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High PCB Concentrations in Free-Ranging Pacific Killer Whales, *Orcinus orca*: Effects of Age, Sex and Dietary Preference

P. S. ROSS†*, G. M. ELLIS‡, M. G. IKONOMOU†, L. G. BARRETT-LENNARD§ and R. F. ADDISON†
†*Institute of Ocean Sciences, P.O. Box 6000, Sidney, BC, Canada V8L 4B2*
‡*Pacific Biological Station, Hammond Bay Rd, Nanaimo, BC, Canada V9R 5K6*
§*Zoology Department, University of British Columbia, 6270 University Blvd, Vancouver, BC, Canada V6T 1Z4*

Blubber biopsy samples were obtained for contaminant analysis from two discrete populations of killer whales (*Orcinus orca*) which frequent the coastal waters of British Columbia, Canada. Detailed life history information for the fish-eating 'resident' population, comprising two distinct communities, and the marine mammal-eating 'transient' killer whale population, provided an invaluable reference for the interpretation of contaminant concentrations. Total PCB concentrations (sum of 136 congeners detected) were surprisingly high in all three communities, but transient killer whales were particularly contaminated. PCB concentrations increased with age in males, but were greatly reduced in reproductively active females. The absence of age, sex and inter-community differences in concentrations of polychlorinated- dibenzo-*p*-dioxins (PCDDs) and- dibenzofurans (PCDFs) may have partly reflected low dietary levels, but more importantly, metabolic removal of dioxin-like compounds in killer whales. While information on toxic thresholds does not exist for PCBs in cetaceans, total 2,3,7,8-TCDD Toxic Equivalents (TEQ) in most killer whales sampled easily surpassed adverse effects levels established for harbour seals, suggesting that the majority of free-ranging killer whales in this region are at risk for toxic effects. The southern resident and transient killer whales of British Columbia can now be considered among the most contaminated cetaceans in the world. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: killer whales; marine mammals; polychlorinated biphenyls; PCB; dioxins; PCDD; furans; PCDF; British Columbia.

*Corresponding author. Tel.: +1-250-363-6806; fax: +1-250-363-6807.
E-mail address: rosspe@pac.dfo-mpo.gc.ca (P.S. Ross).

Introduction

The killer whale, *Orcinus orca*, is widely distributed in the world's oceans, but represents a particularly important natural symbol of the north-eastern Pacific Ocean to the peoples of western Canada and north-western United States. Two sympatric populations of killer whales, with fundamentally different dietary preferences, frequent coastal waters of British Columbia and adjacent areas (Fig. 1) (Ford *et al.*, 1998). Maximum longevity in males is estimated to be 50–60 years, and in females, 80–90 years, with adult female members of matriarchal communities bearing one calf every 3–5 years (Olesiuk *et al.*, 1990). 'Resident' killer whales have been documented to spend up to 12 months per year in the coastal waters of British Columbia, Washington and Alaska, and feed on fish, principally salmonids (Ford *et al.*, 1998). During the winter, residents are sighted with much less frequency in near-shore areas, and there is little information on their distribution. The resident population of killer whales comprises two communities, or subpopulations, referred to as the northern and southern residents, which numbered 212 and 89 individuals, respectively, in a 1998 census (GME, unpub. data; see also Ford *et al.* (1994), Olesiuk *et al.* (1990)). Also frequenting these coastal waters is the more elusive 'transient' killer whale population, for which 219 individuals have been catalogued (Ford and Ellis, 1999). Transients consume pinnipeds and cetaceans almost exclusively, but no fish (Ford *et al.*, 1998). Their movements are poorly understood, but there is no indication that transients have any seasonality to their distribution.

Many persistent and toxic industrial and agricultural chemicals produced in the twentieth century have been shown to bioaccumulate in lipid tissues of animals occupying high trophic levels in aquatic food webs. Fish-eating marine mammals inhabiting the industrialized

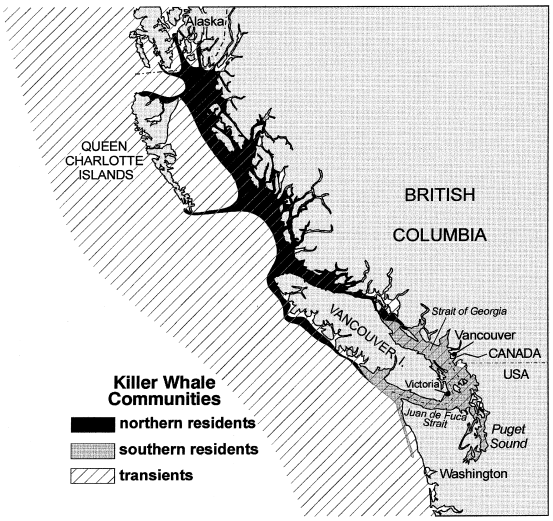

Fig. 1 Two populations of killer whales, the fish-eating residents and the marine mammal-eating transients, frequent the coastal waters of British Columbia, Canada, and the states of Alaska and Washington. The resident population comprises two communities: the northern and southern residents. Adapted from Ford et al. (1994), Ford and Ellis (1999).

coastal waters of northern Europe, the Mediterranean Sea, and the St. Lawrence estuary in eastern Canada, have been found to be particularly contaminated with polychlorinated biphenyls (PCBs) and dichlorodiphenyl-trichloroethane (DDT) (Blomkvist *et al.*, 1992; Kannan *et al.*, 1993; Muir *et al.*, 1996). There is increasing evidence that elevated contaminant concentrations, and PCBs in particular, have caused reproductive impairment, skeletal abnormalities, immunotoxicity and endocrine disruption in some pinniped populations, based on the 'weight of evidence' from field and semi-field studies (Bergman *et al.*, 1992; Brouwer *et al.*, 1989; De Swart *et al.*, 1996; Gilmartin *et al.*, 1976; Helle *et al.*, 1976; Ross *et al.*, 1996; Ross, 2000). As a result of relative ease of sampling and access to larger numbers of samples, pinnipeds have been better studied than cetaceans.

Whereas confounding factors can generally be accounted for in toxicological studies of the more manageable pinnipeds, such is rarely the case in studies of cetaceans. Age and sex, for example, exert a considerable effect on the concentrations of lipophilic chemicals in marine mammals. Males become increasingly contaminated as they grow older, while females off-load contaminants to their offspring during pregnancy and lactation (Addison and Brodie, 1987; Borrell *et al.*, 1995). Past studies of cetaceans where such factors have been accounted for have relied either on samples from stranded animals of questionable quality, on limited numbers of variable-age individuals taken as by-catch in the fisheries sector, or on samples from animals harvested by the whaling industry. More recently, biopsies taken from free-ranging cetaceans have provided information on contaminant concentrations in healthy individuals with minimal invasiveness (Fossi *et al.*, 1992; Marsili and Focardi, 1996). However, the sex and age of the animal sampled are not obvious for most cetaceans, and animals can at best be grouped into age categories, with sex determined later by DNA analysis.

While variable age, sex or condition of cetaceans sampled often interfere with the ability to generate conclusive evidence of toxicity due to organochlorine chemicals, evidence is mounting that free-ranging whales and dolphins are at risk for toxic responses and effects including mixed function enzyme induction (Marsili *et al.*, 1998), endocrine disruption (Subramanian *et al.*, 1987), immunotoxicity (Lahvis *et al.*, 1995) and reproductive impairment (Béland *et al.*, 1993).

Killer whales that frequent the Pacific coastal waters of North America have been the subject of an intensive photo-identification study since 1973, and more anecdotal studies dating back to the 1950s. As a result, the sex and approximate age of virtually all resident killer whales and a large proportion of the transients are known, as are putative relationships among individuals, pods, and populations (Bigg *et al.*, 1990; Ford *et al.*, 1994; Ford and Ellis, 1999; Matkin *et al.*, 1999). In addition, an ongoing genetic study of British Columbia killer whales, based on DNA samples from 175 identified, individuals indicates that interbreeding between the northern and southern residents is rare at most, and has probably not taken place between residents and transients for many generations (LBL, unpub. data). Thus, lifetime dietary habits and feeding areas of individuals can be determined by their group membership.

The free-ranging killer whale communities of British Columbia therefore presented a unique opportunity to obtain samples for contaminant analysis using minimally-invasive biopsy darting techniques. The population identity and detailed individual life histories of these whales enabled a comprehensive interpretation of age- and sex-specific PCB, polychlorinated- dibenzo-*p*-dioxin (PCDD) and- dibenzofuran (PCDF) concentrations in the samples obtained.

Materials and Methods

Sampling

Blubber biopsies were collected for contaminant analysis from 47 killer whales of both sexes, various known ages, and two distinct populations, comprising three major communities, that frequent the waters of British Columbia, Canada, and adjacent areas (i.e. northern and southern residents; transients). These biopsies were collected from killer whales in the coastal waters of central British Columbia (primarily in the region 50°40′N–53°00′N) between 1993–96. Sampling of the southern residents ($n = 6$ samples) was limited because of high vessel traffic in the summer feeding grounds of this population in the Straits of Georgia and Juan de Fuca. A more comprehensive set of samples was

collected from the transients ($n = 15$ samples) and the northern residents ($n = 26$ samples).

A light-weight pneumatic dart system was designed and used for the purpose of obtaining small samples of skin (0.1–0.2 g) from the free-ranging killer whales for the primary purpose of DNA research (LBL, unpub. data), but the attached blubber samples proved valuable for contaminant analysis. The variable-power dart projector and its stainless-steel, 6.4 mm diameter tip, as well as a full description of the sampling procedure, is described elsewhere (Barrett-Lennard *et al.*, 1996). Briefly, samples were collected from small boats at a distance of approximately 5–25 m, following a visual confirmation of the individual's identity based on versions of photographic catalogues of residents (Ford *et al.*, 1994) and transients (Ford and Ellis, 1999) which are continually updated by the participating researchers. The floating and untethered darts which fell from the region immediately posterior to, and below, the dorsal fin of the animal following impact were collected and samples extracted from the dart tip. Skin was removed from the sample and stored for separate DNA studies (LBL, unpub. data), and blubber was placed in pesticide grade hexane-rinsed glass vials with aluminium foil-covered caps and stored at −20°C for contaminant analysis.

Congener-specific PCB, PCDD and PCDF analysis

Blubber samples were analysed for congener-specific PCBs, PCDDs and PCDFs, as well as lipid content. Thawed blubber samples were ground in a porcelain mortar and pestle with 200 g of anhydrous sodium sulphate and spiked with a mixture of $^{13}C_{12}$-labeled PCBs, PCDDs, and PCDFs, as well as pesticides, as supplied by Cambridge Isotope Laboratories (Andover, MA, USA). The blubber-sodium sulphate mixture was transferred to an extraction column and extracted with 250 ml of 1:1 dichloromethane/hexane (DCM/hex) from a glass column by gravity flow. The extract was evaporated to dryness and the residue weighed and related to the original sample weight in order to determine the lipid content of the samples. Subsequently the residue was re-suspended in 1:1 DCM/Hex and divided quantitatively into two aliquots. The larger aliquot (75% of the extract) was subjected to sample-clean-up for PCB, PCDD and PCDF determinations while the remaining aliquot was archived for future pesticide determinations. Details on the sample clean-up and fractionation methodologies utilized, preparation of the silica gel, alumina and carbon fibre columns are described elsewhere (Rantaleinen *et al.*, 1998). Analyses of cleaned-up samples for PCDDs, PCDFs, mono-ortho (MO-), di-ortho (DO-) and non-ortho (NO-) PCBs were conducted by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Details on the instrumental analysis conditions used, the quantification protocols, the criteria used for congener identification and the quality assurance/quality control (QA/QC) measures undertaken for the HRGC/HRMS analysis of all the analytes of interest are described elsewhere (Ikonomou *et al.*, 1998; Rantaleinen *et al.*, 1998).

Although 195 PCB peaks were measured (out of a theoretical total of 209 congeners), many congeners were not detectable in samples of killer whale blubber. Total PCB concentration was calculated as the sum of the concentrations of the 136 congeners that were detectable in at least 70% of the samples. Where congeners were detected in less than 100%, but more than 70%, of blubber samples, the minimum detection limit substitutions were made. Congeners that were detected in less than 70% of the samples were not included in calculations. All results are expressed on a lipid weight (lw) basis. A mean lipid value from killer whale blubber samples analysed (64.3%) was used in lipid-based contaminant determinations for those samples (primarily the transient killer whale samples) that were not analysed for lipid content.

Total Toxic Equivalents (hereafter referred to as TEQ_{98}) to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were calculated for all dioxin-like PCBs (MO PCBs 105, 114, 118, 123, 156, 157, 167, and 189; NO PCBs 77, 81, 126 and 169;) and 2,3,7,8-Cl substituted PCDDs ($n = 7$) and PCDFs ($n = 10$) using the most recent international Toxic Equivalency Factors (referred to hereafter as TEF_{98}) (van den Berg *et al.*, 1998).

Statistics

Inter-community differences in contaminant levels for both males and females were assessed using a one-way analysis of variance (ANOVA), and where significant, were followed post-hoc by a Tukey's Honest Significant Difference (HSD) Test. Sex-related differences within communities were assessed using an unpaired *T*-test. A finite population correction was applied to the error for southern resident male mean contaminant concentrations (sem $\times \sqrt{(1-n/N)}$), since the percentage of the population sampled exceeded 5%. Age- and sex-based regression lines and associated statistics for PCB concentrations in the northern resident killer whales were fitted using Sigma Plot (SPSS, Chicago, USA).

Results and Discussion

Community differences

Surprisingly high total PCB concentrations were found in most killer whale samples, relative to marine mammals studied from different parts of the world, including the industrialized areas of northern Europe and eastern North America (Colborn and Smolen, 1996; Muir *et al.*, 1996; Ross *et al.*, 1996). Significant differences were apparent both within, and among, communities (Fig. 2). While PCB concentrations (all results expressed as mean ± standard error of the mean, or sem) in the northern residents appeared high relative to other studies of marine mammals (adult males: 37.4 ± 6.1 mg kg^{-1} lw; adult females:

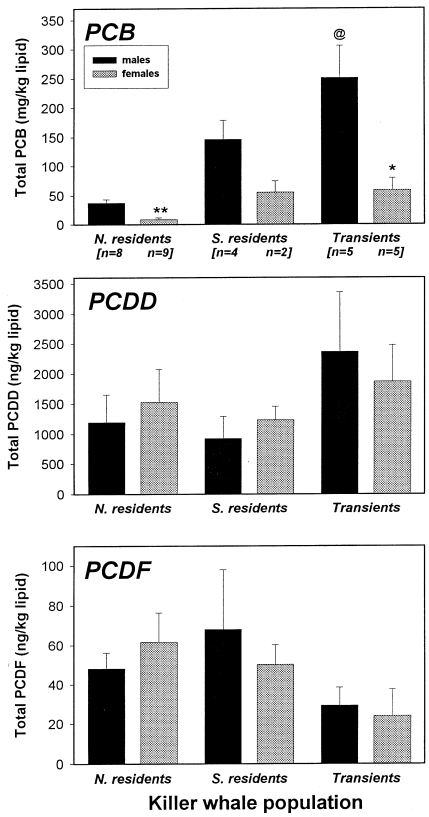

Fig. 2 Mean total PCB ± sem (mg kg^{-1}; sum of the 136 congeners detected), PCDD (sum of all peaks) and PCDF (sum of all peaks) in adult male and female killer whales from northern resident, southern resident and transient communities. Significant inter-community differences existed for PCBs in males (ANOVA; $p < 0.05$), which was due to differences between northern residents and transients (Tukey's HSD test; @ $p < 0.05$). Significant intra-community (i.e., male–female) differences were observed in northern residents and transients (*T*-test; $*p < 0.05$; $**p < 0.01$). No intra- or inter-community differences could be detected for PCDDs or PCDFs (ANOVA). Mean ages for the respective sample groups were 35 and 31 years for northern resident males and females; 35 and 19 years for southern residents; and 24 and 30 years for transients. Sample size for each group is indicated between brackets.

9.3 ± 2.8 mg kg^{-1} lw), they were generally lower than those observed in the southern residents (males: 146.3 ± 32.7 mg kg^{-1} lw; females: 55.4 ± 19.3 mg kg^{-1} lw), and the transients (males: 251.2 ± 54.7 mg kg^{-1} lw; females 58.8 ± 20.6 mg kg^{-1} lw).

Samples obtained from six stranded killer whales from south-western British Columbia and northern Washington in the late 1980s (Jarman *et al.*, 1996), and from two adults described as 'open ocean' individuals in 1986 (Ono *et al.*, 1987), also contained high PCB concentrations (range 9.1–61.5 mg kg^{-1} lw, $n = 6$; and 360–410 mg kg^{-1} lw, $n = 2$, in the respective studies), although variable sample quality, limited background information and different analytical techniques preclude a direct comparison with our results. Mean total PCB concentrations in both the transients and southern residents in our study greatly exceeded those measured in the highly contaminated St. Lawrence beluga whales, *Delphinapterus leucas* (males: 78.9 mg kg^{-1} lw; females: 29.6 mg kg^{-1} lw) (Muir *et al.*, 1996). Of the comprehensive reports of PCBs in cetaceans, only the western Mediterranean striped dolphin, *Stenella coeruleoalba*, with a median concentration of 282 mg kg^{-1} lw (range 100–500 mg kg^{-1} lw) in biopsies collected from free-ranging individuals (Aguilar and Borrell, 1994a), appears to fall into the same range as the transient killer whales in our study.

Dietary contaminant concentrations and trophic position appear to play major roles in the accumulation of PCBs in the three killer whale communities. Both resident killer whale communities have a strong preference for adult salmon (96% of their estimated total diet), with chinook (*Oncorhyncus tshawytscha*) being the salmon species most frequently identified in their prey (Ford *et al.*, 1998). The elevated PCB concentrations observed in southern resident killer whales relative to their northern counterparts might be the result of ingesting small amounts of highly contaminated prey items near the industrialized areas of south-western British Columbia and north-western Washington State. However, chinook salmon spend most of their time in the open Pacific Ocean, and their high trophic level relative to other salmonids may also cause them to accumulate high concentrations of PCBs. In this case, the atmospheric deposition of PCBs into the North Pacific Ocean may represent an important route for food chain contamination in this region of the world. Concentrations of PCBs in stocks of chinook salmon returning from the Pacific Ocean to Puget Sound have been shown to be relatively contaminated with PCBs (mean of 2.2 mg kg^{-1} lw) (O'Neill *et al.*, 1998).

Interestingly, high PCB concentrations found in sea otters (*Enhydra lutris*) and Bald eagle (*Haliaeetus leucocephalus*) eggs from the Aleutian Islands could reflect contamination by local military installations, but may also point to atmospherically transported PCBs of Asian origin (Anthony *et al.*, 1999; Estes *et al.*, 1997). Relatively high PCB levels have also been observed in tissues and eggs of black-footed albatross (*Diomedea nigripes*) on Midway atoll, likely reflecting food chain bioaccumulation from the north Pacific Ocean (Auman *et al.*, 1997; Jones *et al.*, 1996). PCB concentrations in air and surface water samples collected during a global study did not reveal unusually high inputs into the north Pacific relative to other ocean sites, although Asian sources of persistent organochlorine pollutants were apparent (Iwata *et al.*, 1993).

Transient killer whales, on the other hand, have a diet that consists almost exclusively of marine mammals. Harbour seals (*Phoca vitulina*), Steller sea lions (*Eumetopias jubatus*), Dall's porpoises (*Phocoenoides dalli*) and harbour porpoises (*Phocoena phocoena*), represented 53%, 13%, 12% and 11% of observed predatory events, respectively, in a recent study (Ford *et al.*, 1998).

Because their movements are wide-ranging and they do not appear to regularly frequent industrialized coastal areas, the higher degree of PCB contamination in transients most likely reflects their elevated trophic position relative to residents. Many of the pinniped and cetacean species frequenting the Pacific coastal waters of North America consumed by transients are known to be more contaminated with these chemical contaminants than species at lower trophic levels (Hong *et al.*, 1996; Jarman *et al.*, 1996; Ross *et al.*, 1998).

While dietary sources appear important for PCB accumulation in killer whales, a structure-related preferential accumulation of certain types of congeners may also contribute to the high PCB concentrations observed in these long-lived animals. A selective retention of non-planar, or globular, PCBs as a result of low cytochrome P450 2B (CYP 2B)-type activity is thought to be characteristic of cetaceans (Goksoyr, 1995; Norstrom *et al.*, 1992; Tanabe *et al.*, 1988; Watanabe *et al.*, 1989).

Despite the sharply contrasting diets of resident and transient killer whales (i.e., fish vs marine mammals), the mean congener-specific PCB profiles were remarkably similar among the three communities studied (Fig. 3). Typical of patterns observed in other studies of cetaceans, profiles were dominated by the higher chlorinated congeners, with most of the lower chlorinated congeners being absent or present at very low levels. The recalcitrant PCBs 153 and 138 dominated the patterns in all three killer whale communities, consistent with other studies of PCBs in cetaceans (Jarman *et al.*, 1996; Muir *et al.*, 1996). The sum of congeners 52, 101, 118, 153, 138, and 180 accounted for $48.7 \pm 0.4\%$ (mean $\pm$ sem), $48.4 \pm 0.2\%$ and $56.1 \pm 0.4\%$ of the total PCB concentrations in the male northern residents, southern residents and transients, respectively (ANOVA $p < 0.001$; Tukey's HDS test: $p < 0.001$ for both resident communities compared to transients). The contribution of PCB 153 to total PCB increased from northern residents $(16.1 \pm 0.3)\%$ to southern residents $(19.0 \pm 0.3)\%$ to transients $(25.5 \pm 0.8)\%$ (ANOVA: $p < 0.001$; Tukey's HSD test: $p < 0.05$ for all combinations), possibly reflecting pattern differences in their respective diets, or indicating that metabolism of some of the less recalcitrant PCB congeners increased with increasing degree of contamination.

While PCB concentrations were high relative to other marine mammals studied, total PCDDs (sum of concentrations from an average of 13 peaks in killer whale samples) and PCDFs (sum of an average 10 peaks), including both 2,3,7,8-substituted and -unsubstituted congeners, did not appear high in any of the killer whale communities, with many congeners being undetectable. There were no significant differences among communities for either of these classes of compounds (ANOVA; results not shown). This is perhaps surprising, because dioxin- and furan-producing bleach kraft pulp mills have historically introduced large quantities of these compounds into the coastal waters of British Columbia, leading to localized contamination and subsequent fisheries closures (Macdonald *et al.*, 1992).

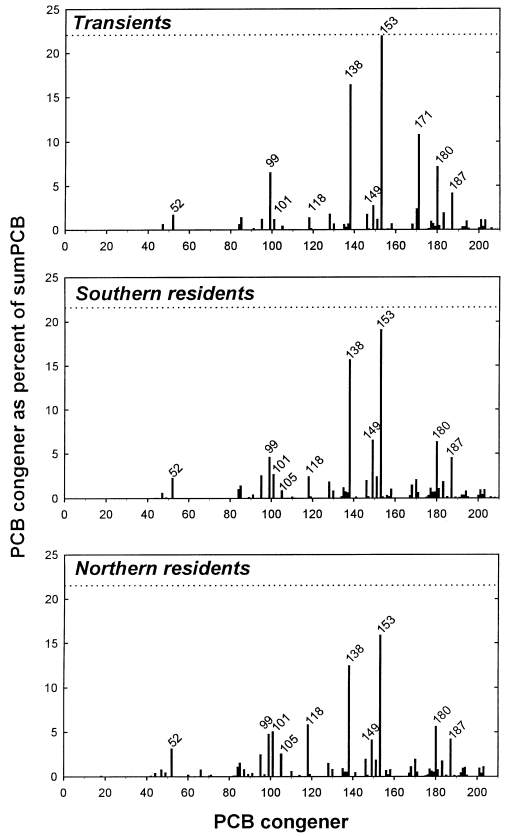

Fig. 3 Mean PCB congener profiles in killer whale blubber samples for the northern resident, southern resident and transient communities, expressed as a percentage of the total PCB concentration. Dotted lines have been placed at the transient PCB 153 level in the plots of the two resident populations for comparative purposes.

Effects of age and sex

A relatively large number of samples (i.e. $n = 26$) from individuals spanning a broad age distribution were collected from the northern resident killer whales, allowing us to examine the roles of age and sex in contaminant accumulation in killer whales. Adult females exhibited a dramatically different age-related pattern of total PCB accumulation compared to the continuous increase observed with age in immature whales of both sexes, and adult males (Fig. 4). Total PCB concentration began to decline markedly at the estimated average age of first calving (defined here as 15 years (Olesiuk *et al.*, 1990), unless proven otherwise by observations of a female with a calf). Low concentrations were observed until the reproducing females reached approximately 50 years of age, at which point PCB concentrations once again increased. Although a considerable amount of variation was observed in the age-related increase in

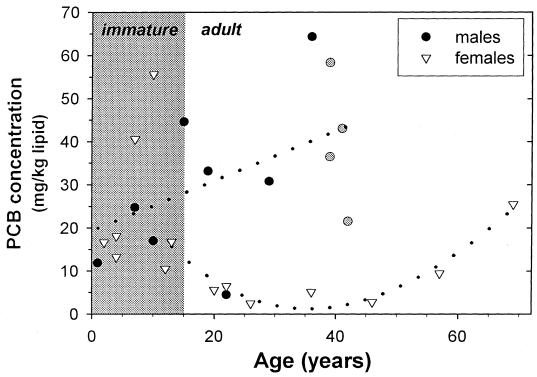

Fig. 4 Total PCB (sum of the 136 detectable congeners) concentrations (mg kg^{-1} lw) in northern resident killer whales are plotted against age. Regression lines approximating these relationships are plotted for (i) males and immatures ($y = y_0 + ax$; $y = 19.44 + 0.5769 \times$ age; regression $r^2 = 0.23$, $p < 0.05$; slope (a) $p < 0.01$; y_0 $p < 0.001$); and (ii) adult females ($y = y_0 + ae^{-bx} + cx$; $y = -270.1 + 310.4e^{-.01724\times\text{age}} + 2.901 \times$ age; regression $r^2 = 0.94$, $p < 0.01$; y_0, a, b, c are not significant). Male killer whales are represented by closed circles (those for which minimum age estimates only are available are lightly shaded), and females by triangles.

PCB concentrations in immatures and males, possibly reflecting individual differences in dietary preference and physiology, the relationship observed was described by a linear function, whereas the relationship for adult females approximated a polynomial exponential decay function:

$$[\text{PCB}]_{\text{males+immatures}} = 19.44 + 0.5769 \times \text{age}, \quad (1)$$

$$[\text{PCB}]_{\text{females}} = -270.1 + 310.4e^{-0.01724\times\text{age}} + 2.901 \times \text{age}. \quad (2)$$

These relationships serve as a general guide for describing age- and sex-related PCB concentrations, but a greater sample size would be required to more accurately describe some of the variation introduced by, for example, multiple calving during the reproductive life of females. The age- and sex-related patterns of total PCB accumulation in the northern resident killer whales are similar to those described in both pinnipeds (Addison and Brodie, 1977; Muir *et al.*, 1988) and cetaceans (Aguilar and Borrell, 1994b; Borrell *et al.*, 1995), although our study represents the first detailed examination of this phenomenon using minimally invasive techniques in live marine mammals.

Generally, cetacean females off-load the majority (>60%) of their organochlorine burden to their calf during reproduction, with most of this transfer taking place during lactation (Borrell *et al.*, 1995; Tanabe *et al.*, 1982). The increase in PCB concentration in older females most likely reflects reproductive senescence, since resident killer whales have been reported to cease reproduction at the approximate age of 40–45 years (Olesiuk *et al.*, 1990). The two older females sampled in our study were confirmed to be post-reproductive (GME, unpub. obs.). A similar pattern has been observed in short-finned pilot whales (*Globicephala macrorhynchus*), where females had increasing total PCB and DDE concentrations later in life, at a time of presumed reduced reproductive activity (Tanabe *et al.*, 1987). A positive correlation between PCB concentration and age in female beluga whales sampled postmortem in the St Lawrence estuary may have been partly due to the large number of post-reproductive animals examined (Muir *et al.*, 1996).

In contrast to PCBs, there were no age- or sex-related patterns in total PCDD or PCDF concentrations in the northern resident killer whales (results not shown; all samples combined: mean total PCDD 1050 ± 258 ng kg^{-1} lw; PCDF 55 ± 6 ng kg^{-1} lw). Together with the observations of low total PCDD and PCDF concentrations in samples from all three killer whale communities noted previously, and lack of concentration differences among them, these results may partly reflect a limited food chain biomagnification, or low dietary concentrations of these compounds. However, these results more likely indicate minimal bioaccumulation in killer whales, as a consequence of the metabolism and excretion of planar 'dioxin-like' compounds.

The preferential ability to metabolize and eliminate 'dioxin-like' compounds, including some of the PCBs, PCDDs and PCDFs, has been inferred from observed pattern differences between predator and prey in both pinniped and cetacean species (Boon *et al.*, 1994, 1997; De Swart *et al.*, 1995; Kannan *et al.*, 1989; Tanabe *et al.*, 1988). These observations are supported by studies which have characterized the presence and activity of cytochrome P450 1A (CYP 1A) detoxifying enzymes in marine mammals (Goksoyr, 1995; Watanabe *et al.*, 1989). Low to undetectable concentrations of PCDDs and PCDFs have also been observed in other studies of cetaceans where animals sampled were considered to be relatively contaminated with PCBs (Jarman *et al.*, 1996; Muir *et al.*, 1996). An examination of TEQ_{98} profiles for the PCBs, PCDDs and PCDFs among the broadly sampled northern residents provides further evidence of a metabolic removal of 'dioxin-like' compounds in killer whales (Table 1). Firstly, TEQ_{98} concentrations for the PCDDs, PCDFs and NO PCBs were very low in immatures, adult males and females compared to the more globular MO PCBs. Secondly, there were no differences in the TEQ_{98} concentrations for the NO PCB congeners, total PCDDs or total PCDFs among immatures, males and females.

As expected from the age- and sex-related patterns observed for PCBs (Fig. 4), total TEQ_{98} in the northern resident killer whales were highest in adult males, followed by the immatures and the adult females (Table 1). PCBs contributed up to 97% of the total TEQ_{98}, although adult females had a proportionately higher contribution of the PCDDs and PCDFs than did the immatures or the adult males. Total PCB

TABLE 1

Mean (±standard error of the mean; sem) Toxic Equivalents (TEQ; ng/kg lipid) to 2,3,7,8-TCDD for individual PCB congeners ([1]IUPAC) and total PCDDs and PCDFs quantified in the blubber of immature, adult male and adult female northern resident killer whales.[a]

	PCB congener[(1)]	$TEF_{98}^{(2)}$	Mean $TEQ_{98}^{(2)}$ (ng/kg lipid) ± sem			Statistical differences (a) immatures vs (b) males vs (c) females
			(a) Immatures	(b) Males	(c) Females	
Non-ortho PCBs	77	0.0001	0.03 ± 0.01	0.05 ± 0.03	0.04 ± 0.01	ns
	81	0.0001	0.01 ± 0.001	0.01 ± 0.002	0.005 ± 0.001	ns
	126	0.1	16.67 ± 2.78	21.48 ± 5.42	11.69 ± 1.99	ns
	169	0.01	2.57 ± 0.35	3.23 ± 0.77	1.39 ± 0.15	ns
Sum noPCBs			19.27 ± 2.92	24.77 ± 6.17	13.12 ± 2.06	ns
% of $\sum$PCB TEQ_{98}			8.0 ± 1.7%	5.8 ± 0.8%	19.7 ± 4.1%	ab, bc
Mono-ortho PCBs	105	0.0001	52.43 ± 10.18	73.84 ± 9.57	13.62 ± 5.63	ab,bc
	114	0.0005	13.69 ± 1.93	22.17 ± 3.62	4.68 ± 1.88	ab,bc
	118	0.0001	118.88 ± 21.33	185.85 ± 28.78	37.10 ± 15.99	ab,bc
	123	0.0001	1.11 ± 0.20	1.28 ± 0.35	0.26 ± 0.05	ab,bc
	156	0.0005	55.11 ± 10.31	82.05 ± 11.54	20.65 ± 4.52	ns
	157	0.0005	21.86 ± 6.47	31.22 ± 4.08	6.23 ± 1.67	bc
	167	0.00001	0.75 ± 0.20	1.11 ± 0.14	0.26 ± 0.08	bc
	189	0.0001	1.27 ± 0.37	2.22 ± 0.29	0.93 ± 0.17	bc
Sum noPCBs			265.10 ± 50.20	399.74 ± 54.06	83.74 ± 29.11	ab,bc
% of $\sum$PCB TEQ_{98}			92.0 ± 1.7%	94.3 ± 0.8%	80.35 ± 4.11%	ab, bc
	Sum PCB TEQ_{98}		284.37 ± 49.44	424.51 ± 58.37	96.86 ± 28.87	ab,bc
			95.6 ± 0.9%	97.2 ± 0.3%	82.8 ± 2.8%	ab,bc
	Sum PCDD TEQ_{98}		6.45 ± 0.78	6.73 ± 1.18	9.50 ± 1.18	ns
			2.5 ± 0.4%	1.7 ± 0.3%	11.0 ± 1.8%	ab,bc
	Sum PCDF TEQ_{98}		5.16 ± 0.94	4.31 ± 0.67	4.58 ± 1.01	ns
			2.3 ± 0.6%	1.1 ± 0.1%	6.2 ± 1.4%	ab,bc
Sum TEQ_{98}			295.42 ± 49.05	435.54 ± 60.22	111.64 ± 29.10	ab,bc

[a] The recent international TEFs from 1998 (TEF_{98}) are used[2] (van den Berg, *et al*., 1998). Percentage contributions to the subtotal and total TEQ_{98} are given for each PCB subclass, as well as the PCDDs and PCDFs. When significant differences within groups were detected using one-way ANOVA ($p < 0.05$), a Tukey's HSD test was used to determine which of the immatures, males and females differed from each other ($p < 0.05$).

TEQ_{98} were an average of 23% lower than had they been estimated using the previous listing of TEFs (Ahlborg *et al*., 1994), mainly due to the omission of the DO PCBs (170 and 180) in the most recent TEQ_{98} listing (van den Berg *et al*., 1998). Of the PCBs, the MO PCBs contributed the most to the PCB TEQ_{98}, with PCBs 118, 156 and 105 contributing approximately 80% to the total PCB TEQ_{98} in northern residents. The dominant contribution of PCBs to the total TEQ_{98}, and the MO PCBs, in particular, is consistent with observations in other studies of cetaceans (Jarman *et al*., 1996; Kannan *et al*., 1993; Tanabe *et al*., 1989).

PCB loads in resident killer whale populations

Since detailed age information and total population numbers are available for the two resident killer whale communities, we estimated the total mass of PCBs in these communities (Table 2). For the broadly sampled northern residents, we first applied the regression equations generated previously for immatures plus adult males [1] and adult females [2] to estimate concentrations for known-age individuals based on the life tables for the community (Bigg *et al*., 1990) (updated by GME, unpub. data).

While detailed life history information was also available for the southern residents (Bigg *et al*., 1990)

TABLE 2

Total estimated PCB loads (kg) in resident killer whale populations frequenting British Columbia coastal waters were modeled using life tables for the two communities.[a]

	Northern residents (kg)	Southern residents (kg)	Totals (kg)
Immatures	0.75 (103)	0.96 (40)	1.71 (143)
Adult males	1.70 (39)	2.04 (12)	3.74 (51)
Adult females	0.34 (70)	1.65 (37)	1.99 (107)
Total	2.79 (212)	4.65 (89)	7.44 (301)

[a] Estimates were derived from age- and sex-dependent regression equations for the better-studied northern residents, and a corrected extrapolation of these relationships to the southern residents. Total numbers of killer whales in the two communities of residents and their respective age categories are listed in parentheses.

(updated by GME, unpub. data), the limited number of samples precluded the use of regression equations to accurately estimate the total PCB load for the community. We therefore extrapolated the model generated for the northern residents to the southern residents by using the mean ratios between observed PCB concentrations in the six southern residents (four males, two females) to values predicted for their age-matched northern resident counterparts. In this manner, PCB concentrations in southern residents were estimated to be approximately four times higher in males, and six times higher in females, than in northern residents. The limited sample numbers used to develop this relationship underline the need for caution in the interpretation of this extrapolation and preclude further statistical evaluation, but this exercise is used as a general guide for estimating population contaminant loads.

For both communities of residents, we assumed a mean 26% total body lipid content for killer whales (using a value of 29% body weight for blubber in killer whales (Christensen, 1982) and assuming an 80% lipid blubber content, with lipid from muscle and viscera contributing to approximately 2.5% of body weight, as described for pilot whales (Lockyer, 1993)). We then used a relationship established between body weight (bw; kg) and length (L; cm) for killer whales (Bigg and Wolman, 1975):

$$bw = 0.000208(L)^{2.577},$$

and approximate age-length relationships documented for killer whales (Ford, pers. comm.) to model total PCB loads for the immatures, adult males, and adult females of the two resident communities:

$$\text{PCB load} = \sum_{1-N} [bw \times 0.26 \times \text{ equation (1) or (2);} \\ \text{or (1)} \times 3.6 \text{ or (2)} \times 5.9].$$

Using this model, we estimated the total PCB load in the 212 killer whales of the northern resident community to be 2.79 kg, of which the majority (61%) could be found in adult males, which represent the minority (18%) of this community (Table 2). The total PCB load in the 89 whales of the southern resident community was estimated to be 4.65 kg, of which the majority (44%) can be found in adult males, which represent the minority (13%) of this community (Table 2). We estimate that a total 7.44 kg of PCBs is present in the resident killer whales of British Columbia. These figures may be useful in efforts to model the transport and fate of persistent contaminants in the north Pacific Ocean, and provide a means of comparing total loads in different wildlife populations or different trophic levels.

Toxicological implications of high PCB concentrations in killer whales

While significant differences in total PCB concentration were apparent among males sampled from the three killer whale communities, no differences could be detected for total TEQ_{98} (results not shown). Southern residents (845 ± 195 ng kg^{-1} lw in adult males and 456 ± 27 ng kg^{-1} lw in adult females) and transients (699 ± 142 ng kg^{-1} lw in adult males and 338 ± 53 ng kg^{-1} lw in adult females) appeared to have high total TEQ_{98} concentrations relative to northern residents (Table 1; 436 ± 60 ng kg^{-1} lw in adult males and 1 12 ± 29 ng kg^{-1} lw in adult females), but this was not significant (ANOVA). Metabolic removal of 'dioxin-like' compounds may have caused a concentration-related reduction in total TEQ_{98} in more contaminated animals (i.e. transients), as has been inferred in other studies (Boon *et al.*, 1997; Tanabe *et al.*, 1988). Alternatively, limited sample size and the variable ages of the animals sampled in each population may have masked inter-community differences.

To eliminate the confounding effect of age on contaminant accumulation in males and females, and allow a comparative assessment of risk among the three killer whale communities, we generated age-adjusted estimates of total TEQ_{98}. In an approach similar to that used to model total PCB loads in southern residents, we extrapolated total TEQ_{98} regression equations generated for the better-studied northern residents to both the southern residents and the transients. The median ages observed in the southern resident community (males 12 years; females 25 years) were used to estimate age-matched TEQ_{98} concentrations for all three communities. Age-adjusted total TEQ_{98} were estimated for northern residents using:

$$[TEQ_{98}]_{male} = 250.1 + 6.127 \times (12 \text{ years}), \quad (3)$$

$$[TEQ_{98}]_{female} = -2700 + 3031\ e^{(-0.01556 \times 25 \text{ years})} + 28.14 \times 25 \text{ years} \quad (4)$$

and adjusted for southern residents by $[TEQ_{98}]_{male} = 1.75 \times$ equation (3) and $[TEQ_{98}]_{female} = 5.27 \times$ equation (4), and for transients by $[TEQ_{98}]_{male} = 2.19 \times$ equation (3) and $[TEQ_{98}]_{female} = 4.73 \times$ equation (4) (Fig. 5). An independent regression analysis of observed TEQ_{98} vs age for male and female transients ($n = 15$) generated age-adjusted TEQ_{98} values that were within 4% of those estimated using the extrapolative model applied above for males, and 14% for females, thereby supporting the extrapolative approach used for the southern residents.

The high total PCB and TEQ_{98} concentrations observed in killer whales from the three communities studied, and the transients in particular, are cause for concern. While it is virtually impossible to directly assess whether these contaminant levels are adversely affecting free-ranging killer whales, low to moderate concentrations of both the 'dioxin-like' and the non-'dioxin-like' PCBs, PCDDs and PCDFs are known to cause immunotoxicity, neurotoxicity, reproductive impairment and endocrine disruption in laboratory animals (Brouwer *et al.*, 1998; Vos and Luster, 1989) and wildlife species

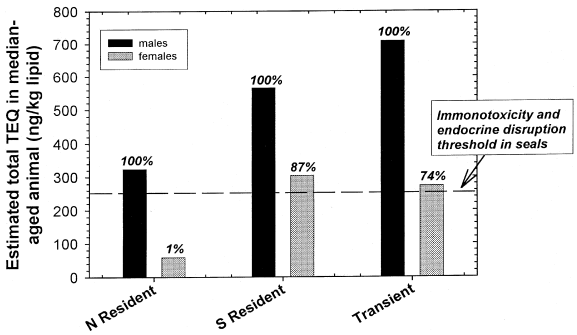

Fig. 5 Estimated total dioxin Toxic Equivalents (TEQ_{98}; ng kg^{-1} lw) in 12-year-old male and 25-year-old female killer whales from each of the three communities studied. These represent the median ages for the southern resident community, with the age-adjusted estimates allowing for a more direct comparative evaluation of risk among the three communities. Numbers above each bar represent the estimated percentage of males and females from the three free-ranging killer whale communities ($N = 212$, 89 and at least 219 individuals, respectively) in British Columbia that exceed the toxicity threshold established previously for harbour seals (De Swart et al., 1995; Ross et al., 1995).

(Colborn *et al.*, 1993; Fry, 1995; Guillette *et al.*, 1995; Luebke *et al.*, 1997).

Two captive feeding studies found that harbour seals fed fish from contaminated areas exhibited reproductive impairment and reduced plasma retinol and thyroid hormone levels (Brouwer *et al.*, 1989; Reijnders, 1986), and impaired immune function (for reviews see (De Swart *et al.*, 1996; Ross *et al.*, 1996)). In the latter, mainly immunotoxicological study, blubber biopsies taken two years after the start of the experiment revealed that seals in the 'contaminated' group which exhibited toxic effects had accumulated a total PCB concentration of 16.8 mg kg^{-1} lw or 209 ng kg^{-1} lw total TEQ (De Swart *et al.*, 1995; Ross *et al.*, 1995). We adjusted this threshold upwards to 255 ng kg^{-1} TEQ_{98} by using the updated TEF_{98} values and by extrapolating the percent contributions of congeners analyzed in killer whales to estimate those 'dioxin-like' PCB congeners not measured in the immunotoxicological study of seals. Most of the killer whales sampled in our study easily surpassed this toxicity level established for seals. In fact, we predict that virtually all of the male killer whales from the three communities and most of the female southern residents and transients exceed the toxic threshold established for harbour seals (Fig. 5).

Although experimental studies are generally limited to the use of laboratory animals, and occasionally captive pinnipeds, there are some indications that free-ranging cetaceans are being adversely affected by contaminants. An inverse relationship between T-cell function and total PCB TEQ was observed in blood samples obtained from five bottlenose dolphins, *Tursiops truncatus*, in the Gulf of Mexico (Lahvis *et al.*, 1995). A negative correlation was observed between DDE concentrations and plasma testosterone concentrations in Pacific Dall's porpoises (Subramanian *et al.*, 1987). A positive relationship was found between skin benzo(a)pyrene monooxygenase activity and total blubber PCB and DDT concentrations in biopsies obtained from fin whales (*Balaenoptera physalus*) in the Mediterranean Sea (Marsili *et al.*, 1998). Higher concentrations of total PCBs in striped dolphins that succumbed to an epizootic of dolphin morbillivirus in the Mediterranean Sea compared to biopsies taken from healthy animals may have reflected a contaminant-related immunotoxicity (Aguilar and Borrell, 1994a). While exact age was not taken into account and may have played a confounding role in these studies, results underline the need for more research. High contaminant concentrations have been implicated in a low recruitment rate and a large number of tumors in the St. Lawrence population of belugas, which has been protected since 1962 (De Guise *et al.*, 1995). In addition, *in vitro* exposures of leucocytes suggest that current contaminant exposure levels in the population may be high enough to cause immunotoxicity (De Guise *et al.*, 1998).

Together with the stresses of past live-fisheries for aquaria (Bigg and Wolman, 1975), heavy boat traffic, and food supply pressures, high PCB concentrations are likely to present an added risk for the killer whale communities of British Columbia. Stable PCB concentrations in a number of environmental compartments in the industrialized world since the mid-1980s (Addison and Smith, 1998; Olsson and Reutergardh, 1986; Tanabe *et al.*, 1994), and the limited ability of cetaceans to metabolize many of the higher chlorinated PCBs (Boon *et al.*, 1997), ensure that PCBs will continue to present a risk to free-ranging cetaceans for some time. While further research into the well-documented populations of killer whales in British Columbia may shed some light on the effects of these contaminant levels on their reproductive success, the immunotoxic potential of PCBs may render these populations more vulnerable to unpredictable scales and frequencies of disease events.

Conclusions

By using minimally invasive biopsy techniques to collect blubber samples from three communities of healthy, free-ranging, Pacific killer whales for which detailed life history information was available, we found that PCB accumulation was strongly related to age, sex and dietary preference (trophic level). PCB, PCDD and PCDF patterns in the three communities suggest that a structure-related selective retention and/or metabolic removal of certain congeners play a major role in contaminant accumulation in killer whales. The marine mammal-eating transient killer whales were particularly contaminated with persistent PCBs, and, together with the southern residents, represent some of the most contaminated cetaceans studied in the world. Profiles of total TEQ_{98} suggest that the PCBs present the greatest 'dioxin-like' risk to killer whales, and are present at levels in the majority of both resident and transient

individuals which surpass those found to be immunotoxic and endocrine disrupting in harbour seals. While future studies may be able partially to assess the toxic impact of these chemicals on Pacific killer whales, a 'weight of evidence' approach which combines results from laboratory animal studies with results from semi-field and field studies of marine mammals (Ross, 2000), suggests that current concentrations of PCBs represent a significant toxicological risk to the populations in British Columbia.

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