mean 40.2 months) (Martin and Rothery 1993, Borrell *et al.* 1995).

THE STUDY PROGRAMME

Study Protocols and Logistics

The study schedule for the research described here resolved itself into five phases: the familiarisation with marine mammal dissection skills (with Department of Conservation staff and veterinarian at Massey University, November 1994); the collection of samples from two mass strandings (February 1995 - February 1996) with the addition of further material taken by DoC staff at strandings from December 1992 to February 1995; familiarisation with analytical skills and the actual PCB analysis (at Institute of Environmental Science, May 1997 to June 1997) and finally the preparation of tooth and reproductive samples (at Victoria University of Wellington, June to November 1997).

Field sampling, health and safety protocols were developed in consultation with DoC and Museum of New Zealand staff and an experienced health nurse and carried out under DoC permit. The principal tissues sampled were blubber (for PCB levels), teeth (for age) and reproductive organs (for sexual maturity status). Only carcasses of ‘good condition’ were sampled, following the guidelines of the Smithsonian Institution Scientific Event Alert Network (Geraci and Lounsbury 1993). A strict procedural order was followed for each carcass, beginning with an overall assessment of condition, through recording of sex, measurement of body length, sampling of blubber, teeth samples, testis slices and ovary collection. Subsequently, the samples were frozen or formalin preserved for study and analysis.

The sex of the individual pilot whales was determined from the configuration of the genital fold: either the genital and anal openings are separated by a bridge of tissue, as in males, or the closer-spaced openings in females are encompassed by a more prominent genital fold and flanked by small mammary slits. Ages were determined from annual growth layer groups (GLGs) in the teeth, as revealed by decalcified and stained longitudinal sections. The degree of sexual maturity was determined from histology of the reproductive organs: ovaries were screened for corpora, thus indicating previous ovulation, and testis sections were examined for developmental stages of spermatogenesis.

PCB levels were determined from blubber samples by ultra-trace congener-specific analysis. This involved complex extraction and clean-up procedures. These principally included, in order, ASE extraction, purification by DCM/H_20 and H_2SO_4/ H_20 partitioning, absorbent column chromatography, fractioning into coplanar and non-coplanar congeners, and separate analysis of the fractions for PCBs by High Resolution Gas Chromatography-High Resolution Mass Spectrometry.

Sources and Reliability of Data in Cetacean Contaminant and Life History Monitoring Studies

Cetaceans can provide much information on pollutant residues and life history parameters from single or mass strandings, direct or indirect catches and biopsies from free-ranging individuals, each sample source having its own advantages and limitations (Aguilar 1995). Data from single stranding individuals are particularly prone to bias: the sampled individuals may have been in impaired health; the sex- and age- composition profile may not equate with that of the 'healthy' population; there may have been loss of pollutants post-mortem. Mass strandings can be relatively rare and are highly unpredictable. Harvested animals are good sample sources but are limited to rather few species and locations on a world scale. Biopsies taken from living, apparently healthy animals are difficult to obtain and provide relatively limited information.

In all of the above modes of sample collection there is the problem of limited sample size. Large samples are difficult to obtain and sampling is typically opportunistic rather than systematic. Accessibility of study animals and time available to take samples are major limiting factors. Small sample sizes impact on the comparability of data.

Though the biological data of sex, age and nutritional status should be part of normal monitoring procedure, nutritional status is difficult because reliable condition indices have not been developed for most cetaceans. As a general measure of the nutrition status, it is generally recommended to monitor the blubber lipid content in standardised body locations (Aguilar *et al.* 1995).

The criteria of environmental occurrence and potential toxicity are of importance in the selection of compounds to be analysed. In the past there has been considerable variation in this (McFarland and

Clarke 1989, Safe 1990). Though quantification of environmentally abundant PCBs may be the prime focus, other persistent organochlorines such as DDT, dieldrin and dioxins exist. The possible synergistic and cumulative effects of all of these are currently poorly known and need to be considered (IWC in press). Much recent effort has focussed on improvement of analytical skills, particularly the accuracy of contaminant identification and quantification assessed against reliable standards. There is also a need for congener specific analysis aimed at assessing toxicity and health effects.

FINDINGS OF THIS STUDY

1) PCBs were detected in every long-finned pilot whale individual examined, indicating the widespread presence of these contaminants in the New Zealand marine environment. In these cetaceans PCB levels were relatively low though highly variable (mean: 311 ng/g range: 33-931 ng/g) and, notably, 2-3 orders of magnitude lower than in North Atlantic long-finned pilot whales. The variability of individual levels was seemingly related to differences in sex, age and sexual maturity.

2) Trophic (feeding) level and proximity to the coast were identified as major determining factors in the accumulation of PCB residues. These open ocean odontocetes feed at an intermediate trophic level and exhibit higher PCB levels than open ocean baleen whales that feed at a lower trophic level. Conversely, PCB levels in long-finned pilot whales were below levels previously detected in the New Zealand Hectors dolphin which is known to feed inshore at a higher trophic level.

3) Life-history parameters (age, growth, reproductive status) could be described for the first-time for Southern long-finned pilot whales, though limited by sample size. They proved to closely match those determined in Northern Hemisphere pilot whales: i.e. 14.3 yr and 494 cm (age and length at sexual maturity respectively) for males, and 8.7 yr and 379 cm for females.

4) Contrary to most previous cetacean research on PCB accumulation, significant PCB age trends were identified for both male and female long-finned pilot whales. Males accumulate PCBs throughout whole of their lives. In females, PCB residues increase prior to sexual maturity, decrease throughout active parturition, but increase significantly again as females subsequently age.

5) The female PCB age trend further indicated a limited period of active parturition, a finding not before reported for long-finned pilot whales, the New Zealand female then becoming reproductively senescent at 20-30 years. Those Northern Hemisphere populations so far studied have exhibited active reproduction in females throughout life. It is unclear whether this difference can be attributed to the existence of distinct northern and southern forms.

6) The male PCB age trend strongly suggests that amongst the up to 4 years old males sampled in this study there were first born calves, these having received exceptionally high PCB burdens during gestation and lactation from their primiparous mothers. The specific PCB congener profile in these young males was distinctly different from all other individuals sampled, presumably as a result of selective maternal transfer of different PCBs.

7) As in other marine mammals, long-finned pilot whales show a structure-related PCB capacity to metabolise PCB congeners. From this study these whales also appeared to exhibit a reduced capacity to metabolise meta-para and certain ortho-meta non-substituted PCB congeners.

8) Despite high variability in PCB levels, actual profiles of these were consistent, strongly suggesting that over the 5 year sampling period, the whales had been exposed to a common PCB contaminant source. PCB profiles were also consistent with those of other open ocean cetaceans, suggesting that despite differences in migrational and dietary patterns, these species were being exposed to the same common contaminant source.

Continuity of the PCB contamination pattern over time, space and species points to distant, possibly even Northern Hemisphere source(s), rather than local PCB emissions. In addition the abundance of the more volatile, chlorinated PCB congeners in the residues analysed suggests that atmospheric transport is likely to be the mode of dissemination of these contaminants into the Southern Ocean.

It is noted that the overall effects of the southward shift of PCB usage to the tropical belt of developing countries may well have been underestimated. PCB exposure in the Southern Hemisphere is likely to increase in the long term, especially the long-range transport of lower chlorinated PCB congeners from these and other sources.

9) The relatively low levels of PCB contaminants determined in this study appear to be below those at which PCB related adverse health effects might be expected. The mean TEQ concentrations of 12.3. pg/g are below levels associated with dioxin-like adverse effects, particularly reproductive effects, in marine mammals. However, a PCB dose-effect relationship has not yet been established for these and sensitivity to these contaminants remains to be determined.

CONSERVATION-RELATED SIGNIFICANCE OF THIS STUDY

The above findings confirm an earlier suggestion that PCBs can be useful tracers for the investigation of age-related reproductive parameters in marine mammals (Tanabe *et al.* 1987). No previous study has reliably used PCB data in this type of application. However, the clarity of the identified PCB age trend imposes limitations on this approach. In the Northern Hemisphere, regional differences in contaminant exposure and/or diet spectrum may explain the high variability in PCB levels, and thus limit the tracing of age-related reproductive parameters. In contrast, New Zealand open ocean cetaceans do not appear to be subjected to such regional differences and hence PCB age trends might be reliably used as indicators of these parameters.

In addition, displaying low contaminant levels, Southern Ocean open ocean cetaceans could be used as a tool to investigate and infer baseline biological parameters at low exposure levels or physiological 'normality' in cetaceans. However, because sample sizes are likely to be limited at any one time, continuing and directed efforts at monitoring PCB levels as tracers are likely to prove invaluable.

In this context, the long-finned pilot whale would appear to be ideal as a study species for the following four specific reasons:

- it is an abundant open ocean odontocete displaying low PCB and TEQ levels;

- the species tends to mass strand frequently on accessible beaches in groups of mixed ages and sexes and can thereby provide a reasonably consistent and diverse sampling source;

- contemporary studies on the Northern Hemisphere population from the Faroese drive fishery and mass strandings would allow a first dose-effect study in a cetacean species by comparison of the physiological responses of the two populations.

- In addition to acting as a model for investigating the conservation-related biology of other marine mammals in general, these studies could serve to evaluate the well-being of long-finned pilot whales themselves. This species is one of the few great whales that are not subject to harvesting provisions of the IWC. They are caught as a matter of customary practise in the Northern Hemisphere, but have gained protection elsewhere by various national legislation (in New Zealand by the Marine Mammals Protection Act 1978). However, being a wide-ranging oceanic species, and not being provided for by IWC requirements, their populations are not readily amenable to monitoring and associated management.

FUTURE RESEARCH AND ACTION

A major step towards the conservation of marine mammals was taken by the establishment of the Southern Ocean whale sanctuary. While this has moderated the effects of direct exploitation, there are other important conservation issues that have not been seriously addressed. These include bycatch, entanglement, noise pollution, disturbance by tourism and exposure to chemical contaminants.

The simple monitoring of concentrations of chemicals in marine mammal tissues alone is insufficient to determine the complexity of effects (if any) of pollutants on populations. A recent IWC workshop on chemical pollution and cetaceans (IWC in press) recommended a multidisciplinary approach to assessing the inherent risks. It suggested that the determination of tissue concentrations be accompanied by the monitoring of appropriate biomarkers, pathological examination and evaluation of any alterations in reproductive biology and early development, as well as the regular collection of a range of biological data.

Given the present lack of knowledge of dose-effect relationships between cetacean health and chemical pollutants, the IWC workshop further recommended that priority be given to carefully defined and designed studies on particular species and in areas where relevant data would be most forthcoming. Examples would best be drawn on the one hand from highly polluted areas and on the other from pristine or relatively unpolluted areas, and species that tend to accumulate pollutants. The relevance of long-finned pilot whales and the Southern Ocean to the above recommendations is clear.

The importance of research was further emphasised by a recent IWC conference in Oman approved by consensus a resolution on increasing research into potential threats to cetaceans, including pollution. The action directed commission scientists to place a higher priority on non-lethal research on environmental effects and urges them to collect and share this information with other scientific bodies (Reuters, 20 May 1998).

New Zealand is thus well placed to respond to the above recommendations. Long-finned pilot whales would represent an ideal species to be studied in the longer term in this context. To further our understanding of pollution burdens, their effects, and their biological and conservation significance the following initiatives are recommended:

1) Long-term monitoring of mass strandings in New Zealand for persistent pollutants and life-history parameters. Routine sampling should wherever possible include at least blubber, teeth and reproductive organs; blubber samples to be screened for a wide range of environmentally significant compounds, including PCBs, PCDDs and PCDFs.

2) Development of an interdisciplinary project to assess molecular, endocrine, enzymatic, histological and gross morphological indices in New Zealand long-finned pilot whales along with contaminant studies to infer baseline biological parameters or physiological 'normality'. Equivalent biological and contaminant data from Northern Hemisphere whales to be taken and compared by similar protocols so as to assess dose-effect relationships.

3) Promotion internationally of the significance of New Zealand as being in a prime position to play a major role in the conservation of marine mammal species by furthering the above national initiatives. Publication of the results of this study would contribute to this end.

4) Every suitable opportunity to be taken to improve public awareness of the all-pervasive nature of PCBs and their biological significance, especially for marine mammals, and that New Zealand and the ocean around it is not isolated from distant as well as immediate sources of these and like environmental contaminants.

APPENDIX I: DETAILS OF THE ACTUAL STUDY

METHODS

Sample Collection

From 1992 to 1996 long-finned pilot whale carcasses from New Zealand mass strandings were sampled for blubber, teeth and reproductive organs. Over this period 7 mass strandings were recorded (5 in Golden Bay, 1 in Otago, and 1 in Wellington) and a total of 61 blubber samples (24 males and 37 females), 35 tooth samples (16 males and 19 females) and 13 samples of reproductive organs (8 males and 5 females) were collected. Of the 61 carcasses sampled for blubber, 26 were sampled for teeth and 13 of those for reproductive organs. A further 7 tooth samples were collected from badly decomposed carcasses. Prior to February 1995 blubber and tooth samples were randomly collected by Department of Conservation staff from 4 strandings. From February 1995 to February 1996, blubber, tooth and reproductive samples were collected from almost all whale carcasses from 3 strandings. All sampled whales were measured for length and were sexed by macroscopic examination of external genitalia.

PCB Analysis

PCB residues in blubber tissue were determined by ultra-trace congener specific analysis, following the methodology of Buckland *et al.* (1990) and Hannah *et al.* (1993). $^{13}C_{12}$ PCB congeners were added to each sample, prior to extraction, to be used as internal standard compounds for the calculation of PCB congener concentrations within the sample. Blubber samples were then extracted with 1:1 acetone/hexane by Accelerated Solvent Extraction (ASE). Extracts were dried by passage through anhydrous Na_2SO_4, concentrated to near dryness and re-dissolved in 50 ml of hexane. A 2.5 ml portion of the extract was removed for lipid determination. The remaining extract, after any sub-sampling, was transferred to a separating funnel and washed five 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 on a column of NaOH/silica gel and H_2SO_4/silica gel. The eluate was subsequently chromatographed on Florisil to isolate the three coplanar PCB congeners. Extracts were analysed for PCBs by High Resolution Gas Chromatography-High Resolution Mass Spectrometry (HRGC-HRMS) on a VG 70S mass spectrometer.

Age Determination

Tooth samples were available for 35 whales. Age determination of these whales was based on the reading of annual growth layer groups (GLGs – see IWC 1980) in the tooth, a method most commonly used for ageing of odontocete whales. Decalcified and stained longitudinal thin sections of teeth were prepared following Lockyer (1993a). Teeth were cut longitudinally and tooth halves decalcified in 10% formic acid. Teeth were then fixed in 10% formalin and subsequently sectioned at 25 µm on a freeze microtome at -30°C. Sections were stained with Delafield's Haematoxylin, rinsed with water, blued with 5% ammonia and again rinsed with water, before partial dehydration in 50% and 70% alcohol. Sections were then floated onto 5% gelatin smear coated slides and permanently mounted.

Tooth sections were examined microscopically for dentinal GLGs (x10-x30) and cemental GLGs (x40-x100) in plain transmitted light. Counts of dentinal GLGs were recorded for all teeth. Where the reading of dentinal GLGs proved difficult, cemental layers were also examined. Each tooth was examined by at least two readers. Several random samples of duplicate readings were made. The mean value of all readings was given as the best age estimate for each individual.

Male and female Laird/Gompertz growth equations calculated from this study (see Section on 'Age- and Sex-related PCB Accumulation') were used to predict age in the 33 whales which had not been sampled for teeth.

Growth Modeling

The Laird/Gompertz growth model (Laird 1969) was found to best describe growth in the Faroe Island long-finned pilot whale (Bloch *et al.* 1993). It was applied in this study so as to allow for a detailed comparison of growth parameters between Faroese and New Zealand pilot whales.

Assessment of Reproductive Status

Sexual maturity was determined for 13 whales through gonadal examination. Assessment of sexual maturity was based on the histological examination of reproductive organs, following Harris *et al.* (1972). In males, sexual maturity was indicated by the presence of sperm in the testicular tissue. In females it was indicated by scaring of the ovarian tissue which is understood to be the result of previous ovulations. Histological slides of testes were prepared using standard techniques. Samples were sectioned and the sections were embedded in paraffin and stained with haematoxylin and eosin. Whole ovaries were fixed in formalin.

Testicular slides were microscopically examined (x125-x500). Functional maturity was defined as the stage where 100% of seminiferous tubules are mature, i.e. contain sperm. Whole ovaries were examined macroscopically for surface scars and were then hand-sectioned in 2 mm slices for internal examination. Sexual maturity was considered to be reached at first ovulation.

In the light of the findings in of Section 'Age, Growth and Reproductive Parameters', Faroese estimates of age (ASM) and length (LSM) at attainment of sexual maturity were adopted for the remaining 48 whales for which reproductive tissue had not been obtained. As Bloch *et al.* (1993) found body length to be a better indicator of the onset of sexual maturity in long-finned pilot whales, Faroese LSM values were used to predict sexual maturity.

RESULTS AND DISCUSSION

PCB Levels

PCB levels were detected in all 61 long-finned pilot whale blubber samples. PCB residues ranged from 33 to 931 ng/g (ppb) with a mean value of 311 ng/g ±26 (SE).

Comparison of Northern and Southern Hemisphere Populations

The mean PCB level in this study was two to three orders of magnitude lower than PCB levels recorded in long-finned pilot whales from the North Atlantic.

Especially noteworthy were the high PCB concentrations found in long-finned pilot whales from Rhode Island and Maine on the east coast of the United States (78 ppm-Taruski *et al.* 1975), from the French coast (189 ppm-Alzieu and Duguy 1979) and from the UK (42 ppm-Martin *et al.* 1987). The combined average of PCB levels in Newfoundland (5.45 ppm-Muir *et al.* 1988) and Faroese pilot whale populations (19.5 ppm-Simmonds *et al.* 1994, 22.8 ppm-Borrell *et al.* 1995) was at 20.4 ppm still some two orders of magnitude higher than the New Zealand mean (0.31 ppm).

An overlap in the PCB range was observed in New Zealand and Newfoundland animals (0.52-14.7 ppm, *n*=14-Muir *et al.* 1988). However, due to the small sample size of the Newfoundland study, as well as differences in sample structure and sum of congeners analysed it was not clear whether this overlap might be attributable to similarly low PCB exposure levels in Newfoundland and New Zealand or rather be the result of technical discrepancies between studies.

Comparison with Other New Zealand Cetaceans

PCB levels in New Zealand long-finned pilot whales were in the mid-range of PCB contamination currently reported in New Zealand cetaceans.

PCB concentrations in this study were lower than previously reported in the inshore carnivorous Hector's dolphin (0.28-10.2 ppm; Hannah *et al.* 1993, Jones *et al.* 1996) but higher than reported in offshore filter feeding baleen whales (0.002-0.02 ppm; Jones *et al.* in press). Findings correspond well with the range of PCBs levels described for other offshore living carnivorous cetaceans (0.05-4.71 ppm; Jones *et al.* in press), including beaked whales and offshore dolphins.

Differences in PCB levels between New Zealand cetaceans appeared to be related to species differences in food habit as well as feeding proximity to the coast. The effect of trophic level and feeding location on PCB levels in cetaceans has been noted previously (Tanabe *et al.* 1983, Aguilar and Borrell 1988, Borrell 1993).

This study supported prior findings that offshore living cetaceans of intermediate trophic levels and intermediate body length, such as pilot whales, carry comparatively much higher PCB concentrations in their tissue than offshore living baleen whales, which are massive but feed mostly on planktonic crustaceans situated low in the food chain (Tanabe *et al.* 1983, Aguilar and Borrell 1988, Borrell 1993).

Results of this study further tended to confirm that offshore feeding odontocetes such as the pilot whale, presumably being less exposed to pollutants than inshore species, carry lower levels of PCBs than inshore feeding odontocetes such as the Hector's dolphin (Tanabe *et al.* 1983, Borrell 1993).

Age, Growth and Reproductive Parameters

Tooth based age readings ranged from less than 1 to 31 years in males (*n*=16) and less than 1 to 35 years in females (*n*=19). Based on tooth derived age estimates and body length data, Laird/Gompertz growth curves were calculated by iterative least square analysis. Male and female Laird/Gompertz growth curves along with tooth derived age estimates are displayed in Figure 1. Growth equations were subsequently used to predict age in pilot whales of this study which had not been sampled for teeth (*n*=33). Thus, maximum ages of pilot whales from New Zealand strandings were ascertained. The oldest female recorded was 35 years the oldest male 40 years. Maximum recorded length in females was 470 cm and 584 cm in males.

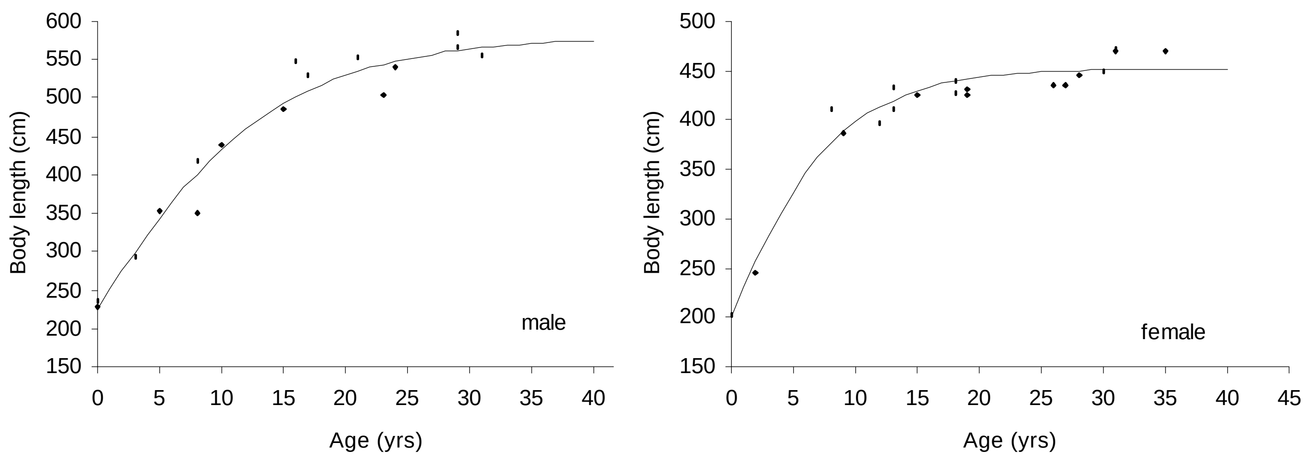

Figure 1. Tooth derived age of long-finned pilot whales by body length with fitted growth curves calculated after Laird/Gompertz for males (*n*=16) and females (*n*=19)

Based on microscopic examination of histological slides, 3 testes were identified as immature and 5 as mature. The youngest sexually mature male observed was 15 years of age and 485cm in length while the oldest immature male was 8 years old and 349 cm long. The present limited data suggested sexual maturity in males to be reached somewhere between these measurements.

Following an external and internal macroscopic examination of ovaries, 1 immature female and 4 mature females were identified. The youngest mature female observed was 10 years old with a body length of 399 cm while the only observed immature female was 2 years old and 245 cm long. Similarly limited data gave a preliminary indication of female sexual maturity being reached between these measurements.

Due to paucity of reproductive data it was not feasible to produce a more defined estimate of average age (ASM) and length (LSM) at attainment of sexual maturity in the New Zealand long-finned pilot whale.

Comparison of Northern and Southern Hemisphere Maximum Recorded Age and Body Length

Maximum recorded ages and lengths of long-finned pilot whales from New Zealand strandings were below those observed in previous pilot whale drive-fishery based studies (Sergeant 1962, Kasuya *et al.* 1988, Bloch *et al.*1993) but were generally consistent with those observed in previous stranding based studies. It has been suggested that underestimation of maximum ages and lengths in stranding based studies might be attributable to small sample size limitations rather than genuine differences in population structure (Bloch *et al.* 1993).

Comparison of Northern and Southern Hemisphere Growth Patterns

Age/length data and growth curves of New Zealand long-finned pilot whales were found to correspond with the overall growth pattern described for North Atlantic animals (Sergeant 1962, Martin *et al.* 1987, Kasuya *et al.* 1988, Bloch *et al.* 1993, Sigurjónsson *et al.* 1993). Detailed comparison of New Zealand Laird/Gompertz growth parameters with findings of Faroe Island animals did not reveal any significant growth differences (T-tests, $p>0.05$). For Faroese and Icelandic animals, a pattern of protracted growth until old age has been suggested (Bloch *et al.* 1993, Sigurjónsson *et al.* 1993). Whether this might be true for New Zealand pilot whales is unclear.

Comparison of Northern and Southern Hemisphere Sexual Maturity Data

Knowledge of New Zealand reproductive parameters is limited to few data and comparisons have to be taken with caution. Preliminary results indicate correspondence with North Atlantic ASM and LSM values in general and Faroese estimates in particular (Sergeant 1962, Bloch 1989-90, Kasuya *et al.* 1988, Bloch *et al.* 1993, Desportes *et al.* 1993, Desportes 1994, Desportes *et al.* 1994).

Faroe Island estimates of ASM and LSM by Bloch *et al.* (1993), Desportes *et al.* (1993), Desportes (1994) and Desportes *et al.* (1994) constitute the most reliable estimate of North Atlantic long-finned pilot whale reproduction to date. In the Faroe Islands attainment of sexual maturity was estimated at 14.3 years ±0.5 and 493.4 cm ±4.6 in males and 8.7 years ±0.2 and 378.5 cm ±0.61 in females. Maturity data of this study were compared with Faroese ASM and LSM estimates in Figure 2. Data of this study matched Faroese ASM and LSM values in all cases except one mature male (485 cm length) was below the given LSM but within the appropriate confidence interval. Despite the disparity in sample size, the compatibility of Faroese ASM/LSM and New Zealand data suggested a correspondence in reproductive life history data.

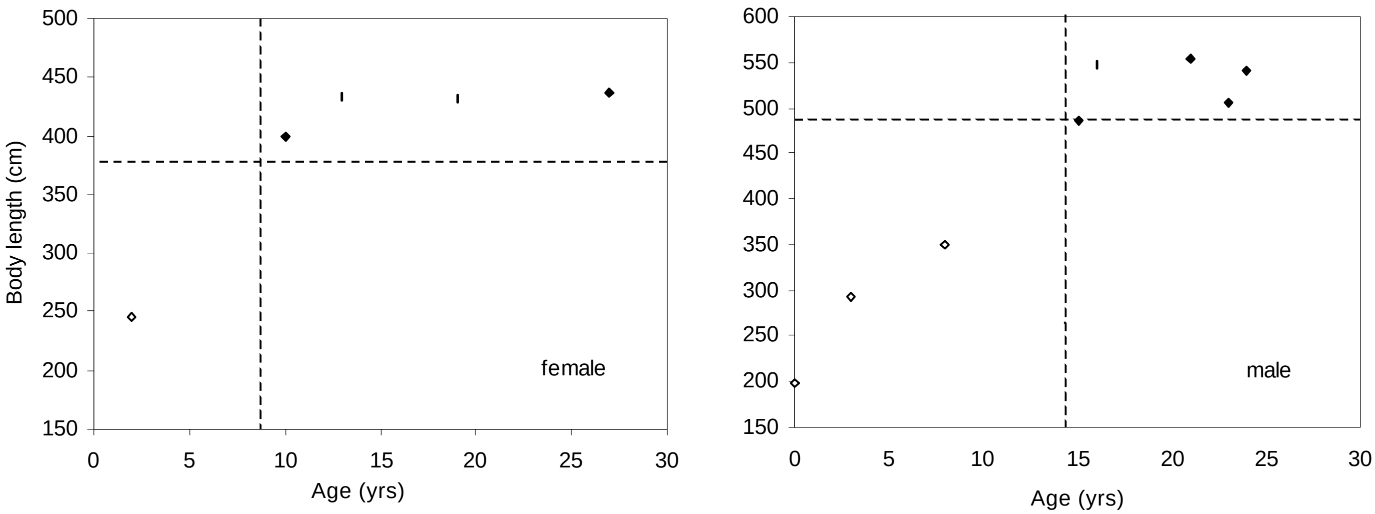

Figure 2. Maturity data for female (n=5) and male (n=8) long-finned pilot whales of this study with superimposed Faroese ASM and LSM values. *Symbols:* filled square-mature; filled triangle-immature.

Age- and Sex-related PCB Accumulation

PCB Levels by Sex and Maturity

PCB levels in immature males, mature males and immature females were not significantly different from each other (Kruskal-Wallis tests, p>0.05) but were significantly higher than PCB levels in mature females (Kruskal-Wallis tests, p<0.05).

Results tend to confirm previous findings that mature females carry lower levels of PCBs, which is likely to be explained by their reproductive investments, leading to maternal transfer of PCBs to their offspring via pregnancy and lactation (Borrell *et al.* 1995, Westgate *et al.* 1997).

Female PCB Age Trend and Reproductive Parameters

A distinct age trend of PCB levels, comprising three phases, was evident in female long-finned pilot whales off New Zealand (Figure 3). In phase one PCB levels increased with age up until about the age of nine years, before decreasing with age in the second phase up to about 25 years. In the third phase PCB levels again increased with age after about 25 years. It was argued that the three phases reflected the reproductive status of female long-finned pilot whales off New Zealand.

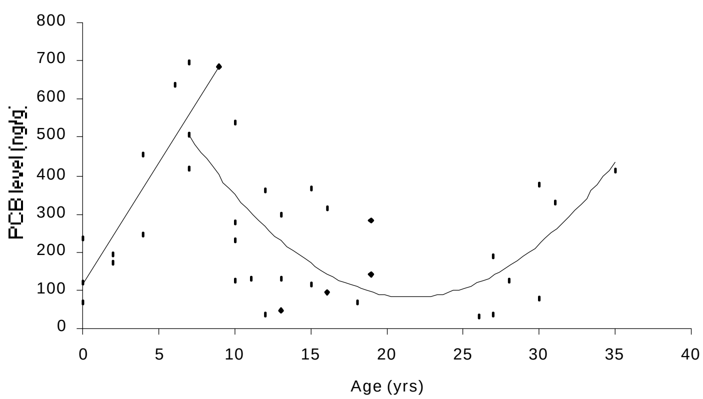

Figure 3. Relationship between PCB concentration and age in female long-finned pilot whales.
Symbols: cicles = immature females; filled circles = mature females.

Regression equations, and r^2- and *p*-values describing the relationship between PCB concentration and age in female long-finned pilot whales as illustrated in Figure 3.

Immature females:	∑PCB = 63.37 (age) + 115.29 $r^2 = 0.81$ $p < 0.001$
Mature females:	∑PCB = 1.98 (age)^2 – 85.12 + 1006.8 $r^2 = 0.45$ $p < 0.05$

Phase one corresponded with a period of female immaturity and indicated an age of sexual maturity

(ASM) around 9 years. Thus, the PCB age trend supported the hypothesis, based upon analysis of reproductive data in this study, that the Faroese female pilot whale ASM of 8.7 years ±0.2 (Bloch *et al.* 1993) best described the ASM for New Zealand female pilot whales.

A period of active parturition was covered by phase two, where PCB levels declined as females transferred PCBs to their offspring during pregnancy and lactation. A decline of PCB concentration with age was also noted for female Faroese long-finned pilot whales (Borrell *et al.* 1995) and for female short-finned pilot whales off Japan (Tanabe *et al.* 1987).

The third and final phase of the female PCB age trend in this study is thought to reflect reproductive senescence (i.e. complete cessation of reproductive activity following menopause) in female pilot whales off New Zealand from 20-25 years of age. Similarly, Kasuya and Marsh (1984) and Marsh and Kasuya (1986) observed reproductive senescence in female short-finned pilot whales off Japan based upon the study of reproductive organs. They suggested that short-finned pilot whales become senescent between 25-30 years of age which is thought to be responsible for increased PCB concentrations in higher age classes observed by Tanabe *et al.* (1987). In contrast Martin and Rothery (1993) reported, on the basis of reproductive data, that Faroese female long-finned pilot whales are reproductively active throughout their whole lives so that PCB concentrations constantly decreased in mature Faroese females (Borrell *et al.* 1995). Consequently, based on the dissimilarity in the PCB age-trends of old females, findings of this study would suggest a possible difference in the reproductive activity of New Zealand and Faroese female long-finned pilot whales.

In the light of the reproductive life history described for North Atlantic long-finned pilot whales, the possibility that New Zealand females also reproduce throughout life should not be fully discounted. Lowered reproductive rate and higher offspring mortality (calves may not even live long enough to complete or start lactation) observed in old Faroese females, could also be expected to result in an increase in PCB concentrations in old aged females. Such PCB increase in old aged Faroese females might have been masked by the high variability of PCB levels observed in the female sample.

Male PCB Age Trend and First Born Calves

In male pilot whales from this study considerable variation in PCB levels among calves was noted (82-931 ng/g). It was hypothesized that three of these male calves which had amongst the highest PCB levels in all samples in this study, were first born calves. The hypothesis was based upon research by Borrell *et al.* (1995) which found that female pilot whales transferred about 96% of their PCB body burden to their offspring so that primiparous females transferred greater amounts of PCBs than females with later offspring. While estimated maternal transfer rates apply equally to calves of both sexes, the variability of PCB levels amongst female calves from this study (69-453 ng/g) was comparatively low and first-born female calves did not appear to be included in the sample. The 3 male calves thought to be first-borns displayed a high blubber lipid content (68-85%) suggesting the high PCB levels observed did not appear to result from emaciation.

Reproductive PCB transfer loads were estimated for mature females of reproductively active age from this study and PCB concentrations for respective calves were predicted. This analysis indicated that young females from the age of 7 would transfer a PCB load three and a half times higher than older females around 25 years of age. When comparing actual PCB levels measured in the 3 male calves argued to be first-borns with predicted PCB levels in calves from those females, the 3 male calves with unusually high PCB levels in this study matched neatly with predicted PCB concentrations in calves of young mothers, supporting the hypothesis that those 3 male calves were first-born calves. Considering that the actual maternal transfer rate may not be as high as 96% this would appear to be even more likely.

When the 3 (first-born) male calves were omitted from the analysis as explained outliers, a slow increase of male PCB levels with age was apparent (Figure 4). While Tanabe *et al.* (1987) reported a similar increase in PCB levels for short-finned male pilot whales off Japan, in Faroese long-finned pilot whale males high variability in PCB levels was observed and no relationship with age identified (Borrell *et al.* 1995). Westgate *et al.* 1997 referred to regional dietary differences and/or exposure levels as possible explanations for high variability of PCB levels observed in some Northern Hemisphere cetaceans. In general, however, PCB concentrations have been reported to increase with age in male cetaceans (Aguilar and Borrell 1988, Westgate *et al.*1997). A significant increase in male PCB concentrations with age, as detected in this study, has so far been described for only a few cetacean species, including the harbour porpoise and the beluga whale (Westgate *et al.*1997).

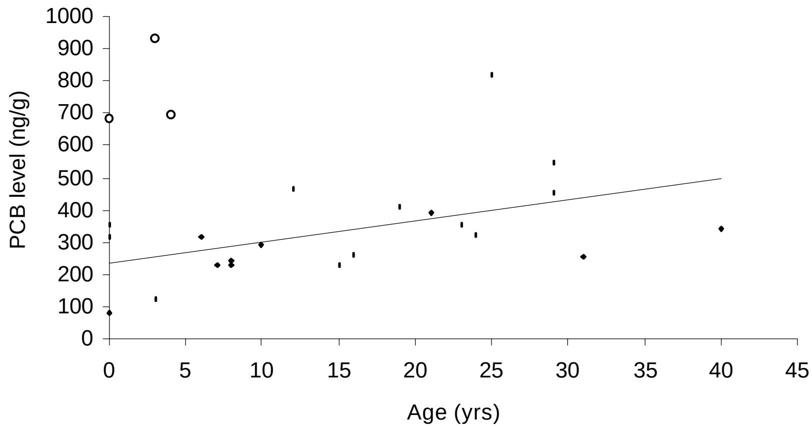

Figure 4. Relationship between PCB concentration and age in male long-finned pilot whales, excluding three first-born calves. *Symbols*: Open circles = first born calves; filled circles = immature and mature males.

Regression equation and r^2- and *p*-value describing the relationship between PCB concentration and age in male long-finned pilot whales as illustrated in Figure 4.

Males:	$\sum PCB = 6.55 (age) + 233.82$	
	$r^2 = 0.24$	$p < 0.05$

PCB Accumulation Profiles and Possible Health Effects

PCB Profile and its Implications for Metabolism

The mean PCB congener profile of New Zealand pilot whales (Figure 5) tended to confirm previous findings that lower chlorinated PCBs are less prominent in marine mammals, which is likely to be explained by their ability to metabolise and eliminate certain lower chlorinated PCB congeners from the body (Tanabe *et al.* 1988, Boon *et al.* 1992).

Pilot whales appeared to be generally capable of metabolising mono-*ortho* substituted PCB congeners with adjacent non-chlorinated carbons at *ortho* and *meta* positions. These include PCBs #28, #105, #114, #123, #156 and #157, which in the PCB profile were present at relatively low concentrations. PCBs with adjacent non-chlorinated carbons at *ortho* and *meta* positions but *ortho* chlorine substitution at two or more positions (PCBs #99, #138 and #170) were of relatively high abundance in the PCB profile indicating that this structural group appears to be less readily metabolised in pilot whales.

Relatively high abundance of PCBs #153, #180 and #187 in the PCB profile of this study suggested that pilot whales are less capable of metabolising PCB congeners with *ortho* chlorination but lacking non-substituted adjacent carbon atoms. New Zealand pilot whales also appeared to be less capable of metabolising *meta-para* non-substituted PCB congeners indicated by high abundance of PCBs #52 and #101 in the PCB profile.

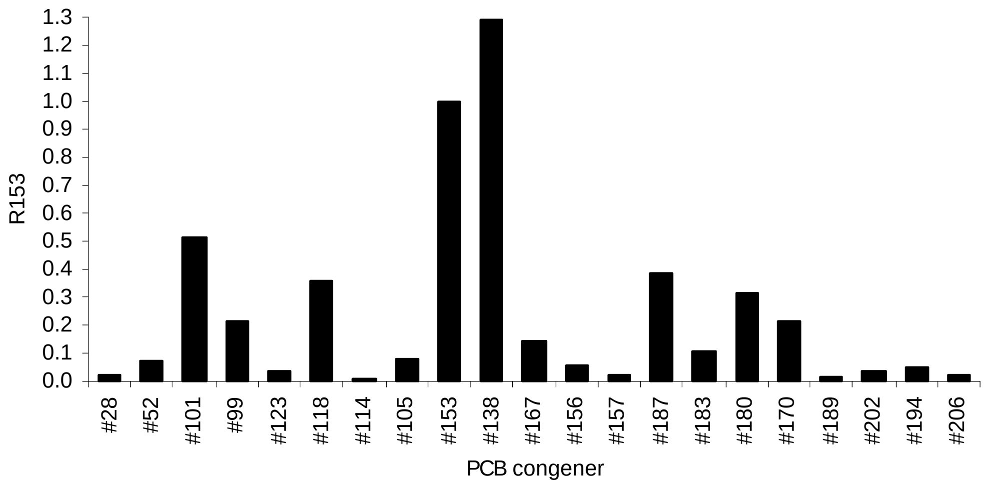

Figure 5. Mean profile of non-coplanar PCBs in 61 pilot whale blubber samples (non-*ortho* congeners not displayed due to low concentration). Concentrations are normalised to that of PCB #153 (R^{153} = concentration of PCB congener/concentration of PCB #153).

The above characteristics of the pilot whale PCB congener profile tended to confirm previous findings of a selective metabolism of *ortho-meta* non-substituted PCB congeners in marine mammals, indicating that metabolism of these compounds strongly depends on the degree of *ortho*-chlorination. Relatively high abundance of PCBs #52, #101 and #118 in New Zealand as well as United Kingdom, United States and Newfoundland pilot whales suggested that long-finned pilot whales may also exhibit a lower capacity to metabolise *meta-para* (#52, #101) as well as certain *ortho-meta* (#118) non substituted PCB congeners. In contrast such congeners have been reported to be readily metabolised in seals and sea lions (Tanabe *et al.* 1988, Boon *et al.* 1992). Abundance of these congeners confirmed findings by Tanabe *et al.* (1988) of a reduced metabolic capacity in small cetaceans, suggesting that these might lack the ability to metabolise *meta* and *para* non-chlorinated PCB compounds.

Boon *et al.* (1994) and Muir *et al.* (1996a) hypothesized that high PCB exposure may enhance the metabolic capacity in marine mammal species, resulting in lower proportions of lower chlorinated, less persistent PCB compounds in the PCB profile. Whether this occurs in pilot whales is not so clear.

To investigate this further, PCB levels and profiles of pilot whales from this study and from Newfoundland, the U.S. and the U.K. were compared. Whereas mean PCB levels in pilot whales according to geographical area ranked as U.K. (36.9 ppm) > U.S. (7.9 ppm) > Newfoundland (5.45 ppm) > New Zealand (0.31 ppm), the relative abundance of lower chlorinated PCB congeners (i.e. the combined R^{153} of PCBs #28, #52, #101, #105 and #118) by population ranked as New Zealand < U.K. < U.S. < Newfoundland. New Zealand pilot whales which had lowest overall PCB levels exhibited the lowest relative contribution of lower chlorinated PCBs to the PCB profile and findings did not appear to confirm a PCB level enhanced metabolism for the New Zealand pilot whale. However, inverse ranking of U.S., U.K., and Newfoundland pilot whale populations by PCB levels and relative abundance of lower chlorinated PCBs in the profile seemed to provide some support for a PCB level enhanced metabolism in these populations.

PCB Profiles and their Implications for Contaminant Sources

A cluster analysis performed on PCB congener data of New Zealand pilot whales indicated a close relatedness of PCB congener profiles. Differences in profiles by stranding, stranding year, stranding location, sex or age were not apparent. While Aguilar *et al.* (1993) reported a certain heterogeneity in the pollution profile of Faroese pilot whales, indicating some segregation between the schools studied, such heterogeneity was not apparent in the pollution profile of New Zealand animals. The consistency of PCB congener profiles in this study seemed to suggest a common contaminant source for the pilot whales analysed over the 5 year sample collection period.

The above cluster analysis was repeated, including PCB congener data from Jones *et al.* (in press) for other New Zealand cetaceans. PCB profiles of all New Zealand open ocean cetaceans, including baleen whales and offshore odontocetes, were closely related to each other. In their PCB congener profiles an abundance of lower chlorinated congeners was present. The principal source of these lower chlorinated PCBs is believed to be long-range atmospheric transport (Jones *et al.* in press): lower chlorinated PCBs evaporate at warmer places of the globe, travel through the atmosphere and condense in colder areas. This process is assumed to be responsible for the redistribution of PCB compounds from high usage areas of the Northern Hemisphere and the tropics to remote Arctic and Antarctic environments (Simonich and Hites 1995, Mackay and Wania 1995).

Consistency of PCB congener profiles in pilot whales of this study and other New Zealand open ocean cetaceans along with evidence of atmospheric transport in PCB profiles seemed to indicate a common PCB contaminant source of a distant origin for New Zealand open ocean cetaceans.

TEQ Levels and their Implications for Hazard Assessment

Planar chlorinated hydocarbons (PCHs) such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and some PCBs exert common toxic responses similar to those observed for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic PCH congener (Safe 1990). Toxic Equivalency Factors (TEFs) can be used to calculate the biological potency of PCH mixtures relative to 2,3,7,8-TCDD (WHO in press). Using this method, the total concentration of TCDD-Equivalents (TEQs) were calculated.

According to data previously reported for New Zealand cetaceans (Jones 1995) it appears that in New Zealand delphinids the contribution of PCDD and PCDF compounds to total TEQ ranges from 2-13% with a mean value of 9%. PCBs on average thereby account for 91% of total TEQ. In this study, the PCB contribution to total TEQ was calculated as 11.2 pg/g ±0.93 (SE). Assuming a PCDD/PCDF contribution set at 9% of PCB-TEQ, the total TEQ in New Zealand pilot whales was estimated to be 12.3 pg/g ±1.0 (SE) with a range of 1.82-43.7 pg/g.

Total TEQ concentrations in this study (1.82-43.7 pg/g) were considerably below TEQ levels associated with deleterious effects in marine mammals. Reijnders (1986) and Ross *et al.* (1995) have suggested pollution associated decreased fecundity and impaired immune function in harbour seals from the North Atlantic which exhibited an estimated total TEQ of 208.7 pg/g. Similarly, occurrence of tumors and decreased fecundity in the beluga whale from the St. Lawrence estuary has been associated with organochlorine pollution in animals exhibiting 290-1070 pg/g TEQ (Muir *et al.* 1996b).

Epidemiological evidence has suggested that planar chlorinated hydrocarbons (PCHs) may be implicated in a number of bioeffects in free-ranging populations (Table 1). However, few epidemiological studies have demonstrated a cause-effect association between accumulation of these compounds and impaired immunity, development of cancer and lowered reproductive success in marine mammals (Addison *et al.* 1989, Kuiken *et al.* 1994).

Table 1. Epidemiological evidence of contaminant-related bioeffects among marine mammal populations

Effect	Location	Species	Source
Abortions Premature pupping	California, USA	California sea lion (*Zalophus californianus*)	De Long *et al.* 1973
Tumors Decreased fecundity	Québec, Canada	Beluga whale (*Delphinapterus leucas*)	Martineau *et al.* 1987 De Guise *et al.* 1994, 1995
Hormone disruption Decreased fecundity	Wadden Sea, Netherlands	Common seal (*Phoca vitulina*)	Reijnders 1986 Brouwer *et al.* 1989
Abortions Impaired reproduction	Baltic Sea	Ringed seal (*Phoca hispida*)	Helle *et al.* 1976a, 1976b
Reduced testosterone levels	North Pacific Ocean	Dall's porpoise (*Phocenoides dalli*)	Subramanian *et al.* 1987
Impaired immune function	North Sea	Harbour seal (*Phoca vitulina*)	Ross *et al.* 1995, 1996 De Swart *et al.* 1995, 1996
Impaired immune function	Mediterranean Sea	Striped dolphin (*Stenella coeruleoalba*)	Kannan *et al.* 1993
Skeletal lesions	Baltic Sea	Harbour seal (*Phoca vitulina*) Ringed seal (*Phoca hispida*)	Bergman *et al.* 1992

The only study that has come close to establishing a cause-effect relationship has been a controlled feeding experiment with harbour seals fed with fish from the Baltic and the Atlantic Ocean (Reijnders 1986). This was the first experimental evidence that organochlorine levels compared to those of free-ranging seals may affect seal reproduction and immune function, but even this technically difficult experiment did not yield unequivocal conclusions, as the diets differed in lipid and therefore caloric content and the relative contribution of other pollutants was not considered in the analysis.

Various studies have demonstrated that the most sensitive processes to the effects of PCHs involve reproduction and embryonic development (Peterson *et al.* 1993, Colborn *et al.* 1993, Fry 1995). To further assess the possible adverse effects of TEQ levels on the reproduction of the pilot whale, a hazard index was calculated, based on TEQ toxicity data of the mammalian species most sensitive to the reproductive toxicity of PCHs - the mink (*Mustella vison*) (Table 2). Following Giesy *et al.* (1994) the hazard index was calculated as the lowest/highest pilot whale TEQ concentration (lipid wt) divided by a reference dose, the no-observable-adverse-effect-level (NOAEL) TEQ, in this case the NOAEL reported by Tillit *et al.* (1996) for mink. The hazard index (HI) gives an indication of the likelihood of the occurrence of adverse reproductive effects. Lowest-observable-adverse-effect-levels (LOAEL) are generally ten fold higher than NOAELs and thus it is unlikely that adverse effects will be seen until the HI exceeds 10 (Giesy *et al.* 1994, Tillit *et al.* 1996).

The HI calculated for the pilot whale (0.01-0.29, Table 2) was far below levels where adverse effects would be expected. Findings thus seemed to suggest that the TEQ levels present in these pilot whales were probably not causing adverse reproductive effects and thus not negatively affecting the maintenance of the population. However, as the sensitivity of animals to dioxin-like toxicity varies greatly between species, it is not known whether the sensitivity of marine mammals is comparable to that of terrestrial mammals.

Table 2. Hazard index (HI) for TCDD toxic equivalents (TEQs) in pilot whale blubber

Sample	**Contaminant**	**Concentration**	**Reference dose**	**HI**
Pilot whale	TCDD TEQs	2.4-58.3 pg/g lipid wt[a]	200pg/g lipid wt[b]	0.01-0.29

[a] equals blubber TEQ of 1.82-43.7 pg/g wet wt with average blubber lipid content of 74.9%
[b] TEQ-NOAEL value of mink (10 pg/g liver wet wt-Tillit *et al.* 1996) based on 5% liver lipid content

APPENDIX II

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