ELSEVIER

The Science of the Total Environment 281 (2001) 183–203

the Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

www.elsevier.com/locate/scitotenv

Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (*Orcinus orca*) from Prince William Sound, AK

Gina M. Ylitalo[a,*], Craig O. Matkin[b], Jon Buzitis[a], Margaret M. Krahn[a], Linda L. Jones[a], Teri Rowles[c], John E. Stein[a]

[a] *United States Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Services, Northwest Fisheries Science Center, 2725 Montlake Blvd. East, Seattle, WA 98112-2097, USA*
[b] *North Gulf Oceanic Society, 60920 Mary Allen Ave., Homer, AK 99603, USA*
[c] *United States Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Services, Office of Protected Resources, 1315 East-West Highway, Silver Spring, MD 20910, USA*

Received 24 October 2000; accepted 10 March 2001

Abstract

Certain populations of killer whales (*Orcinus orca*) have been extensively studied over the past 30 years, including populations that use Puget Sound, WA, the inside waters of British Columbia, Southeastern Alaska and Kenai Fjords/Prince William Sound, Alaska. Two eco-types of killer whales, 'transient' and 'resident', occur in all of these regions. These eco-types are genetically distinct and differ in various aspects of morphology, vocalization patterns, diet and habitat use. Various genetic and photo-identification studies of eastern North Pacific killer whales have provided information on the male–female composition of most of these resident pods and transient groups, as well as the approximate ages, reproductive status and putative recruitment order (birth order) of the individual whales. Biopsy blubber samples of free-ranging resident and transient killer whales from the Kenai Fjords/Prince William Sound, AK region were acquired during the 1994–1999 field seasons and analyzed for selected organochlorines (OCs), including dioxin-like CB congeners and DDTs. Concentrations of OCs in transient killer whales (marine mammal-eating) were much higher than those found in resident animals (fish-eating) apparently due to differences in diets of these two killer whale eco-types. Certain life-history parameters such as sex, age and reproductive status also influenced the concentrations of OCs in the Alaskan killer whales. Reproductive female whales contained much lower levels of OCs than sexually immature whales or mature male animals in the same age class likely due to transfer of OCs from the female to her offspring during gestation and lactation. Recruitment order also influenced

* Corresponding author.

PII: S0048-9697(01)00846-4

the concentrations of OCs in the Alaskan killer whales. In adult male residents, first-recruited whales contained much higher OC concentrations than those measured in non-first-recruited (e.g. second recruited, third recruited) resident animals in the same age group. This study provides baseline OC data for free ranging Alaskan killer whales for which there is little contaminant information.

Keywords: Killer whales; Transients; Residents; Organochlorines; Chlorobiphenyls (CBs); DDTs; Toxic equivalents (TEQs)

1. Introduction

Organochlorines (OCs) are persistent chemical contaminants that frequently occur in the marine environment. Many of these compounds, including chlorobiphenyls (CBs) and DDTs, are highly lipophilic and can bioaccumulate in relatively high concentrations in top level predators of the marine food web through trophic transfer. Because many of these contaminants are toxic to humans and wildlife, open uses and the manufacture of CBs in the US was ceased in 1977 (Beeton et al., 1979) and use of DDT was banned for use in the US in 1972 (Ahmed, 1991). However, several of these compounds continue to be used as agricultural and industrial chemicals in other parts of the world, including countries from South America and Asia (Schmidt, 1998). OCs enter the marine environment via several sources (i.e. atmospheric transport, landfill runoff) and are found in environmental samples from all over the world, including remote, non-industrial areas such as Alaska, the Canadian Arctic, Greenland (AMAP, 1998; Barrie et al., 1992; Iwata et al., 1993; Muir et al., 1992).

Killer whales (*Orcinus orca*), the largest species in the Delphinidae family, are relatively long-lived animals with mean life expectancies of approximately 50 years for females and 30 years for males (Olesiuk et al., 1990), and maximum life expectancies of 80–90 years for females and 50–60 years for males. These animals are abundant in coastal waters and high latitudes, with well-studied populations occurring in Puget Sound, the inside waters of British Columbia, Southeastern Alaska and Kenai Fjords/Prince William Sound, Alaska. Two eco-types of killer whales, 'transient' and 'resident', occur in all of these regions (Bigg, 1982; Bigg et al., 1990; Dahlheim, 1997; Matkin et al., 1999a). These eco-types are genetically distinct (Hoelzel et al., 1998; Barrett-Lennard, pers. comm.) and differ in various aspects of morphology, vocalization patterns and habitat use (Bigg, 1987; Morton, 1990; Jurk, pers. comm.). The social structure of resident killer whale populations from the Eastern North Pacific appears complex. The residents travel in large groups called pods, which center on mature females and are considered matriarchal societies. The resident killer whales in the Kenai Fjords/Prince William Sound region travel in stable pods of 6–36 individuals composed of females and their descendents (Matkin et al., 1999a,b). Pod membership is supported by pod-specific vocal dialect (Bigg et al., 1990; Ford, 1991). In contrast, transient whales from this region travel in smaller, more fluid groups than residents (typically composed of 1–7 individuals) (Matkin et al., 1999b). Although transient groups may consist of a female whale and her offspring, some transient groups are composed of only males (Matkin et al., 1999b). Genetic and photo-identification studies of Alaskan killer whales have provided information on the male–female composition of most of these resident pods and transient groups, as well as the approximate ages, reproductive status and putative recruitment order of the individual whales (Heise et al., 1992; Dahlheim, 1997; Hoelzel et al., 1998; Matkin et al., 1999a,b).

The resident and transient killer whales from the Kenai Fjords/Prince William Sound, AK region have distinct dietary preferences and feed at different trophic levels. Saulitis et al. (2000) found that Prince William Sound transients feed on marine mammals, primarily harbor seals and Dall's porpoise while the sympatric resident whales eat fish, primarily salmon. Transients often feed along shorelines and in glacial areas while residents most often forage offshore (Sheel,

pers. comm.). Because of differences in diet and habitat use by resident and transient killer whales, differing contaminant levels might be expected in the two killer whale eco-types. Relatively high levels of contaminants might be expected in killer whales, especially transients, since they are top level predators in Eastern North Pacific waters.

Little comprehensive contaminant data are available for Alaskan killer whales, especially free-ranging animals. As part of a collaborative study between the Environmental Conservation Division at the Northwest Fisheries Science Center and the North Gulf Oceanic Society, blubber samples were acquired by biopsy from free-ranging killer whales in the Prince William Sound/Kenai Fjords region from 1994 to 1999 to determine if killer whales have levels of toxic OC contaminants that could negatively affect the whales. From these data, the influences of diet as well as biological factors including sex, reproductive status and recruiment (birth) order on contamination concentrations were assessed.

2. Materials and methods

2.1. Whale identification

Using a photo-identification catalogue (Matkin et al., 1999b), each killer whale was visually identified prior to obtaining a biopsy sample. In addition, identification photographs were taken of the individual at the time of sampling to confirm its identity or for later identification using the method of Bigg et al. (1986). Each biopsied killer whale is listed in Table 1, based on eco-type (resident or transient) and pod or group membership. Information on sex, approximate age (e.g. <34, 28?), reproductive status (i.e. sexually immature, reproductive female, sexually mature male) and putative recruitment order (i.e. first-recruited, non-first-recruited) were determined using methods detailed in Matkin et al. (1999b). Reproductive status of each killer whale was based on age, association analysis and direct observation (Matkin et al., 1999a,b). Killer whales <15 years of age were classified as sexually immature (except AI04, a female known to have given birth at age 13), females ≥ 15 years of age were grouped as reproductive and males ≥ 15 years of age were designated as sexually mature. Putative recruitment order of certain resident killer whales was inferred by direct observation (Matkin et al., 1999a,b).

2.2. Field sample collection

Biopsy samples for chemical contaminant analyses were collected from 77 individual free-ranging killer whales from the Kenai Fjords/Prince William Sound region (Fig. 1) during the months May–September, 1994–1999. Biopsy samples of free-ranging marine mammals have been used for various chemical and biological studies, including genetic and biomarker analyses, isotopic studies of diets and chemical contaminant monitoring (Woodley et al., 1991; Fossi et al., 1997; Todd et al., 1997; Worthy and Abend, 1997; Hoelzel et al., 1998). This sampling technique provides fresh tissues of marine mammals, such as killer whales, which do not commonly strand in the North Pacific (Matkin and Saulitis, 1994). One small core containing skin and blubber (approx. 1.6 cm in length and 0.5 cm in diameter) was obtained from each animal using a 22-caliber rifle powered by air pressure that propelled a lightweight plastic/aluminum dart containing a bevelled tubular sterile stainless steel tip (Barrett-Lennard et al., 1996). The sterilized dart was aimed at the saddle patch region of the upper back of the whale and fired at a range of 8–20 m. The floating dart was retrieved from the water using a long-handled dipnet. The blubber portion of the biopsy sample was excised using a solvent-rinsed scalpel, placed in a solvent rinsed glass vial and stored at -20°C until chemical analysis.

2.3. Analytical techniques

Biopsy blubber samples of killer whales were analyzed by a high-performance liquid chromatography/photodiode array (HPLC/PDA) method (Krahn et al., 1994) that was developed to rapidly measure concentrations of dioxin-like CBs and other selected OCs in various tissues of commercially and recreationally important ma-

rine species (Ylitalo et al., 1999). Briefly, blubber (0.1–0.4 g), hexane/pentane (1:1 v/v), sodium sulfate (5 g) and a surrogate standard (1,7,8-trichlorodibenzo-*p*-dioxin, 250 ng) were homogenized for 2 min. The sample mixture was then centrifuged and the extract was decanted into a 50-ml concentrator tube. The homogenization step was repeated and the extracts were combined. A 1-ml aliquot of sample extract was removed for lipid analyses and the remaining sample extract was reduced in volume to ~1 ml. The sample extract was loaded onto a gravity-flow cleanup column, comprised of a glass wool plug, silica gel, basic silica gel and acidic silica gel, to separate the desired analytes from other interfering compounds (e.g. lipids, aromatic compounds). The analytes were eluted from the cleanup column with 14-ml hexane/methylene chloride (1:1 v/v)

Table 1
Life history information: whale pod or group identification, recruitment order[c], site, sex and age of killer whales from Kenai Fjords and Prince William Sound, AK samples from 1994 to 1999

Resident pod	Sex[a]	Recruitment order[b]	Age[c]	Resident pod	Sex[a]	Recruitment order[b]	Age[c]
AB[d]				**AJ**[d]			
AB03	Male, sm	Unknown	28?	AJ02	Male, sm	First recruited	29?
AB04	Male, sm	Non-first recruited	28?	AJ03	Female, r	Unknown	22?
AB05	Male, sm	First recruited	> 31	AJ04	Female, r	Unknown	19?
AB10	Female, r	Unknown	> 50	AJ08	Female, r	Unknown	> 44
AB11	Male, sm	Non-first recruited	19?	AJ10	Male, sm	Non-first recruited	16?
AB17	Female, r	Unknown	> 34	AJ13	Female, r	Unknown	21?
AB24	Male, sm	First recruited	28?	AJ16	Male, sm	First recruited	28?
AB26	Female, r	Unknown	18?	AJ19	Male, sm	Non-first recruited	19?
AB27	Female, si	Unknown	15?	AJ21	Male, sm	Non-first recruited	22?
AB35	Male, sm	Non-first recruited	18?	AJ39	Female, si	Non-first recruited	2
AB39	Female, si	First recruited	11	AJ41	Female, si	Non-first recruited	1
AB40	Male, si	Non-first recruited	6				
AD[d]				**AK**[d]			
AD02	Male, sm	Unknown	30?	AK02	Female, r	Unknown	> 36
AD05	Female, r	Unknown	> 38	AK03	Female, nr	First recruited	> 53
AD13	Male, sm	First recruited	> 35	AK09	Female, si	Non-first recruited	11
AD14	Female, nr	Unknown	> 48	AK10	Female, si	Non-first recruited	7
AD16	Female, r	Unknown	> 31	AK13	Female, si	Non-first recruited	3
AD19	Male, sm	First recruited	16?	AK14	Female, si	Non-first recruited	1
AD28	Juvenile, si	Non-first recruited	3	AK15	Female, si	First recruited	1
AE[d]				**AN10**[d]			
AE01	Male, sm	First recruited	> 31	AN01	Male, sm	First recruited	> 31
AE02	Female, r	Unknown	> 20	AN03	Male, sm	First recruited	> 34
AE03	Male, sm	Non-first recruited	17?	AN07	Male, sm	Non-first recruited	26?
AE06	Male, sm	First recruited	16?	AN08	Female, r	Unknown	22?
AE09	Male, sm	First recruited	> 31	AN10	Female, r	Unknown	> 25
AE10	Female, r	Unknown	> 21	AN12	Female, r	Non-first recruited	16?
AE11	Female, r	Unknown	> 24	AN35	Female, r	Unknown	> 24
AE14	Male, sm	Unknown	18?	AN46	Male, si	Non-first recruited	5
AE15	Male, si	First recruited	6				
AE16	Male, si	First recruited	5				
AE20	Female, si	Non-first recruited	1	**AS**[d]			
				AS?	Unknown	Unknown	Unknown
AI[d]				AS12	Male	Unknown	Unknown
AI02	Male, sm	Non-first recruited	26?				
AI03	Female, r	Unknown	> 46				
AI04	Female, r	Unknown	13	**AX**[d]			
AI06	Male, sm	Non-first recruited	19?	AX31	Unknown	Unknown	Unknown

Table 1 (*Continued*)

Transient group	Sex[a]	Recruitment order[b]	Age[c]	Transient group	Sex[a]	Recruitment order[b]	Age[c]
AT1[d]				**GOA**[e]			
AT03	Female, si	Unknown	15?	GOA	Unknown	Unknown	Unknown
AT06	Male, sm	Unknown	> 23	AT32	Male, sm	Unknown	Unknown
AT09	Female, r	Unknown	> 30	AT101	Female	Unknown	Unknown
AT10	Male, sm	Unknown	15	AT102	Female, r	Unknown	Unknown
AT13	Male, sm	Unknown	> 38	AT103	Male, si	First recruited	1
AT17	Male, sm	Unknown	> 33	AT105	Female	Unknown	Unknown
AT18	Female, nr	Unknown	> 20				

[a]Abbreviations: nr, non-reproductive; r, reproductive; si, sexually immature, sm; sexually mature.
[b]Putative recruitment (birth) order.
[c]Age at time of sampling.
[d]Prince William Sound, AK.
[e]Gulf of Alaska.

and collected into a clean 50-ml concentrator tube. The HPLC internal standard (1,2,3,4-tetrachlorodibenzo-*p*-dioxin, 250 ng) was added to each sample and the solvent volume was reduced by nitrogen to ~ 150 μl.

Eight dioxin-like congeners (CBs 77, 105, 118, 126, 156, 157, 169, 189) were resolved from other selected CBs (CBs 101, 128, 138, 153, 170, 180) and chlorinated hydrocarbons (*o*,*p*′-DDD, *p*,*p*′-DDD, *p*,*p*′-DDE, *o*,*p*′-DDT, *p*,*p*′-DDT) by HPLC on two Cosmosil PYE analytical columns, connected in series and cooled to 16°C. The congeners were measured by an ultraviolet (UV) photodiode array detector and were identified by comparing their UV spectra (200–310 nm) and retention times to those of reference standards in a library. The analyte purity was confirmed by comparing spectra within a peak to the apex spectrum. In some cases, certain CB congeners co-elute with other CBs with the HPLC/PDA method. For example, CB 101 co-elutes with CBs 99/149/196 and possibly with others, CB 153 co-elutes with CB87 and CB 170 co-elutes with CB194.

The HPLC system was calibrated daily. A sample set consisted of 11–14 field samples, a method blank and quality assurance samples. Method blanks contained no more than five analytes that exceeded 4 times the method detection limit (MDL), unless the analyte was not detected in the associated blubber samples of the set. Approximately 10% of the whale blubber samples were analyzed in duplicate to measure precision of the method and the laboratory quality assurance criteria were met for all analytes detected in the blubber samples. To monitor the accuracy of our HPLC/PDA method, a National Institute of Standards and Technology (NIST) control whale blubber sample was analyzed with each sample set and results met laboratory criteria (Wise et al., 1993). The limits of detection (LOD) for the CB congeners ranged from < 0.46 to < 14 ng/g, wet weight. The LOD for the DDTs ranged from < 2.5 to < 17 ng/g.

The mass of each biopsy blubber sample was small (< 0.50 g), therefore, the entire sample was used for OC analyses. In order to determine lipid content of each sample, a 1-ml aliquot of each sample extract was set aside for lipid analysis using thin layer chromatography coupled with flame ionization detection (TLC/FID) (Shantha, 1992). Each lipid sample extract was spotted on a Chromarod (Type SIII) and developed in a solvent system containing 60:10:0.02 hexane/diethyl ether/formic acid (v/v/v). Various classes of lipids (i.e. wax esters, triglycerides, free fatty acids, cholesterol and polar lipids) were separated based on polarity, with the non-polar compounds (i.e. wax esters) eluting first, followed by the more polar lipids (i.e. phospholipids). The lipid concentrations were determined using an Iatroscan Mark 5 (Iatron Laboratories, Tokyo, Japan), operated

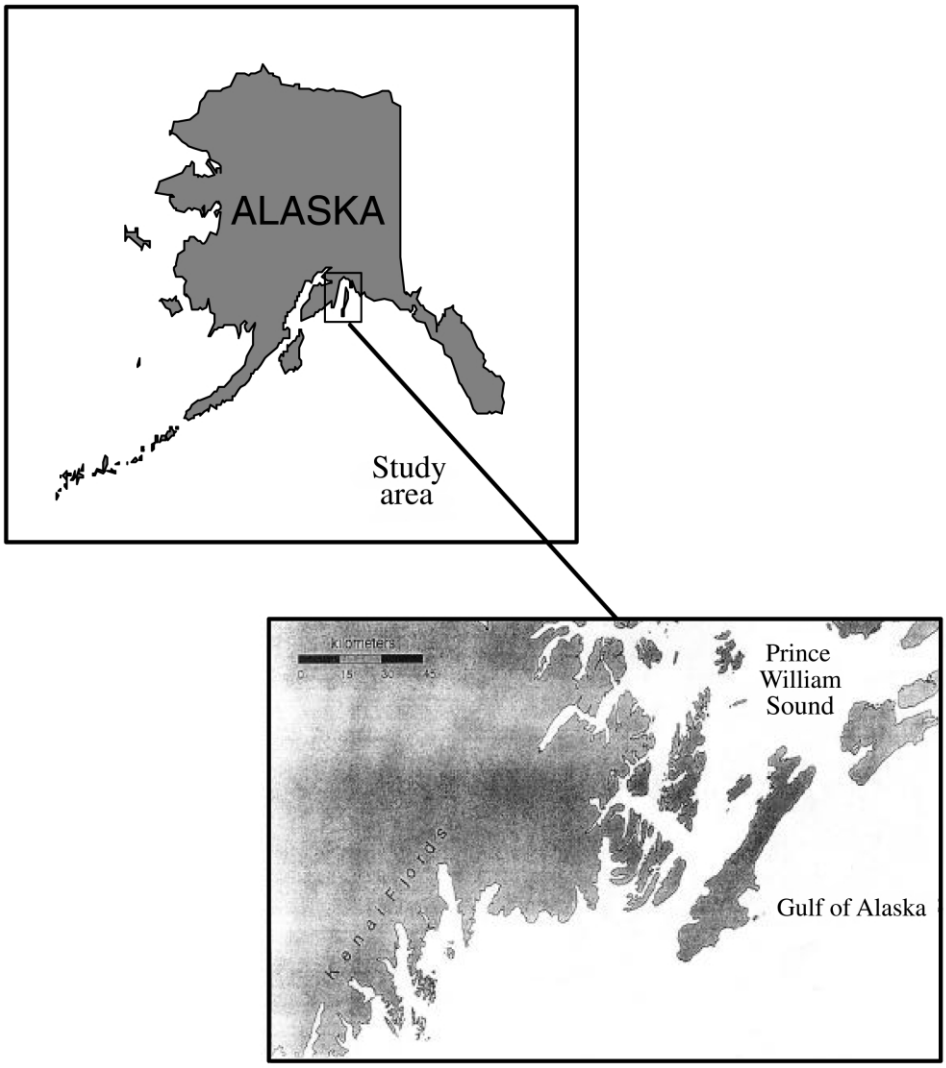

Fig. 1. Killer whale study region in Kenai Fjords/Prince William Sound, AK.

with a hydrogen flow rate of 160 ml/min and air flow of 2000 ml/min. Data were acquired and analyzed on a 386 PC compatible computer using TDatascan software (RSS Inc., Bemis, TN). A four-point linear external calibration was used for quantitation. Total lipid concentrations were calculated by adding the concentrations of the five lipid classes for each sample and were reported as percent total lipid. Duplicate TLC/FID analyses were performed for each sample extract and the mean value was reported.

2.4. Calculated values

Total CB (∑CB) concentrations were calculated using the following formula: ∑CBs = ∑concentrations of selected CBs (based on individual response factor) + ∑concentrations of other CB congeners (calculated by summing areas of peaks identified as CBs and using an average CB response factor). Summed DDT (∑DDTs) concentrations were calculated by adding the concentrations of five DDTs (o,p'-DDD, p,p'-DDD,

p,*p*′-DDE, *o*,*p*′-DDT, *p*,*p*′-DDT) determined by our HPLC/PDA method. Summed DDT and total CB concentrations were reported as ng/g, wet weight or lipid weight.

To assess the toxic potency of the dioxin-like CBs in the whale blubber samples, CB TEQs were calculated according to the method of Safe (1990) using an additive model of toxicity. In this method, the molar concentration of each dioxin-like CB congener was multiplied by the appropriate toxic equivalency factor (TEF), recommended recently by World Health Organization for human and wildlife health (Van den Berg et al., 1998). The following TEF values, which are based on several in vivo and in vitro studies, including human, mammalian and avian investigations, were used for TEQ calculations: CB77 (0.0001); CB105 (0.0001); CB118 (0.0001); CB126 (0.1); CB156 (0.0005); CB157 (0.0005); CB169 (0.01); and CB189 (0.0001). The CB TEQs are reported as pg/g, wet weight or lipid weight.

The TEQs calculated for the blubber of killer whales in the current study are conservative values. For example, only concentrations of dioxin-like CBs are determined by our HPLC/PDA method while concentrations of other dioxin-like compounds, such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are not. As a result, the TEQs calculated for the killer whale samples are based solely on dioxin-like CBs and do not include PCDDs and PCDFs that, if detected, would increase the TEQ values of the biopsy samples. However, several marine mammal contaminant studies have shown that PCDDs and PCDFs contribute much less (usually $< 15\%$) to the TEQ values compared to the dioxin-like CBs in cetaceans from various parts of the world (Kannan et al., 1989; Jarman et al., 1996; Addison et al., 1999; Jimenez et al., 2000; Ross et al., 2000). This is because dioxin-like CBs are often found in much higher concentrations than are the polychlorinated dibenzodioxins (PCDDs) or dibenzofurans (PCDFs) in the marine environment even though dioxin-like CBs are only 0.00001–0.1 times as toxic as TCDD (Van den Berg et al., 1998). Furthermore, our HPLC/PDA method has higher limits of detection (LODs) for certain congeners, especially the non-*ortho*-substituted CBs (e.g. CBs 77, 126, 169) compared to the LODs of more comprehensive analytical methods (i.e. high-resolution gas chromatography/mass spectrometry). In addition, irrespective of analytical method, the small masses (< 0.5 g) of the killer whale biopsy samples also contribute to higher LOD for certain mono-*ortho*- and non-*ortho*-substituted congeners. Consequently, the TEQs determined in the killer whale biopsy samples were conservative values because sample size is small, and they did not include PCDDs, PCDFs or any dioxin-like congeners (e.g. CBs 77, 126, 169) that were below the LOD in the TEQ calculation.

2.5. Statistical analyses

Lipid concentrations were arcsine square root transformed and OC concentrations were log transformed to increase the homogeneity of variance. Stepwise regression analysis was used to evaluate relationships among OC exposure (e.g. CB congeners, DDT, DDT metabolites, ∑CBs, ∑CB TEQs) and life-history parameters (e.g. age, sex, birth order, see Table 1) in killer whales to determine the life-history parameters (e.g. ecotype, sex, reproductive status, birth order) that most closely correlated to OC concentrations. Analysis of variance (ANOVA) and the Tukey–Kramer honestly significant difference test (HSD) were used to compare mean concentrations of OCs between transient and resident whales. For resident whales, analysis of variance and the Tukey–Kramer HSD test were used to determine differences in mean concentrations of OCs among three reproductive groups [sexually immature whales (both males and females), reproductive females, sexually mature males]. The level of significance used for all statistical tests was $P \leq 0.05$. All statistical analyses were completed using JMP Statistical Software (SAS Institute, Inc., Cary, NC).

3. Results

Although wide ranges of OC levels were measured in the killer whale biopsy samples, signifi-

cantly higher OC concentrations were measured in blubber of transient killer whales compared to the levels found in the blubber of residents based on both wet weight and lipid weight values (Tables 2 and 3). For example, the mean ΣDDTs concentration in transient whales was approximately 25 times as great as the mean level in resident whales. Similar results were observed for ΣCBs, with transient whales containing a mean ΣCB concentration more than 15 times the mean level in residents.

The most abundant OC analyte (Table 3) measured in blubber of Alaskan killer whales was the DDT metabolite, *p,p′*-DDE, with concentrations (wet weight) ranging in resident whales from 150–22 000 to 21 000–210 000 ng/g in transients. This DDT metabolite accounted for approximately 80% of ΣDDTs measured in resident

Table 2
Mean concentrations ($X \pm$ SD ng/g, wet weight or ng/g, lipid weight) of dioxin-like CBs and other selected CBs in biopsy blubber of resident and transient killer whales from the Kenai Fjords/Prince William Sound, AK region

Whale form	Dioxin-like CB congeners[a]					
	105*	118*	156*	157	189*	
Resident ($n = 64$) (ng/g, wet wt.)	50 ± 54 e (ND–230)	200 ± 220 (13–940)	14 ± 14 b (ND–60)	NR[c]	NR	
Resident ($n = 64$) (ng/g, lipid wt.)	170 ± 160 e (ND–850)	710 ± 700 (60–3400)	49 ± 36 b (ND–140)	NR	NR	
Transient ($n = 13$) (ng/g, wet wt.)	320 ± 290 (19–890)	1600 ± 1400 (100–4400)	54 ± 31 (4.3–120)	I[d]	17 ± 12 a (ND–39)	
Transient ($n = 13$) (ng/g, lipid wt.)	1200 ± 970 (260–3700)	6200 ± 4900 (1400–18 000)	220 ± 100 (58–430)	I[d]	76 ± 40 a (ND–160)	
	Other selected CB congeners					
	101[b]*	128*	138*	153/87*	170/194*	180*
Resident ($n = 64$) (ng/g, wet wt.)	620 ± 620 f (ND–2900)	66 ± 68 d (ND–300)	310 ± 370 (16–1700)	660 ± 680 f (ND–2900)	70 ± 74 c (ND–340)	170 ± 200 (12–1000)
Resident ($n = 64$) (ng/g, lipid wt.)	2200 ± 2100 f (ND-11 000)	270 ± 310 d (ND–2100)	1100 ± 1100 (64–4500)	2300 ± 2100 f (ND–9000)	250 ± 210 c (ND–820)	610 ± 540 (44–2500)
Transient ($n = 13$) (ng/g, wet wt.)	8600 ± 6100 (870–22 000)	1300 ± 820 (120–2600)	5300 ± 3500 (460–12 000)	9900 ± 6500 (1100–24 000)	1100 ± 560 (110–2000)	2900 ± 1600 (240–5600)
Transient ($n = 13$) (ng/g, lipid wt.)	35 000 ± 23 000 (12 000–92 000)	5100 ± 2700 (1600–11 000)	21 000 ± 11 000 (6200–46000)	40 000 ± 23 000 (15 000–100 000)	4400 ± 1800 (1500–8300)	12 000 ± 4400 (3200–19 000)

Asterisk indicates significant concentration differences between resident and transient whales based on both wet weight and lipid weight values; Tukey-Kramer HSD test, $P < 0.05$. Letter after $X \pm$ S.D. value referes to the number of samples (other than reported value in whale form column) where OCs were detected: a ($n = 10$); b ($n = 45$); c ($n = 57$); d ($n = 61$); e ($n = 62$); and f ($n = 63$).

[a] Mean values of CBs 77, 126 and 169 are not reported because they were not detected in any killer whale biopsy blubber samples.

[b] Other CB congeners (e.g., 99,149, 183, 196) may also be present (see Materials and Methods section).

[c] NR = not reported because analyte detected in fewer than 50% of total samples.

[d] I = concentration of analyte not determined due to interference with coeluting compound on PYE column.

Table 3
Mean concentrations ($X \pm$ SD ng/g, wet weight or ng/g, lipid weight) of DDTs, HCB, ΣCBs, ΣDDTs, ΣTEQs[a] and lipid[b] in biopsy blubber samples of resident killer whales from the Kenai Fjords/Prince William Sound, AK region

Whale form	DDTs					ΣCBs*	ΣTEQs*¶	ΣDDTs*	% Lipid
	o,p′-DDD*	*p,p′*-DDD*	*p,p′*-DDE*	*o,p′*-DDT*	*p,p′*-DDT*				
Resident ($n = 64$) (ng/g, wet wt.)	66 ± 48h (ND–180)	200 ± 200j (ND–980)	3100 ± 4100 (150–22 000)	380 ± 380j (ND–2000)	97 ± 120i (ND–570)	3900 ± 4500 (270–27 000)	29 ± 33 (1.5 ± 150)	3800 ± 4700 (190–26 000)	28 ± 9.8 (9.8–59)
Resident ($n = 64$) (ng/g, lipid wt.)	230 ± 170h (ND–900)	700 ± 570j (ND–2300)	11 000 ± 12 000 (670–56 000)	1400 ± 1300j (ND–6500)	320 ± 310i (ND–1800)	14 000 ± 13 000 (1100–65 000)	100 ± 98 (5.9–470)	13 000 ± 14 000 (730–64 000)	– –
Transient ($n = 13$) (ng/g, wet wt.)	940 ± 880 (80–2800)	2700 ± 2300 (110–9000)	71 000 ± 54 000 (4300–210 000)	6600 ± 5100 (690–16 000)	1400 ± 1000g (ND–3100)	59 000 ± 43 000 (4900–140 000)	220 ± 190 (14–580)	83 000 ± 63 000 (5200–240 000)	24 ± 9.5 (7.4–43)
Transient ($n = 13$) (ng/g, lipid wt.)	3800 ± 3300 (950–11 000)	11 000 ± 7800 (1500–32 000)	280 000 ± 180 000 (58 000–750 000)	26 000 ± 18 000 (9200–62 000)	5500 ± 3200g (ND–10 000)	230 000 ± 130 000 (66 000–500 000)	860 ± 640 (190–2400)	320 000 ± 210 000 (70 000–860 000)	– –

Asterisk indicates significant concentration differences between resident and transient whales based on both wet weight and lipid weight values; Tukey-Kramer HSD test, $P < 0.05$. Letter after $X \pm$ SD value refers to the number of samples (other than reported value in whale form column) where OCs were detected: g ($n = 12$), h ($n = 43$), i ($n = 54$), j ($n = 60$).

[a] ΣTEQs reported as pg/g.

[b] Lipid concentration reported as % lipid.

whales and 86% of ΣDDTs in the transient whales.

The moderately chlorinated *ortho*-substituted congeners (i.e. CBs 138, 153) were the predominant CBs measured in the killer whale blubber (Table 2). Similar to the ΣCB and ΣDDT concentration data, we also found much higher concentrations of individual CBs in transient whale blubber compared to those in the residents. For example, CB 118 concentrations (based on lipid weight) ranged from 60 to 3400 ng/g in residents and 1400 to 18 000 ng/g in transients. Dioxin-like CBs (e.g. CBs 105, 118, 156, 157, 189) were also determined in whale blubber samples, with the mono-*ortho*-substituted congeners being most abundant. In addition, a greater number of dioxin-like congeners were measured in the transient whales compared to the number of these congeners found in the residents. The two mono-*ortho*-substituted dioxin-like congeners (CBs 157 and 189) were measured in 60% of the transient whale samples but were detected in less than 20% of the resident samples. The most toxic CB congeners, the non-*ortho*-substituted congeners, CBs 77, 126 and 169, were not detected in any of the tissue samples analyzed, with the LOD ranging from 0.46 to 14 ng/g, wet weight.

The mean total CB TEQs (ΣTEQs) concentrations (based on wet and lipid weights) in transient animals were significantly higher than the levels in resident whales (Table 3). However, the relative proportions of dioxin-like congeners contributing to the total mean CB TEQs in resident and transient killer whales were similar (Fig. 2). Because concentrations of the non-*ortho*-substituted CBs were below the LOD in all killer whale blubber samples, the mono-*ortho* substituted dioxin-like congeners were the only contributors to CB TEQs in these samples. Although the mono-*ortho*-substituted CB congeners contributed approximately 7% to the mean total CB concentration in resident animals and 13% to the mean ΣCBs in transients, these CBs contributed 100% of the toxic potency to the mean ΣTEQ in these resident and transient whales. Furthermore, CB 118 was the largest contributor to the total CB TEQs in both eco-types of killer whales, contributing approximately 67% to the CB TEQs in resident animals and 72% to the CB TEQs in transients.

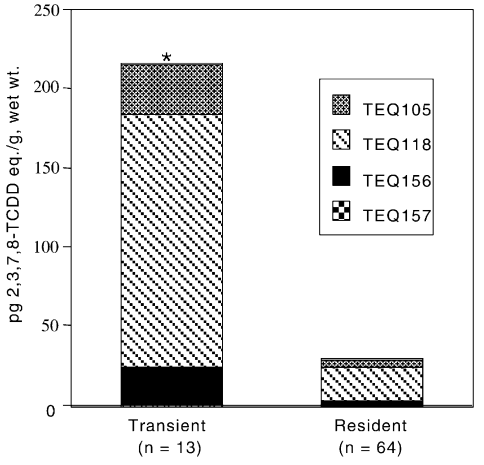

Fig. 2. Mean CB toxic equivalents (pg TCDD eq./g, wet weight) measured in biopsy blubber samples of transient and resident killer whales from Kenai Fjords/Prince William Sound, AK. Bar with asterisk indicates significantly higher concentrations using Tukey–Kramer honestly significant difference test, $P < 0.05$.

A wide range of lipid concentrations was measured in the killer whale biopsy samples (Table 3), with levels ranging from 7.4 to 59%. The lipids measured in biopsy blubber samples consisted primarily of neutral lipids (e.g. triglycerides, non-esterified free fatty acids). The lipid concentrations ($27 \pm 9.9\%$) of resident killer whales were not significantly different than the lipid levels in the transients ($23 \pm 11\%$).

The OC concentrations in resident killer whales were examined based on age and sex (Fig. 3). For example, concentrations (based on lipid weight) of eight CB congeners (CBs 101, 105, 118, 128, 138, 153, 156 and 180) in reproductive female resident killer whales (≥ 13 years of age) were significantly lower than those measured in immature resident whales (males $<$ age 15 and females < 13) or sexually mature male resident animals ($=$ age 15). However, no differences in concentrations of these congeners were found between the immature whales and mature male animals. Similar results were observed for DDTs except *p,p′*-DDT. The mean concentration of *p,p′*-DDT in mature adult male whales was sig-

nificantly higher than the mean level in reproductive females. However, we found no significant differences in mean *p,p′*-DDT concentrations between immature residents and mature males or immature residents and reproductive females.

The concentrations of ΣCBs, ΣDDTs and ΣCB TEQs measured in mother-offspring groups are shown in Table 4. We found that, in both resident and transient whales, concentrations of OCs were higher in the killer whale offspring compared to the levels in the corresponding mother (Table 4). In addition, in resident whales, OC concentrations in offspring appeared to be affected by birth order. For example, a first known offspring (AE16) of AE02 contained OC levels that were approximately 9–20 times those in his mother and 3–8 times those measured in the subsequent sibling (AE20). Furthermore, we compared OC levels in sexually mature (≥ age 15) male resident whales (first-recruited and non-first-recruited). Overall, mean concentrations of selected CBs and DDTs in first-recruited animals were roughly 1 order of magnitude higher than in later-recruited whales (Fig. 4). Mean concentrations of ΣCBs, ΣDDTs and ΣCB TEQs in first-recruited whales were approximately 4.0 times those in non-first-recruited animals when based on lipid weight and 2.5 times the mean levels in the non-first-recruited whales when based on wet weight (data not shown). Stepwise regression showed that OC concentrations (based on wet and lipid weights) in sexually mature resident killer whales were more highly correlated to birth order than to age. For example, ΣCBs (ng/g, lipid weight) were much more highly correlated to birth order ($P = 0.0025$) in sexually mature resident males than to age ($P = 0.917$). Similar results were obtained with individual CB congeners and DDT and DDT metabolites as well as ΣDDTs and ΣCB TEQs.

4. Discussion

We analyzed 77 biopsy blubber samples of free-ranging killer whales from the Kenai Fjords/Prince William Sound, AK region for selected toxic organochlorines, including dioxin-like CBs. These killer whales have been extensively studied since the mid-1980s and substantial life history data (i.e. pod membership, eco-type, approximate ages, reproductive status, putative birth order) are known (Matkin et al., 1999a,b). However, few chemical contaminant data are reported for killer whales from the North Pacific (Calambokidis et al., 1984; Jarman et al., 1996; Hayteas and Duffield, 2000; Ross et al., 2000), particularly free-ranging animals from Alaska. Therefore, this unique set of biopsy blubber samples allowed us to determine the concentrations of persistent and toxic OCs in free-ranging killer whales from Kenai Fjords/Prince William Sound, AK and examine the influence of various life history parameters on OC concentrations in these animals.

The concentrations of CBs and DDTs that we measured in blubber of the Alaskan killer whales are much higher than the concentrations in blubber of various other cetaceans and pinnipeds that reside and feed in Alaskan waters (Miles et al., 1992; Varanasi et al., 1992, 1994; Lee et al., 1996; Krahn et al., 1997; O'Hara et al., 1999). For example, Krahn et al. (1999) determined the ΣCBs and ΣDDTs concentrations (based on wet weight) ranged from 3770–6880 to 2090–4850 ng/g, respectively, in adult beluga whales from three Alaskan stocks. These concentrations are comparable to those measured in resident female killer whales but were approximately 1 order of magnitude lower than the contaminant concentrations determined in transient whales. The OC concentrations found in the Alaskan killer whales are similar to those recently reported in pinnipeds and cetaceans that occur in more contaminated waters of the Eastern North Pacific (Lieberg-Clark et al., 1995; Jarman et al., 1996; Hong et al., 1998). For example, the ΣCB levels (based on lipid weight) measured in the Alaskan transient killer whales are similar to those recently reported in biopsy blubber samples of transient killer whales from coastal waters of British Columbia (Ross et al., 2000), whereas the ΣCBs concentrations (based on wet weight) determined in the Alaskan resident whales are similar to those found in blubber of harbor seal pups from Puget Sound, WA (Hong et al., 1996). However, ΣCBs is, in most cases, an estimated value (unless

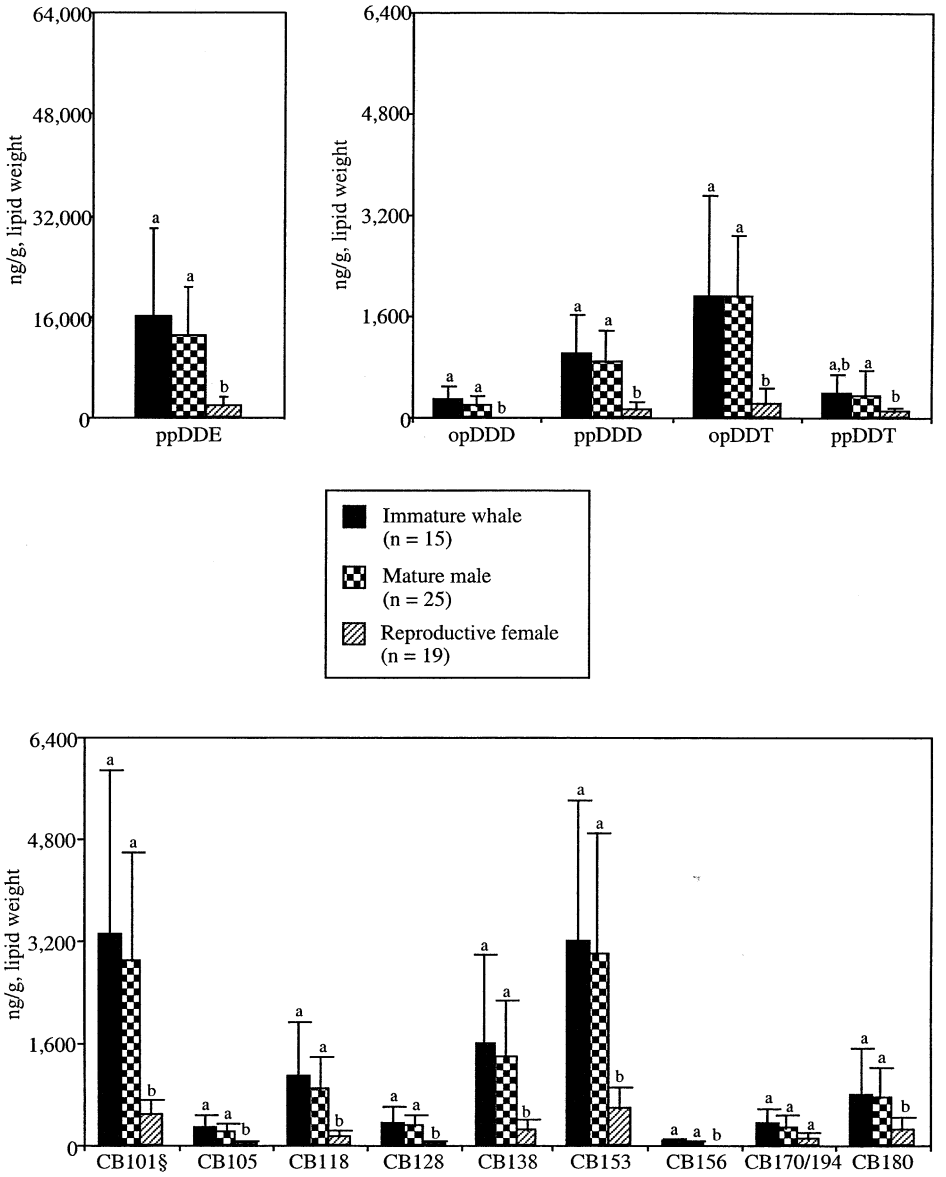

Fig. 3. Mean concentrations of individual CB congeners and DDTs (ng/g, lipid weight) measured in biopsy blubber samples of resident killer whales from Kenai Fjords/Prince William Sound, AK region grouped by reproductive status [i.e. reproductive female, immature animals (both males and females), sexually mature males]. Bars with unlike letters differ significantly using Tukey–Kramer honestly significant difference test, $P < 0.05$. §CB101 co-elutes with CBs 99/149/196 and possibly with other CB congeners.

all 209 congeners are quantitated with appropriate standards) and caution should be used when comparing ΣCBs among different studies because different methods are used to calculate these values. The ΣCBs reported in killer whales from British Columbia waters by Ross et al. (2000) were calculated by summing the concentrations of 136 CB congeners in killer whale biopsy samples. In contrast, the ΣCBs reported in the current study were calculated by summing concentrations

Table 4
Concentrations of total CBs, summed DDTs and total CB TEQs measured in mother and offspring of Alaskan killer whales from Kenai Fjords/Prince William Sound, AK. Age of each whale is reported in Table 1

Pod eco-type	Pod	Pod no.	Relationship	Wet Weight			Lipid Weight		
				ΣCBs (ng/g)	ΣDDTs (ng/g)	ΣCB TEQs (pg/g)	ΣCBs (ng/g)	ΣDDTs (ng/g)	ΣCB TEQs (pg/g)
Resident	AD	AD16	Mother	780	350	13	2700	1200	45
		AD28	Non-first recruited juvenile	3700	3400	35	15 000	14 000	150
	AE	AE02	Mother	1800	1200	16	5500	3600	48
		AE16	First recruited male	27 000	26 000	190	63 000	60 000	440
		AE20	Second recruited female	4700	4500	57	8000	7600	97
		AE10	Mother	530	240	4.7	4800	2200	43
		AE15	First recruited male	15 000	13 000	150	39 000	34 000	390
	AI	AI03	Mother	750	460	9.1	4200	2600	51
		AI04	Reproductive female	1600	1300	14	4300	3600	38
	AJ	AJ04	Mother	570	380	4.7	1900	1300	16
		AJ41	Non-first recruited female	1600	1200	11	5500	4100	38
		AJ13	Mother	1000	320	5.7	3700	1200	21
		AJ39	Non-first recruited female	5200	5200	43	14 000	14 000	120
	AK	AK02	Mother	610	310	6.5	2000	1000	22
		AK09	Non-first recruited female	4600	3700	44	21 000	17 000	200
		AK10	Non-first recruited female	4600	3800	47	16 000	13 000	160
		AK13	Non-first recruited female	7400	5800	72	20 000	16 000	190
	AN	AN10	Mother	540	270	4.2	2600	1300	20
		AN46	Non-first recruited male	4800	4500	45	13 000	12 000	120
Transient	GOA	AT102	Mother	27 000	26 000	190	120 000	120 000	860
		AT103	First recruited male	120 000	240 000	760	430 000	860 000	2700

of selected CBs (based on individual response factor) and concentrations of other CB congeners (calculated by summing areas of peaks identified as CBs and using an average CB response factor). However, because PDA (UV) response factors for CBs vary by only $\pm 15\%$ or so from the average, a reasonable estimate of ΣCBs is obtained using this method.

The DDT metabolite, *p,p′*-DDE was the OC found in the highest concentration in the Alaskan killer whale biopsy samples. Similar to our findings, *p,p′*-DDE was the most abundant OC measured in blubber samples of beluga whales and northern fur seals that reside in waters of the Eastern North Pacific (Mossner and Ballschmiter, 1997). Moderately chlorinated *ortho*-substituted CBs (i.e. CBs 153, 138) were the predominant CB congeners measured in the Alaskan killer whales. These findings are similar to those previously reported in various species of marine mammals from the Eastern North Pacific (Varanasi et al., 1994; Hong et al., 1996; Jarman et al., 1996; Mossner and Ballschmiter, 1997; Beckmen et al., 1999) as well as for pinnipeds and other cetaceans from various parts of the world (Corsolini et al., 1995; Lake et al., 1995; Gauthier et al., 1997; Weisbrod et al., 2000a). CB congeners that contain 5–7 chlorine atoms make up high proportions of certain technical mixtures of CBs (e.g. Aroclor 1254) (Schulz et al., 1989; Schwartz et al., 1993). Because many of these moderately chlorinated congeners are not easily degraded in the

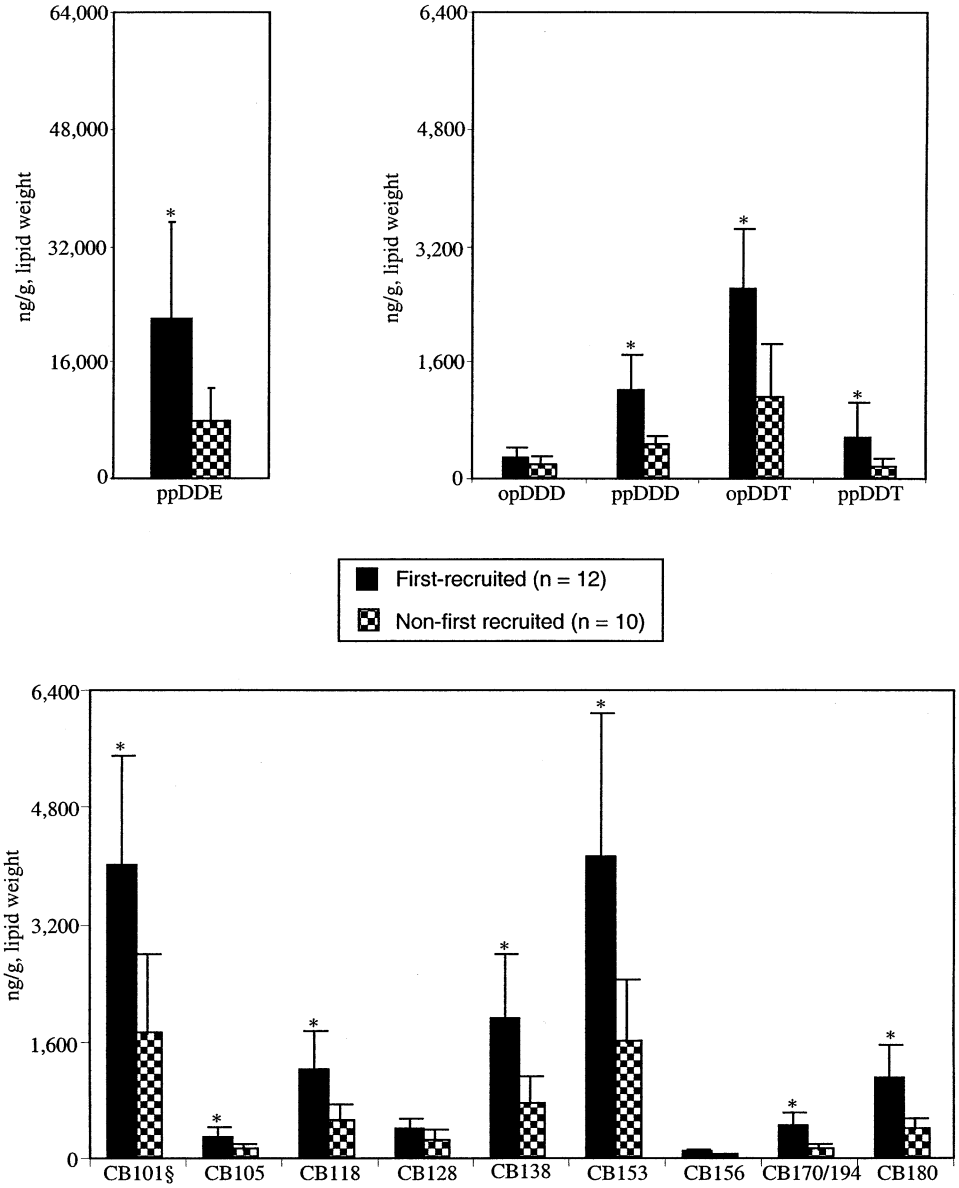

Fig. 4. Mean concentrations of individual CB congeners and DDTs (ng/g, lipid weight) measured in biopsy blubber samples of sexually mature male resident killer whales from Kenai Fjords/Prince William Sound, AK region grouped by birth order (first recruited and non-first recruited animals). Bars with asterisks indicate significantly higher concentrations using Tukey–Kramer HSD (honestly significant difference) test, $P < 0.05$. §CB101 co-elutes with CBs 99/149/196 and possibly with other CB congeners.

environment or eliminated by aquatic organisms as are lower chlorinated CBs, relatively high concentrations of these congeners bioaccumulate in marine animals, especially species at the top of the marine food chain. Furthermore, certain CBs are not as readily metabolized by aquatic organisms as other chlorinated congeners relative to chlorine substitution pattern. Boon et al. (1992) report that harbor seals, cetaceans and polar bears appear to metabolize congeners with vicinal H

atoms in the *ortho*, *meta* positions, even in the presence of one *ortho*-chlorine atom but this metabolic capability is not as apparent in ringed seals. However, irrespective of *ortho*-chlorine substitution, the cetacean species do not seem to metabolize CBs with vicinal H atoms in the *meta*, *para* positions as readily as the seals and polar bears. Based on these data, relatively high concentrations of certain moderately chlorinated CBs are expected to bioaccumulate in marine mammals, especially top level predators such as killer whales.

The relatively high concentrations of CBs and DDTs in Alaskan killer whales are somewhat surprising, but consistent with current information about transport of these compounds to Arctic ecosystems. Studies indicate that certain OCs primarily enter the Alaskan marine ecosystem via atmospheric transport from the lower and middle latitudes (Barrie et al., 1992; Iwata et al., 1993). These compounds can also enter the marine environment from direct input (e.g. transformer spill) into the far northern marine environment but these sources appear to be less significant than atmospheric deposition (Iwata et al., 1993; AMAP, 1998). For example, DDT is a persistent, lipophilic compound that was once widely used in the United States on agricultural crops and to control disease-carrying insects (e.g. malaria-carrying mosquitoes) and has been shown to have various toxic effects on experimental animals and wildlife (e.g. reproductive impairment, potential carcinogen). Consequently, it was banned in the US in the mid-1970s and has been prohibited or restricted for use in several other countries (e.g. Canada, Sweden). However, the compound is still used to control disease-carrying insects in other regions (i.e. Southeast Asia) and appears to be deposited to the pristine Arctic and sub-arctic ecosystems of the eastern North Pacific via atmospheric transport (Barrie et al., 1992; Iwata et al., 1993; AMAP, 1998; Schmidt, 1998).

The CB TEQs calculated for Alaskan transient killer whales are comparable to those determined in transient killer whales from coastal waters of British Columbia (Jarman et al., 1996; Ross et al., 2000) and harbor seals from Puget Sound, WA (Hong et al., 1998). In contrast, the CB TEQ concentrations for these killer whales were much lower than the levels in striped dolphins affected by an epizootic in the Mediterranean Sea (Kannan et al., 1993), common porpoise from the Baltic Sea (Falandysz et al., 1994) and two species of dolphin from the Italian coast (Corsolini et al., 1995). The TEF values (Van den Berg et al., 1998) we used to calculate the CB TEQs are different than those used in the European dolphin studies (Kannan et al., 1993; Falandysz et al., 1994; Corsolini et al., 1995). In the Safe (1990) technique, the TEF values are higher or comparable to those recommended by Van den Berg et al. (1998) and a larger number of CB congeners are used in TEQ calculations. Furthermore, the mono-*ortho* substituted dioxin-like congeners were the only contributors to the TEQs determined in our killer whale study because the non-*ortho*-substituted CB congeners (CBs 77, 126, 169) were below the LOD and PCDDs and PCDFs are not quantitated by the HPLC/PDA method. Therefore, the CB TEQ values determined in this study are conservative values. Ross et al. (2000) report the sum TEQs (based on concentrations of dioxin-like CBs, PCDDs and PCDFs) in biopsy blubber samples of northern resident killer whales from coastal British Columbia. The dioxin-like CBs contribute more than 85% to the sum TEQs in these samples, with the mono-*ortho*-substituted CB congeners contributing more than 80% to the sum CB TEQ ($92.0 \pm 1.7\%$ in immature animals, $94.3 \pm 0.8\%$ in males and $80.35 \pm 4.11\%$ in females) and more than 75% to the sum TEQ. Based on these data, we may be underestimating the ΣCB TEQs in the Alaskan killer whale biopsy samples by approximately 6–20% and the sum TEQs (including CBs, dioxins, furans) by approximately 8–25%.

Lipid concentrations in the killer whale biopsy samples ranged widely and consisted primarily of neutral lipids (e.g. triglycerides, free fatty acids). Previous studies show that blubber of healthy cetaceans is comprised primarily of neutral lipids, such as triglycerides and non-esterified free fatty acids (Kawai et al., 1988; Tilbury et al., 1997). Lipid levels in our samples were comparable to lipid concentrations in biopsy samples of North Atlantic right whales (4.8–25.5%) (Woodley et al.,

1991) and Northwest Atlantic right whales (mean $13 \pm 18\%$ lipid) (Weisbrod et al., 2000b), but are lower than those previously determined in non-biopsy samples of other large cetaceans (Borrell, 1993; Gauthier et al., 1997; Prudente et al., 1997; O'Hara et al., 1999). The average lipid concentration measured in necropsy blubber samples of killer whales ($n = 6$) collected off the coasts of British Columbia and Washington State was 91% ($n = 6$) (Jarman et al., 1996). This discrepancy is probably due to two factors. First, biopsy samples probably contain a higher portion of connective tissue attached to the skin and blubber than the necropsy samples, especially if collected from areas of lower lipid concentration (i.e. the base of the dorsal fin) (Woodley et al., 1991; Gauthier et al., 1997; Weisbrod et al., 2000b). Second, different quantitation methods were used in these lipid determinations. The biopsy blubber samples in our study were quantitated by TLC-FID (see Section 2) while the lipid concentrations in the Jarman et al. (1996) study were determined gravimetrically. Delbeke et al. (1995) found that lipid concentrations determined by TLC-FID were, on average, approximately half those determined by the gravimetric method, and that gravimetric lipid values were overestimated due to interference of non-lipid co-extracts. Therefore, caution should be used when comparing the lipid data from our study with lipid concentration data determined by other quantitation methods.

In this study, diet had important effects on OC accumulation in Alaskan killer whales. Transient whales contained much higher levels of OCs than did the residents. Similarly in coastal British Columbia waters, Ross et al. (2000) reported higher levels of CBs (based on lipid weight) in biopsy blubber samples of transient whales compared to those found in resident whales. Studies of feeding habits of Prince William Sound, AK resident killer whales show that these animals consume predominantly salmon and, to a lesser extent, other fish species (e.g. halibut, herring) (Matkin et al., 1999b; Saulitis et al., 2000). The principal marine mammal species consumed by transient killer whales from Prince William Sound are Dall's porpoise and harbor seals (Saulitis et al., 2000). In general, the prey species of transient whales contain higher OC levels than do resident prey. For example, ΣCBs (based on wet weight) in blubber of harbor seals from Prince William Sound, AK range from 45 to 356 ng/g (Krahn et al., 1997), whereas ΣCBs range from 17 to 50 ng/g in muscle of three salmon species (chum, coho, pink) collected from the same area (Brown, 2000 pers. comm.). Similarly, Ross et al. (2000) reported higher concentrations of CBs in prey of British Columbia transient whales (e.g. harbor seals) compared to the levels in resident whale prey (e.g. salmon) from this area. Therefore, based on their diet, transient whales would be expected to have higher concentrations of persistent contaminants than those found in residents.

Life history parameters such as age, sex and reproductive status influenced the concentrations of OCs in the killer whales. Reproductively active female killer whales contained much lower OC concentrations than sexually mature resident males or immature animals. Furthermore, killer whale offspring had higher OC concentrations than those determined in the corresponding mothers. These results are consistent with those from other marine mammal contaminant studies that report much lower OC burdens in reproductive females than in males in the same age group (Aguilar and Borrell, 1988; Kuehl and Haebler, 1995; Krahn et al., 1999; Tilbury et al., 1999). These studies have shown that the OC concentrations in juvenile animals of both sexes increase until sexual maturity. Males continue to accumulate these lipophilic contaminants throughout their lives. In contrast, a reproductive female's OC levels decrease due to maternal transfer of lipophilic OCs to her offspring during gestation and lactation (Wagemann and Muir, 1984; Aguilar and Borrell, 1994; Beckmen et al., 1999; Krahn et al., 1999). Furthermore, in some odontocetes (e.g. killer whales, pilot whales, short-finned pilot whales), after a female reaches senescence, her OC levels again increase with age (Tanabe et al., 1987; Tilbury et al., 1999; Ross et al., 2000).

Recruitment order also appears to affect the OC levels in killer whales. For example, first-recruited (first-born) adult male resident whales contained significantly higher levels of OCs than were found in non-first-recruited males in the

same age range. Lee et al. (1996) estimated that a female Steller sea lion transfers approximately 80% of her OC burden to her first-recruited offspring during lactation. In another study, it was calculated that a first-recruited offspring of a female fin whale received approximately 1 g ΣCBs and 1.5 g ΣDDTs, but that the levels of these lipophilic contaminants transferred to subsequent offspring gradually decreased to a minimum of 0.2 g ΣCBs and 0.3 g ΣDDTs in old females (Aguilar and Borrell, 1994). It appears that the OC burden transferred from mother to offspring decreases as reproductive females mature, because older females that have gone through several lactation cycles have lower OC burdens (Ridgway and Reddy, 1995). These data suggest that first-recruited marine mammals are likely to be exposed to higher OC burdens than subsequent offspring and, because of these higher OC burdens, may be at higher risk of toxicological effects of these contaminants than later offspring.

We compared OC levels in killer whales to contaminant levels associated with biological and physiological effects in various mammalian species. Concentrations of ΣCBs (based on lipid weight) above 77 000 ng/g, lipid are linked to reproductive dysfunction in ringed seals, harbor seals and otters and immune suppression in Rhesus monkeys (AMAP, 1998). More than 90% of the Alaskan transient killer whales contained ΣCBs above this benchmark concentration whereas, none of the resident animals contained ΣCBs greater than 77 000 ng/g lipid. Using experimental literature data on mink, a critical body residue (EC50) of 160 pg/g (TCDD equivalence/wet weight) is proposed for mink litter size (Leonards et al., 1995). Although none of the resident whales in this study contained ΣCB TEQs above this threshold level, more than half the transients had ΣCB TEQs at or above 160 pg/g, wet weight. These preliminary data suggest that the levels of OCs measured in the Alaskan killer whales could potentially cause various deleterious biological and physiological effects, such as reproductive impairment (Subramanian et al., 1987; Addison, 1989) and immune suppression (Ross et al., 1995, 1996). However, caution should be used in evaluation of the level of risk posed by toxic anthropogenic chemicals from this limited data set. These analyses focused only on OCs and exposure to toxic substances or other human factors, such as petroleum-related hydrocarbons, biotoxins and fishing interactions, may be affecting the health of these killer whales.

5. Conclusion

This study provides baseline chemical contaminant data for free-ranging killer whales in Alaskan waters for which there is little previous information. These Alaskan killer whales contain some of the highest levels of OCs reported in tissues of marine mammals from the eastern North Pacific and are comparable to killer whales from coastal waters of British Columbia. In particular, transient whales had much higher contaminant concentrations than did resident whales because they feed at a higher trophic level than do residents. In addition to diet, biological factors such as age, sex, reproductive status and birth order also affected the concentrations of OCs determined in these animals, with elevated OC concentrations determined in sexually mature male killer whales. Furthermore, presumably first-recruited resident males had significantly higher OC levels than did those in non-first-recruited animals from the same age range. The causal factors for low reproduction and population decline of certain pods (AB pod) and groups (AT1 group) of killer whales from Prince William is not known. The low reproduction and population decline may be a natural cycle, related to human factors (e.g. fishing interactions) or to exposure to natural toxins (e.g. biotoxins) or a combination of environmental and anthropogenic factors. Exposure to toxic OCs may also be the factor or a contributing factor. The highly elevated levels warrant further examination of the relationship of OC exposure to fitness of individual killer whales and possible relationship to population declines.

Acknowledgements

The *Exxon Valdez* Oil Spill (EVOS) Trustee Council funded the collection of the killer whale biopsy samples. Eva Saulitis, Lance Barrett-Lennard and Graeme Ellis provided assistance in biopsy collection and processing. Graeme Ellis also confirmed killer whale identifications. We would like to thank Dr Teri Rowles of the Office of Protected Resources for support under the Marine Mammal Health and Stranding Response Program. In addition, we thank Larry Hufnagle, Jr., Carol Pitzel, Gladys Yanagida, Daryle Boyd, Karen Tilbury, Lyndal Johnson, Dr Edmundo Casillas and Beth Horness for assistance in chemical and data analyses. We also thank Lyndal Johnson and Dr Marilyn Dahlheim for careful review of the manuscript.

References

Addison RF. Organochlorines and marine mammal reproduction. Can J Fish Aquat Sci 1989;46:360–368.

Addison RF, Ikonomou MG, Stobo WT. Polychlorinated dibenzo-*p*-dioxins and furans and non-*ortho*- and mono-*ortho*-chlorine substituted polychlorinated biphenyls in grey seals (*Halichoerus grypus*) from Sable Island, Nova Scotia, in 1995. Mar Environ Res 1999;47:225–240.

Aguilar A, Borrell A. Age- and sex-related changes in organochlorine compound levels in fin whales (*Balaenoptera physalus*) from the Eastern North Atlantic. Mar Environ Res 1988;25:195–211.

Aguilar A, Borrell A. Reproductive transfer and variation of body load of organochlorine pollutants with age in fin whales (*Balaenoptera physalus*). Arch Environ Contam Toxicol 1994;27:546–554.

Ahmed FE. Seafood safety. Washington, DC: National Academy Press, 1991, p. 43.

AMAP. AMAP Assessment Report: Arctic Pollution Issues. Arctic Monitoring and Assessment Program (AMAP), Oslo, Norway, 1998, xii + 859 pp.

Barrett-Lennard LG, Smith TG, Ellis GM. A cetacean biopsy system using lightweight pneumatic darts, and its effect on the behavior of killer whales. Mar Mamm Sci 1996; 12(1):14–27.

Barrie LA, Gregor D, Hargrave B, Lake R, Muir D, Shearer R, Tracey B, Bidleman T. Arctic contaminants: sources, occurrence and pathways. Sci Total Environ 1992;122:1–74.

Beckmen KB, Ylitalo GM, Towell RG, Krahn MM, O'Hara TM, Blake JE. Factors affecting organochlorine contaminant concentrations in milk and blood of northern fur seal (*Callorhinus ursinus*) dams and pups from St. George Island, Alaska. Sci Total Environ 1999;231:183–200.

Beeton AM, Allen JR, Andren AW, Cristol SJ, Durfee RL, Freeman AM, Metcalf RL, Nisbet ICT, Sanders JE, Sodergren A, Zitko V. Polychlorinated biphenyls. Washington, DC: National Academy of Sciences, 1979, p. 182.

Bigg MA. An assessment of killer whale (*Orcinus orca*) stocks off Vancouver Island, British Columbia. Rep Int Whaling Comm 1982;32:655–666.

Bigg MA. Killer whales: a study of their identification, genealogy and natural history in British Columbia and Washington State. Nanaimo, B.C.: Phantom, 1987, p. 79.

Bigg MA, Ellis GM, Balcomb III KC. The photographic identification of individual cetaceans. Whalewatcher: J Am Cetacean Soc 1986;20:10–12.

Bigg MA, Olesiuk PF, Ellis GM, Ford JKB, Balcomb III KC. Social organization and genealogy of resident killer whales (*Orcinus orca*) in the coastal waters of British Columbia and Washington State. Rep Int Whaling Comm Spec Is 1990;12:386–406.

Boon JP, Arnhem EV, Jansen S, Kannan N, Petrick G, Schulz D, Duinker JC, Reijnders PJH, Goksoyr A. The toxicokinetics of PCBs in marine mammals with special reference to possible interactions of individual congeners with the cytochrome P450-dependent monooxygenase system: an overview. In: Walker CH, Livingstone DR, editors. Persistent pollution in marine ecosystems. New York: Pergamon Press, 1992:119–159.

Borrell A. PCB and DDTs in blubber of cetaceans from the Northeastern North Atlantic. Mar Pollut Bull 1993;26(3):146–151.

Calambokidis J, Peard J, Steiger GH, Cubbage JC, DeLong RL. Chemical contaminants in marine mammals from Washington State. NOAA Technical Memorandum, NOS OMS 6, 1984, p. 167.

Corsolini S, Focardi S, Kannan K, Tanabe S, Borrell A, Tatsukawa R. Congener profile and toxicity assessment of polychlorinated biphenyls in dolphins, sharks and tuna collected from Italian coastal waters. Mar Environ Res 1995;40(1):33–53.

Dahlheim ME. A photographic catalog of killer whales, *Orcinus orca*, from the Central Gulf of Alaska to the Southeastern Bering Sea. NOAA Technical Report, NMFS 131, 1997, p. 54.

Delbeke K, Teklemariam T, De La Cruz E, Sorgeloos P. Reducing variability in pollution data: the use of lipid classes for normalization of pollution data in marine biota. Int J Environ Anal Chem 1995;58:147–162.

Falandysz J, Yamashita N, Tanabe S, Tatsukawa R, Rucinska L, Skora K. Congener-specific data on polychlorinated biphenyls in tissues of common porpoise from Puck Bay, Baltic Sea. Arch Environ Contam Toxicol 1994;20:267–272.

Ford JKB. Vocal traditions among resident killer whales (*Orcinus orca*) in coastal waters of British Columbia. Can J Zool 1991;69:1454–1483.

Fossi MC, Savelli C, Marsili L, Casini S, Jimenez B, Junin M, Castello H, Lorenzani JA. Skin biopsy as a non-destructive tool for the toxicological assessment of endangered popula-

tions of pinnipeds: preliminary results on mixed function oxidase in *Otaria flavescens*. Chemosphere 1997;35(8): 1623–1635.

Gauthier JM, Metcalfe CD, Sears R. Validation of the blubber biopsy technique for monitoring of organochlorine contaminants in balaenopterid whales. Mar Environ Res 1997;43(3):157–179.

Hayteas DL, Duffield DA. High levels of PCB and *p,p′*-DDE found in the blubber of killer whales (*Orcinus orca*). Mar Pollut Bull 2000;40(6):558–561.

Heise K, Ellis G, Matkin C. A catalogue of Prince William Sound killer whales 1991. Homer, AK: North Gulf Oceanic Society, 1992, p. 51.

Hoelzel AR, Dahlheim M, Stern SJ. Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and genetic differentiation between foraging specialists. J Heredity 1998;89:121–128.

Hong C-S, Calambokidis J, Bush B, Steiger GH, Shaw S. Polychlorinated biphenyls and organochlorine pesticides in harbor seal pups from the inland waters of Washington State. Environ Sci Technol 1996;30:837–844.

Hong CS, Xiao J, Bush B, Shaw SD. Environmental occurrence and potential toxicity of planar, mono-, and di-*ortho* polychlorinated biphenyls in the biota. Chemosphere 1998;36(7):1637–1651.

Iwata H, Tanabe S, Sakai N, Tatsukawa R. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate. Environ Sci Technol 1993;27:1080–1098.

Jarman WM, Norstrom RJ, Muir DCG, Rosenberg B, Simon M, Baird RW. Levels of organochlorine compounds, including PCDDs and PCDFs, in the blubber of cetaceans from the west coast of North America. Mar Pollut Bull 1996;32(5):426–436.

Jimenez B, Gonzalez MJ, Jimenez O, Reich S, Eljarrat E, Rivera J. Evaluation of 2,3,7,8 specific congener and toxic potency of persistent polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in cetaceans from the Mediterranean Sea, Italy. Environ Sci Technol 2000;34: 756–763.

Kannan N, Tanabe S, Ono M, Tatsukawa R. Critical evaluation of polychlorinated biphenyl toxicity in terrestrial and marine mammals: increasing impact of non-*ortho* and mono-*ortho* co-planar polychlorinated biphenyls from land to ocean. Arch Environ Contam Toxicol 1989;18:850–857.

Kannan N, Tanabe S, Borrell A, Aguilar A, Focardi S, Tatsukawa R. Isomer-specific analysis and toxic evaluation of polychlorinated biphenyls in striped dolphins affected by an epizootic in the Western Mediterranean Sea. Arch Environ Contam Toxicol 1993;25:227–233.

Kawai S, Fukushima M, Miyazaki N, Tatsukawa R. Relationship between lipid composition and organochlorine levels in the tissues of striped dolphin. Mar Pollut Bull 1988;19(3):129–133.

Krahn MM, Ylitalo GM, Buzitis J, Sloan CA, Boyd DT, Chan S-L, Varanasi U. Screening for planar chlorobiphenyl congeners in tissues of marine biota by high-performance liquid chromatography with photodiode array detection. Chemosphere 1994;29(1):117–139.

Krahn MM, Becker PR, Tilbury KL, Stein JE. Organochlorine contaminants in blubber of four seal species: integrating biomonitoring and specimen banking. Chemosphere 1997;34:2109–2121.

Krahn MM, Burrows DG, Stein JE, Becker PR, Schantz MM, Muir DCG, O'Hara TM, Rowles T. White whales (*Delphinapterus leucas*) from three Alaskan stocks, concentrations and patterns of persistent organochlorine contaminants in blubber. J Cetacean Res Manage 1999;1 (3):239–249.

Kuehl DW, Haebler R. Organochlorine, organobromine, metal, and selenium residues in bottlenose dolphins (*Tursiops truncatus*) collected during an unusual mortality event in the Gulf of Mexico, 1990. Arch Environ Contam Toxicol 1995;28:494–499.

Lake CA, Lake JL, Haebler R, McKinney R, Boothman WS, Sadove SS. Contaminant levels in harbor seals from the northeastern United States. Arch Environ Contam Toxicol 1995;29:128–134.

Lee JS, Tanabe S, Umino H, Tatsukawa R, Loughlin TR, Calkins DC. Persistent organochlorines in Steller sea lion (*Eumetopias jubatus*) from the bulk of Alaska and the Bering Sea, 1976–1981. Mar Pollut Bull 1996;32(7):535–544.

Leonards PEG, de Vries TH, Minnaard W, Stuijfzand S, de Voogt P, Cofino WP, van Straalen NM, van Hattum B. Assessment of experimental data on PCB-induced reproduction inhibition in mink, based on an isomer- and congener-specific approach using 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalency. Environ Toxicol Chem 1995;14 (4):639–652.

Lieberg-Clark P, Bacon CE, Burns SA, Jarman WM, Boeuf BJ. DDT in California sea-lions: a follow-up study after 20 years. Mar Pollut Bull 1995;30(11):744–745.

Matkin CO, Ellis G, Olesiuk P, Saulitis E. Association patterns and inferred genealogies of resident killer whales, *Orcinus orca*, in Prince William Sound, Alaska. Fish Bull 1999a;97:900–919.

Matkin CO, Ellis G, Saulitis E, Barrett-Lennard L, Matkin D. Killer whales of Southern Alaska. Homer, AK: North Gulf Oceanic Society, 1999b, p. 96.

Matkin CO, Saulitis EL. Killer whale (*Orcinus orca*) biology and management in Alaska. Marine Mammal Commission, Contract No. T5135023, 1994, p. 46.

Miles AK, Calkins DG, Coon NC. Toxic elements and organochlorines in harbor seals (*Phoca vitulina richardsi*), Kodiak, Alaska, USA. Bull Environ Contam Toxicol 1992;48:727–732.

Morton AB. A quantitative comparison of the behavior of resident and transient forms of killer whale off the central British Columbia coast. Rep Int Whal Comm Spec Issue 1990;12:245–248.

Mossner S, Ballschmiter K. Marine mammals as global pollution indicators for organochlorines. Chemosphere 1997;34(5–7):1285–1296.

Muir DCG, Wagemann R, Hargrave BT, Thomas DJ, Peakall DB, Norstrom RJ. Arctic marine ecosystem contamination. Sci Total Environ 1992;122:75–134.

O'Hara TM, Krahn MM, Boyd D, Becker PR, Philo LM. Organochlorine contaminant levels in Eskimo harvested bowhead whales of Arctic Alaska. J Wildlife Dis 1999;35(4):741–752.

Olesiuk PF, Bigg MA, Ellis GM. Life history and population dynamics of resident killer whales (*Orcinus orca*) in the coastal waters of British Columbia and Washington State. Rep Int Whaling Comm Spec Is 1990;12:209–243.

Prudente M, Tanabe S, Watanbe M, Subramnian A, Miyazki N, Suarez P, Tatsukawa R. Organochlorine contamination in some Odontoceti species from the North Pacific and Indian Ocean. Mar Environ Res 1997;44(4):415–427.

Ridgway S, Reddy M. Residue levels of several organochlorines in *Tursiops truncatus* milk collected at varied stages of lactation. Mar Pollut Bull 1995;30(9):609–614.

Ross PS, de Swart RL, Reijnders PJH, van Loveren HV, Vos JG, Osterhaus ADME. Contaminant-related suppression of delayed-type hypersensitivity and antibody responses in harbor seals fed herring from the Baltic Sea. Environ Health Perspect 1995;103(2):162–167.

Ross PS, de Swart RL, Timmerman HH, Reijnders PJH, Vos JG, van Loveren H, Osterhaus ADME. Suppression of natural killer cell activity in harbor seals (*Phoca vitulina*) fed Baltic Sea herring. Aquat Toxicol 1996;34:71–84.

Ross PS, Ellis GM, Ikonomou MG, Barrett–Lennard LG, Addison RF. High PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca*: effects of age, sex and dietary preference. Mar Pollut Bull 2000;40(6):504–515.

Safe S. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 1990;24:87–149.

Saulitis E, Matkin C, Barrett-Lennard L, Heise K, Ellis G. Foraging strategies of sympatric killer whale (*Orcinus orca*) populations in Prince William Sound, Alaska. Mar Mamm Sci 2000;16(1):94–109.

Schmidt CW. Spheres of influence: no POPs. Environ Health Perspect 1998;107(1):A24–25.

Schulz DE, Petrick G, Duinker JC. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Chlophen mixtures by multidimensional gas chromatography-electron capture detection. Environ Sci Technol 1989;23:852–859.

Schwartz TR, Tillitt DE, Feltz KP, Peterman PH. Determination of mono- and non-*oo′*-chlorine substituted polychlorinated biphenyls in Aroclors and environmental samples. Chemosphere 1993;26:1443–1460.

Shantha NC. Thin-layer chromatography-flame ionization detection Iatroscan system. J Chromatogr 1992;624:21–35.

Subramanian A, Tanabe S, Tatsukawa R, Saito S, Miyazaki N. Reduction in the testosterone levels by PCBs and DDE in Dall's porpoises of Northwestern North Pacific. Mar Pollut Bull 1987;18(12):643–646.

Tanabe S, Loganathan BG, Subramanian A, Tatsukawa R. Organochlorine residues in short-finned pilot whale. Mar Pollut Bull 1987;18:561–563.

Tilbury KL, Stein JE, Meador JP, Krone CA, Chan S-L. Chemical contaminants in harbor porpoise (*Phocoena phocoena*) from the North Atlantic Coast: tissue concentrations and intra- and inter-organ distribution. Chemosphere 1997;34(9/10):2159–2181.

Tilbury KL, Adams NG, Krone CA, Meador JP, Early G, Varanasi U. Organochlorines in stranded pilot whales (*Globicephala melaena*) from the Coast of Massachusetts. Arch Environ Contam Toxicol 1999;37:125–134.

Todd S, Ostrom P, Lien J, Abrajano J. Use of biopsy samples of humpback whale (*Megaptera novaeangliae*) skin for stable isotope (del^{13}C) determination. J Northw Atl Fish Sci 1997;22:71–76.

Van den Berg M, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FXR, Liem AKD, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106(12):775–792.

Varanasi U, Stein JE, Reichert WL, Tilbury KL, Krahn MM, Chan SL. Chlorinated and aromatic hydrocarbons in bottom sediments, fish, and marine mammals in US coastal waters: laboratory and field studies of metabolism and accumulation. In: Livingstone CH, Walker DR, editors. Persistent pollutants in marine ecosystems. New York: Pergamon Press Ltd, 1992:83–115.

Varanasi U, Stein JE, Tilbury KL, Meador JP, Sloan CA, Clark RC, Chan SL. Chemical contaminants in gray whales (*Eschrichtius robustus*) stranded along the West Coast of North America. Sci Total Environ 1994;145:29–53.

Wagemann R, Muir DCG. Concentrations of heavy metals and organochlorines in marine mammals of northern waters: overview and evaluation. Can Tech Rep Fish Aquat Sci 1984;1279:1–97.

Weisbrod AV, Shea D, Moore MJ, Stegeman J. Bioaccumulation patterns of polychlorinated biphenyls and chlorinated pesticides in Northwest Atlantic pilot whales. Environ Toxicol Chem 2000a;19(3):667–677.

Weisbrod AV, Shea D, Moore MJ, Stegeman JJ. Organochlorine exposure and bioaccumulation in the endangered Northwest Atlantic right whale (*Eubalaena glacialis*) population. Environ Toxicol Chem 2000b;19(3):654–666.

Wise SA, Schantz MM, Koster BJ, Demiralp R, Mackey EA, Greenberg RR, Burow M, Ostapczuk P, Lillestolen TI. Development of frozen whale blubber and liver reference materials for the measurement of organic and inorganic contaminants. Fresenius J Anal Chem 1993;345:270–277.

Woodley TH, Brown MW, Kraus SD, Gaskin DE. Organochlorine levels in north Atlantic right whale (*Euba-*

laena glacialis) blubber. Arch Environ Contam Toxicol 1991;21:141–145.

Worthy GAJ, Abend AG. Impact of killer whale predation on harbor seals in Prince William Sound: a preliminary assessment of diet using stable isotope and fatty acid signature analysis on blubber biopsies. Exxon Valdez Oil Spill Restoration Project Final Report, 1997, Restoration Project 96012A–2, p. 26.

Ylitalo GM, Buzitis J, Krahn MM. Analyses of tissues of eight marine species from Atlantic and Pacific Coasts for dioxin-like chlorobiphenyls (CBs) and total CBs. Arch Environ Contam Toxicol 1999;37:205–219.