

## The role of organochlorines in cancer-associated mortality in California sea lions (*Zalophus californianus*)

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### Abstract

Wild California sea lions (*Zalophus californianus*) have an unusually high prevalence of neoplasms (18% of stranded dead adults) and high levels of contaminants. The contribution of organochlorine (OC) tissue burdens to the probability of sea lions dying from carcinoma was explored using a logistic regression model. Levels of PCBs and DDTs were determined in blubber of sea lions diagnosed with metastatic carcinoma and animals that had died from non-carcinoma-related incidents (e.g., gunshot, domoic acid poisoning). Animals with carcinoma had higher mean concentrations (based on wet weight) of PCBs and DDTs (more than 85% and 30% higher, respectively) in blubber than did sea lions without carcinoma; the highest concentrations of OCs in the sea lions affected with carcinoma were measured in the males. Blubber thickness was significantly different between the two groups of sea lions, but after controlling for this difference, there was still a significant effect of PCBs, but not DDTs, on the probability of sea lions dying with carcinoma. Age, sex, mass and length did not affect the probability of dying from carcinoma. Published by Elsevier Ltd.

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### 1. Introduction

Organochlorines (OCs) such as polychlorinated biphenyls (PCBs) and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and DDT metabolites are persistent pollutants that biomagnify in the environment and have been measured in tissues of marine mammals world-wide (O'Shea, 1999). Experimental exposure stud-

ies show effects of these chemicals on the physiology, immune function and reproductive success of pinnipeds (Brouwer et al., 1989; Ross et al., 1996; reviewed in O'Hara and O'Shea, 2001). Furthermore, epidemiological investigations have linked high tissue residues of OCs to increased prevalences of infection and physiological impairment in other marine mammal species (Jepson et al., 1999; Simms et al., 2000; Hall et al., 2003; Jenssen et al., 2003). For example, immunosuppressive effects were observed in captive harbor seals (*Phoca vitulina*) after they were fed Baltic Sea herring that contained high levels of PCBs and other OCs (DeSwart et al.,

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1994; Ross et al., 1995). Belugas (*Delphinapterus leucas*) that stranded in the highly polluted St. Lawrence Estuary had a high prevalence of neoplasms and contained high tissue levels of PCBs and DDTs (Martineau et al., 1999). These whales also exhibited impaired reproductive and immune function (Beland et al., 1993; DeGuise et al., 1994, 1995).

Until recently, evidence of neoplasms in marine mammals has been scarce. There were only seven reports of neoplasms documented in pinnipeds prior to the early 1970s (Mawdesley-Thomas, 1974). By the early 1980s, neoplastic lesions were found in 2.5% of 1500 marine mammals surveyed (Howard et al., 1983). Neoplasms in stranded belugas from the St. Lawrence Estuary have been reported by several researchers, with tumors present in 40% of beluga whales that were found dead between 1982 and 1990 (Martineau et al., 1988; Girard et al., 1991; Beland et al., 1993; DeGuise et al., 1994). An 18% prevalence of neoplasms, the highest to date in a pinniped population, has been reported in adult California sea lions (*Zalophus californianus*) that stranded live along the central California coast (Gulland et al., 1996). The predominant neoplasm was a poorly differentiated carcinoma of urogenital origin, occurring in sexually mature animals of both sexes. These tumors in California sea lions contain cytoplasmic and nuclear virions that are characteristic of a herpesvirus (Lipscomb et al., 2000). King et al. (2002) report that, based on phylogenetic analysis, this herpesvirus appears to be a gammaherpesvirus distinct from other phocid gamma-herpesviruses, and named it otarine herpesvirus-1 (OtHV-1). After establishment of a polymerase chain reaction (PCR) specific for OtHV-1, the authors found that viral DNA was present in all urogenital tumors of California sea lions examined in the study (King et al., 2002). Gammaherpesviruses are associated with neoplasia in several species of animals but other factors, such as genetics, other infections and exposure to chemicals, are often needed for neoplasia to develop in individual animals (Morrison et al., 1996; McKinnell and Carlson, 1997; Lackovich et al., 1999).

Although the etiology and pathogenesis of this neoplasm in California sea lions are unknown, environmental contaminants may play a role. Certain types of chemical contaminants can directly induce carcinogenesis through DNA damage as initiators or complete carcinogens (acting as both initiators and promoters) (Faroon et al., 2001; Glauert et al., 2001; Ludewig, 2001). Environmental contaminants may be indirectly linked to carcinogenesis by acting as promoters by increasing cell proliferation (Faroon et al., 2001; Glauert et al., 2001; Ludewig, 2001) or as immune suppressors by increasing susceptibility to infection by an oncogenic virus (Gauthier et al., 1999). Previous studies show that California sea lions are exposed to OCs (DeLong et al., 1973; Kajiwara et al., 2001) and that some of these com-

pounds are present at tissue concentrations that are associated with impaired immune function in other species of marine mammals (Ross et al., 1995, 1996; Beckmen et al., 2003). Based on these findings, exposure to high levels of immunosuppressive chemical contaminants coupled with infection by an oncogenic virus could potentially increase the incidence of neoplasm in California sea lions.

In the present study, concentrations of selected OCs in blubber of California sea lions diagnosed with carcinoma, as well as in sea lions without carcinoma that died from other causes, were measured to determine whether exposure to contaminants was associated with the presence of carcinoma. We investigated the role of OC contaminants in the probability of sea lions dying of carcinoma, controlling for potentially confounding life history parameters.

## 2. Materials and methods

### 2.1. Sea lion sampling

California sea lions that stranded along the central California coast from 1993 to 2003 were brought to The Marine Mammal Center, Sausalito, CA, for examination. All fresh adult animals that died during this study period at the facility were necropsied and the cause of death was determined as described by Gulland et al. (1996). Blubber samples were collected from California sea lions diagnosed with ( $n = 38$ ) and without ( $n = 38$ ) carcinoma and were analyzed for OCs and lipids. Formalin-fixed tissues were examined histologically to determine whether or not carcinoma was present in these animals. Of the 38 sea lions that died of non-carcinoma causes, three died of leptospirosis, 21 animals died with neuronal necrosis due to domoic acid poisoning (Scholin et al., 2000) and 14 died from other causes (coccidiodomycosis, gunshot, trauma, cerebral hemorrhage, pneumonia, nephritis, pulmonary congestion, retroperitoneal abscesses or renal failure). The life history data (sex, age, length, mass) and body condition index (blubber thickness) were collected at The Marine Mammal Center according to Gulland et al. (1996). Best estimate of age was determined on all sea lions by counting annual growth layers in teeth (Payne, 1987). A blubber sample from each animal was collected aseptically with acetone-washed stainless steel knives and wrapped in Teflon sheets. The blubber samples were stored at  $-40^{\circ}\text{C}$  before transfer to the NOAA Fisheries's Northwest Fisheries Science Center in Seattle for analyses.

### 2.2. OC and lipid analyses

Two methods [gas chromatography with electron capture detection (GC/ECD) and high-performance liquid

chromatography with photodiode array detection (HPLC/PDA)] were used to analyze for PCBs and DDTs in blubber of California sea lions. In addition, lipid concentrations of the sea lion blubber samples were quantified by two methods [gravimetric and thin layer chromatography with flame ionization detection (TLC/FID)]. Blubber of sea lions collected from 1993 to 1998 were analyzed for OCs by GC/ECD (Krahn et al., 1988; Sloan et al., 1993) and lipids by gravimetric analysis whereas blubber of animals sampled after 1998 were analyzed for OCs by HPLC/PDA (Krahn et al., 1994) and lipids by TLC/FID (Krahn et al., 2001; Ylitalo et al., in press). We have found that the OC data obtained by these two methods were in good agreement for a wide range of marine biota (Krahn et al., 1994). Previous studies have found that for many tissues of marine animals, the percent lipid values determined by TLC/FID are comparable to or lower than the values measured gravimetrically (Delbeke et al., 1995; Bergen et al., 2000). In a gray whale (*Eschrichtius robustus*) study, we found that the mean percent lipid values of blubber determined by TLC/FID ( $43 \pm 11\%$ ) were comparable to the values measured gravimetrically ( $48 \pm 22\%$ ) (Krahn et al., 2001).

### 2.3. Analysis by GC/ECD

Briefly, blubber samples (1.0–2.0 g) were weighed and extracted by homogenizing with sodium sulfate and methylene chloride. The methylene chloride extract was filtered through a column of silica gel and alumina and concentrated for further cleanup to remove interfering lipid compounds. Size exclusion chromatography with high-performance liquid chromatography (HPLC) was used to collect the fraction containing the OCs. The HPLC fraction was analyzed for OCs by capillary column GC/ECD. Identification of selected individual PCBs and DDTs was confirmed using gas chromatography/mass spectrometry (GC/MS). The summed PCBs were calculated by summing the concentrations PCBs 18, 28, 44, 52, 66, 101, 105, 118, 128, 138, 153, 170, 180, 187, 195, 206, and 209. The summed DDTs were calculated by adding the concentrations of *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD, *o,p'*-DDE and *o,p'*-DDT. Lipid content in the sea lion blubber samples was determined gravimetrically as described by Sloan et al. (1993).

### 2.4. Analyses by HPLC/PDA

Blubber samples (0.2–0.4 g), hexane/pentane (1:1 v/v), sodium sulfate and a surrogate standard were homogenized and separated from interfering compounds (e.g., lipids, aromatic compounds) on a gravity flow cleanup column that contained neutral, basic and acidic silica gels eluted with hexane/methylene chloride (1:1 v/v).

Prior to the cleanup step, a 1-mL aliquot of each sample extract was removed for lipid quantitation by TLC/FID. Eight dioxin-like congeners (PCBs 77, 105, 118, 126, 156, 157, 169, 189) were resolved from eight other selected PCBs (PCBs 101, 128, 138, 153, 170, 180, 190, 200) and six additional organochlorines [*o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, hexachlorobenzene (HCB)] 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. Purity of each analyte was confirmed by comparing spectra within a peak to the apex spectrum. Concentrations of summed PCBs were calculated using the following formula:  $\sum \text{PCBs} = \sum \text{concentrations of 16 PCBs listed above (based on individual response factor)} + \sum \text{concentrations of other PCB congeners (calculated by summing areas of peaks identified as PCBs and using an average PCB response factor)}$ . Summed DDT 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).

### 2.5. Quality assurance

To monitor the accuracy of our GC/ECD and HPLC/PDA methods, a National Institute of Standards and Technology (NIST) control whale blubber sample or standard reference whale blubber (SRM1945) was analyzed with each sample set and results met laboratory criteria (Wise et al., 1993). Approximately 10% of the sea lion 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. Method blanks also met laboratory criteria.

## 3. Results

The mean, standard deviation, median and ranges for the independent variables used in predicting the probability of carcinoma mortality among the California sea lions, by sex are given in Tables 1 and 2. The Kolmogorov–Smirnov goodness of fit test was used to test whether the observations were consistent with a random sample drawn from a normal OC distribution. All variables were normally distributed except the lipid content and contaminant concentrations. The lipid values were therefore arcsine transformed and contaminants were  $\log_{10}$  transformed and the resulting values used in the final analysis. The distribution of sexes was identical between the two cause of death groups, with 8 males and 30 females in each.

Table 1

Mean, standard deviation, median and ranges for the independent and confounding variables in male California sea lions that died from all causes

	Mean	SD	Median	Minimum	Maximum
<i>Males (n = 16)</i>					
Age (year)	11	2.3	12	6.0	15
Length (cm)	210	30	210	170	300
Mass (kg)	150	46	150	69	250
Blubber thickness (mm)	20	14	16	7.0	55
Percent lipid	45	29	52	2.0	84
Summed PCBs (ng g <sup>-1</sup> , wet wt.)	20,000	18,000	20,000	1700	64,000
Summed PCBs (ng g <sup>-1</sup> , lipid wt.)	77,000	79,000	48,000	2700	270,000
Summed DDTs (ng g <sup>-1</sup> , wet wt.)	76,000	62,000	54,000	9400	200,000
Summed DDTs (ng g <sup>-1</sup> , lipid wt.)	380,000	480,000	170,000	15,000	1,800,000

Table 2

Mean, standard deviation, median and ranges for the independent and confounding variables in female California sea lions that died from all causes

	Mean	SD	Median	Minimum	Maximum
<i>Females (n = 60)</i>					
Age (year)	9.0	2.2	9.0	4.5	15
Length (cm)	160	11	160	130	190
Mass (kg)	73	16	69	50	120
Blubber thickness (mm)	15	11	13	1.0	42
Percent lipid	34	23	37	0.91	78
Summed PCBs (ng g <sup>-1</sup> , wet wt.)	7900	6900	6100	720	39,000
Summed PCBs (ng g <sup>-1</sup> , lipid wt.)	83,000	160,000	24,000	940	860,000
Summed DDTs (ng g <sup>-1</sup> , wet wt.)	30,000	27,000	21,000	1700	120,000
Summed DDTs (ng g <sup>-1</sup> , lipid wt.)	250,000	440,000	82,000	4200	2,300,000

There were no differences between the age, length or mass of the animals that died of carcinoma compared with those that died of non-carcinoma (two sample *t*-tests with separate variances,  $p > 0.05$ ). However, there were significant differences in blubber thickness ( $t = 4.61$ ,  $p < 0.0001$ ) and lipid ( $t = 3.49$ ,  $p = 0.0008$ ) between the two groups. Animals that died from carcinoma had significantly thinner blubber (approximately 50% of that found in the non-carcinoma animals) (Fig. 1). In addition the carcinoma animals had a significantly lower blubber lipid content than the non-carcinoma animals (approximately 60% of that found in the non-carcinoma animals). There was also a significant positive relationship between blubber thickness and lipid content (Fig. 2), regardless of cause of death (least squares regression  $p < 0.0001$ ,  $R^2 = 0.49$ ).

### 3.1. Concentrations of PCBs and DDTs

Two analytical methods were used to determine the blubber lipid content, as well as two methods to measure OC concentrations during this study. However, there was no difference in the mean levels of lipid or these two OC classes when the results were stratified by extraction or determination method (two sample *t*-test, separate variances,  $p > 0.05$ ).

In addition to the correlation between lipid content and blubber thickness in the study animals, there was

a significant negative relationship between  $\log_{10}(\text{PCB})$  wet weight and blubber thickness (Fig. 3, least squares regression  $p < 0.008$ ,  $R^2 = 0.09$ ), although there was a high degree of variability around the regression. There was no difference in the mean concentrations of summed OCs (log transformed summed PCBs or summed DDTs)

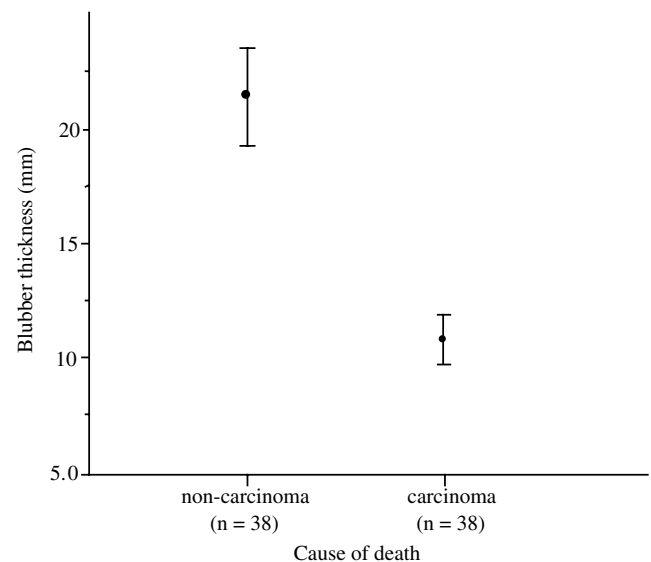


Fig. 1. Mean ( $\pm$ SE) blubber thickness in California sea lions diagnosed with or without carcinoma.

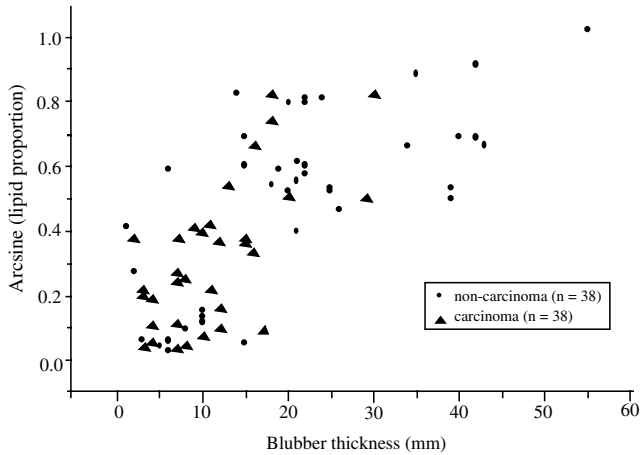


Fig. 2. Relationship between blubber thickness and the proportion of lipid in the blubber, by cause of death in California sea lions.

by sex for each cause of death group, with males and females having comparable concentrations.

Summed PCB and summed DDT concentrations in the blubber of the California sea lions were significantly different between the carcinoma and the non-carcinoma groups (Fig. 4a and b). For example, animals that died from carcinoma had levels of summed PCBs (based on wet weight) almost twice those that died from other causes and concentrations of summed DDTs (based on wet weight) that were approximately 30% higher.

### 3.2. Concentrations of PCBs and cause of death

In order to investigate the relationship between the probability of carcinoma death and summed blubber PCB concentrations, confounding factors such as sex, age, mass, length, blubber thickness and blubber lipid were controlled for, using a logistic regression model with cause of death (carcinoma or non-carcinoma) as the dependent variable (using a generalized linear model fitted with a binomial family and logit link function) and

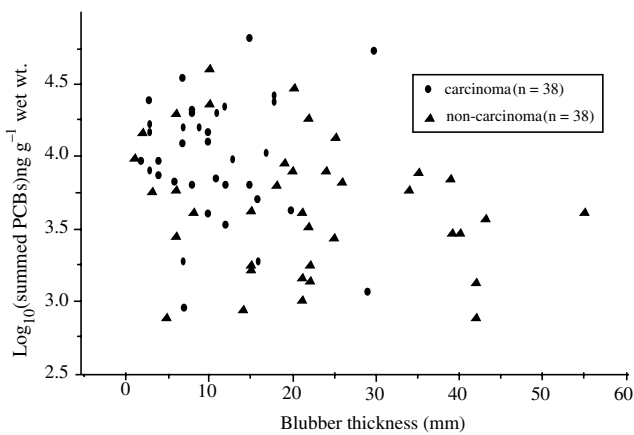
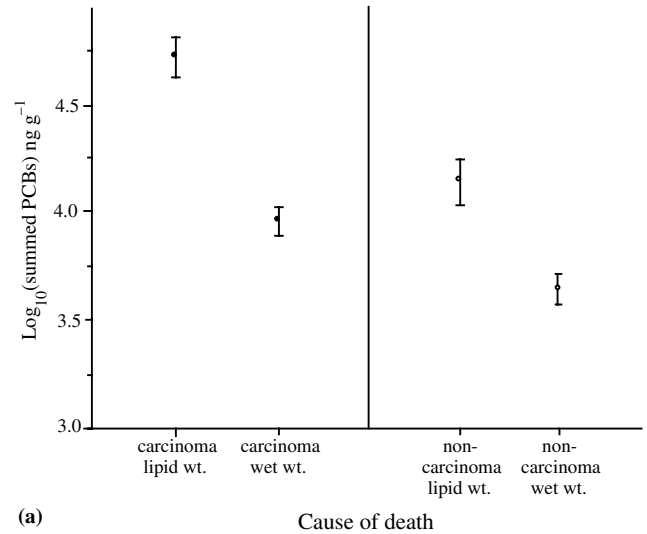
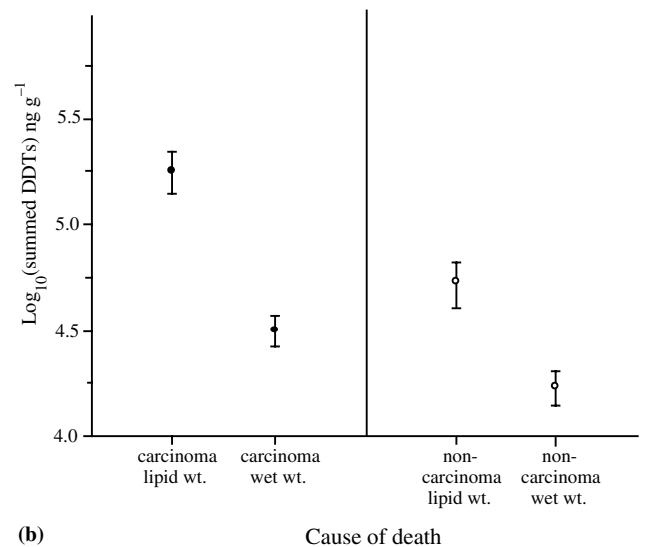


Fig. 3. Relationship between blubber thickness and  $\log_{10}(\text{PCB})$  (ng/g wet weight), by cause of death in California sea lions.



(a)



(b)

Fig. 4. Mean ( $\pm$ SE)  $\log_{10}$  (a) summed PCBs and (b) summed DDTs in blubber by cause of death, on a lipid and wet weight basis.

the confounding factors and blubber PCB concentrations as predictor variables. The different lipid extraction and PCB analysis methods were also included as factors in the model. A global model including all the potential confounding factors was first fitted (and the model fit assessed from the residuals) and then a stepwise regression was conducted. Using Akaike's An Information Criterion method (AIC) the best set of predictor variables was determined, trading off the fit of the model's bias against its precision using the AIC formula  $2 * \log\text{-likelihood} + k * \text{the number of parameters in the fitted model}$  where  $k = 2$ . (Burnham and Anderson, 1998). This model selection procedure indicated that blubber thickness and  $\log_{10}\text{PCB}$  (wet weight) were the two best predictors of the probability of carcinoma death. The model summary is given in Table 3, indicating that after controlling for the difference in blubber



Table 3

Logistic regression model of cause of death (carcinoma or non-carcinoma) against blubber thickness and blubber log<sub>10</sub> (summed PCBs) (ng/g, wet wt.)

	Estimate	Standard error	z-value	Pr(>z)
Intercept	3.34	2.4	1.39	0.16
Blubber thickness	0.098	0.032	3.12	<b>0.002</b>
log <sub>10</sub> (summed PCBs) (wet wt.)	-1.26	0.60	-2.09	<b>0.036</b>

thickness between the two groups, blubber PCBs remains a significant predictor of cause of death in California sea lions. The independent variables in the model together explain approximately 22% of the total deviance. Fig. 5a and b show the fitted logistic model (the probability of carcinoma death in relation to blubber thickness plus summed blubber PCBs) against blubber thickness and summed blubber PCBs (wet weight).

A second model was then constructed using summed blubber PCBs on a lipid weight basis. However, the model selection process indicated that blubber thickness and PCBs on a wet weight basis were better predictors in this dataset, compared to PCBs on a lipid weight basis. Because blubber thickness and lipid content are negatively correlated and there is also a significant relationship between summed PCBs and blubber thickness, including summed blubber PCBs on a wet weight basis together with blubber thickness in the model ensures independence between the predictor variables.

The effect of interactions between confounding variables such as age/sex and blubber thickness/summed PCBs were also tested but were not significant, suggesting the relationship is the same for males and females. This can be seen in Fig. 6, which shows the relationship between ages and summed PCBs (wet weight) with symbol size relating to blubber thickness and fill type relating to cause of death. No clusters are observable within the data. Concentrations of blubber PCBs were not age-related in either sex.

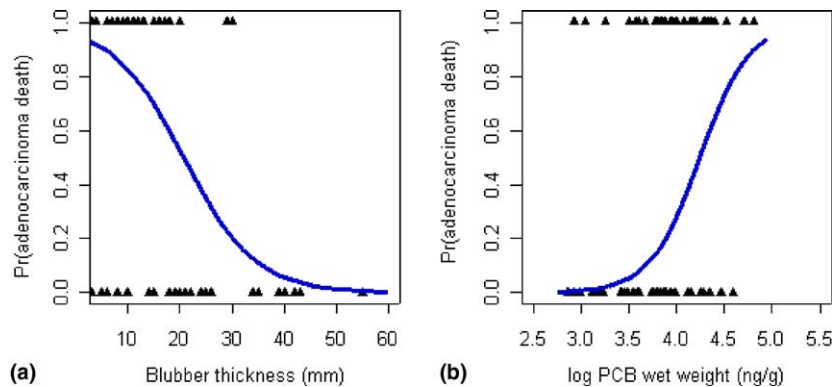


Fig. 5. Relationship between the probability of carcinoma death and (a) blubber thickness and (b) summed blubber PCBs in California sea lions. The triangles show the data points and the lines are predicted from the logistic regression model that incorporates both these predictor variables.

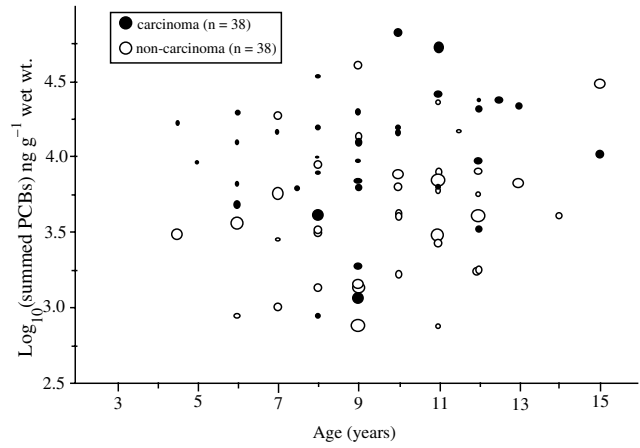


Fig. 6. Age-specific summed blubber PCBs (wet weight) in California sea lions. Symbol sizes are proportional to blubber thickness and coded by cause of death (filled = carcinoma death, open = non-carcinoma death).

3.3. Concentrations of DDTs and cause of death

In contrast to the findings for PCBs, there was no significant relationship between probability of carcinoma death and log summed DDT on a wet weight or lipid weight basis. After controlling for the other confounding factors blubber thickness was the single best predictor and summed DDTs, along with the other independent variables, was not significant.

There was a significant positive linear relationship between blubber summed PCBs and summed DDTs (Fig. 7, least squares regression,  $p < 0.0001$ ,  $R^2 = 0.455$ ) which might suggest there should also be a relationship between levels of summed DDTs and cause of death. However, there are five male sea lions with high blubber PCB concentrations but lower DDT concentrations than would be predicted by this linear relationship. This may be why the relationship with carcinoma death seen for the summed blubber PCBs in this dataset is not repeated for the summed blubber DDTs.

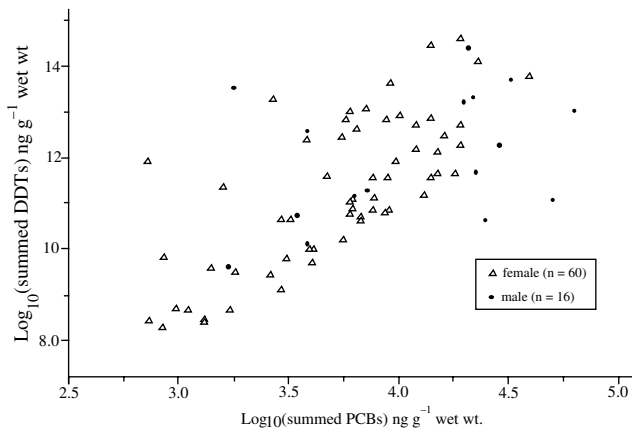


Fig. 7. Relationship between  $\log_{10}$  summed blubber PCBs and  $\log_{10}$  summed blubber DDTs on a wet weight basis, by sex.

#### 4. Discussion

We demonstrate an association between blubber concentrations of summed PCBs and carcinoma in California sea lions, which suggests that these contaminants may play a role in the development of this disease. The blubber levels of summed PCBs reported here are within the range of those reported in other species of marine mammals that showed evidence of physiological effects, including immunosuppression and reproductive dysfunction (Reijnders, 1986; DeSwart et al., 1994; Ross et al., 1996). High levels of OCs including PCBs have been measured in marine sediments and biota samples collected along the central and southern California coast (DeLong et al., 1973; Mearns and Sherwood, 1976; Harmon et al., 1998; Brown et al., 1998; Ylitalo et al., 1999). These contaminants enter the marine environment via several sources including agricultural runoff, atmospheric deposition and constituents of municipal wastewater plants (Bay et al., 2003).

Blubber samples of California sea lions that died from carcinoma contained higher concentrations of PCBs and DDTs than blubber of non-carcinoma animals. However, the comparison of OCs levels between these two groups of animals is confounded by the effect of a number of life history variables on blubber OC concentrations. One of the most important of the confounding factors in studies examining contaminants and disease is the dynamics of lipophilic contaminants in blubber (Hall et al., 1992; Aguilar and Borrell, 1994). It is suspected that body condition can influence OC burdens in the blubber of marine mammals even though the dynamics of blubber OCs during changes in physiological condition of these animals are complex and poorly understood (Aguilar, 1987). Marine mammals can lose weight during various stages of their life cycles due to different stresses such as disease, migration, lactation, moult or reduced prey abundance. The mobiliza-

tion of lipid associated with weight loss could lead to either redistribution of OCs to other tissues, or to retention of OCs in blubber that would result in an increase in OC concentrations. For example, harp seals from the Barents Sea showed seasonal changes in OC levels related to changes in blubber thickness, with the highest OC levels (based on wet weight) found in the thinnest seals (Kleivane et al., 1995).

In the current study, sea lions with carcinoma had lower blubber thickness and increased concentrations of OCs compared to non-carcinoma animals. It is likely that the reduced blubber thickness in the carcinoma sea lions was associated with the occurrence of carcinoma and subsequent weight loss caused by this disease. The logistic regression model indicated that after controlling for differences in blubber thickness, there was still a significant effect of PCBs on probability of death due to carcinoma. Because of the strong correlation between blubber thickness and blubber lipid concentration, including blubber thickness in the model accounts for any differences in blubber lipid between the two groups. Therefore, the model selection process found that the best predictors of death from carcinoma were blubber thickness and PCB levels on a wet weight basis. Using PCBs per wet weight of blubber rather than per lipid weight ensures independence between dependent and predictor variables.

Other variables that we considered in the model were sex, age, mass and length. Our findings of lower OC concentrations in the blubber of adult female sea lions compared to the levels in sexually mature males are consistent with data from other marine mammal contaminant studies that report lower OC burdens in reproductive females than sexually mature males (Aguilar and Borrell, 1988; Kuehl and Haebler, 1995; Krahn et al., 1999; Tilbury et al., 1999; Ylitalo et al., 2001). This is due to the transfer of contaminants from the mother to her offspring during the female's pregnancy and lactation (Addison and Brodie, 1977; Tanabe et al., 1982; Ridgway and Reddy, 1995; Debier et al., 2003).

The lack of correlation between blubber OC concentrations and age in these sea lions was somewhat unexpected, as previous studies on contaminants in marine mammals, including other otariids, report an increase in blubber levels of males, and a decrease in levels in females, with age (Tanabe et al., 1994; Lee et al., 1996; Aguilar et al., 1999; Ross et al., 2000; Conolly and Glaser, 2002). This may be explained by the reproductive history and the feeding ecology of the sea lions in this study. The decrease in blubber OC levels in female marine mammals is explained by transfer of contaminants to her offspring (see above), so the lack of an association between contaminants and age in the female sea lions in this study may be a consequence of them all having produced offspring before sampling. The lack of accumulation of contaminants with age in males suggests

that the legacy from maternal transfer of contaminants to these animals may be more important than the accumulation from feeding in later years. This could be because male California sea lions spend less time feeding along the heavily contaminated California coast than adult females, as they eat limited amounts during the breeding season when they are on the rookeries and migrate north after the breeding season (Mearns and Sherwood, 1976; King, 1983). Additional OC studies on California sea lions sampled during various life history stages (e.g., fetus, nursing pup, weaned pup, subadult, pregnant female) are needed to help clarify our findings.

Epizootiological studies such as this one do not prove the etiology of complex diseases such as neoplasia, yet this study suggests PCBs may play a role in the development of carcinoma in California sea lions. Evidence from mammalian exposure studies suggests OCs are important in the etiology of cancer (Faroon et al., 2001; Glauert et al., 2001; Ludwig, 2001).

Organochlorines, specifically PCBs, are thought to play a role in tumor promotion and also have been shown to cause direct DNA damage through multiple mechanisms (Schilderman et al., 2000). DNA damage plays an important role in cancer initiation as damaged genes cause alterations in cell cycle control (Coltran et al., 1999). Organochlorines are also immunosuppressive (DeSwart et al., 1994). Thus, a possible scenario is that California sea lions are exposed to various types of immunosuppressive contaminants in their environment that may increase the susceptibility of these animals to oncogenic viruses, such as the gammaherpesvirus identified in these tumors (Lipscomb et al., 2000; King et al., 2002).

In conclusion, our findings support previous studies demonstrating exposure of California sea lions to relatively high levels of organochlorines (DeLong et al., 1973; Lieberg-Clark et al., 1995; Kajiwara et al., 2001), and suggest that these chemicals, especially PCBs, may play a role in the multifactorial etiology of carcinoma in these sea lions. They could be affecting carcinoma prevalences by acting as immunosuppressive agents or through genotoxic mutation and tumor promotion. While the findings suggest that there is a relationship between neoplasia and exposure to OCs in California sea lions, caution is warranted when interpreting lipophilic contaminant data on stranded marine mammals. Further studies are needed to investigate the dynamics of OC partitioning in lipid compartments associated with weight loss, as well as studies on the temporal changes in exposure of sea lions to these compounds during their development.

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