



Sentinel California sea lions provide insight into legacy organochlorine exposure trends and their association with cancer and infectious disease



Nistara Randhawa^a, Frances Gulland^{a,b}, Gina M. Ylitalo^c, Robert DeLong^d, Jonna A.K. Mazet^{a,*}

^a Wildlife Health Center, One Health Institute, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

^b The Marine Mammal Center, Sausalito, CA 94965-2619, USA

^c National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northwest Fisheries Science Center, Environmental Fisheries and Sciences Division, 2725 Montlake Boulevard East, Seattle, WA 98112 USA

^d National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center, National Marine Mammal Laboratory, 7600 Sand Point Way NE, Seattle, WA 98115 USA

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ABSTRACT

Background: Organochlorine contaminants (OCs), like polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs), are widespread marine pollutants resulting from massive historical use and environmental persistence. Exposure to and health effects of these OCs in the marine environment may be examined by studying California sea lions (*Zalophus californianus*), which are long lived, apex predators capable of accumulating OCs.

Methods: We evaluated PCB and DDT levels in 310 sea lions sampled between 1992 and 2007: 204 individuals stranded along the coast of central California, 60 healthy males from Washington State, and 46 healthy females from southern California. Lipid-normalized contaminant concentrations were analyzed using general linear models and logistic regression to ascertain temporal trends; differences between stranded and healthy sea lions; and association of organochlorines with sex, age, and presence of cancer or fatal infectious disease.

Results: Concentrations of the contaminants in stranded adults decreased over time in the study period (adjusted for sex, as adult males had higher mean blubber concentrations than adult females and juveniles). Cancer was almost eight and six times more likely in animals with higher summed PCBs and DDTs, compared to those with lower levels (95% CI 5.55–10.51 and 4.54–7.99, respectively). Fatal infectious diseases were similarly seven and five times more likely in animals with higher contaminant burdens (95% CI 4.20–10.89 and 3.27–7.86, respectively). Mean contaminant loads were significantly higher in stranded sea lions than in healthy live captured animals ($p < 0.001$).

Conclusion: Organochlorine contamination has significant associations with health outcomes in California sea lions, raising concerns for humans and other animals eating tainted seafood. While environmental exposure to these organochlorines appears to be decreasing over time based on levels in sea lion tissues, their persistence in the environment and food web for all predators, including humans, and the associated serious health risks, warrant monitoring, possibly through sentinel species like marine mammals.

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Introduction

Legacy organochlorines, such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs), are persistent organic pollutants that are toxic and ubiquitous in the global ecosystem due to their widespread use and long-range environmental transport [1]. Up to 1324 million tons of PCBs were produced globally between 1930 and 1993 [2], 50% in the USA before in-country production was banned in 1977 [3]. The Stockholm Convention in 2001 sought to eliminate the use of 12 persistent organic pollutants, including PCBs and DDTs [4]; however, in the absence of locally appropriate and cost-effective alternatives, DDT continued to be used as an insecticide for vector control

[5]. While the use of DDTs has been banned in the USA since the late 1970s, its reported global use, primarily for indoor residual spraying against malaria vectors, is estimated to be around 4882 tons per year [6].

Organochlorine contaminants (OCs) are highly persistent molecules which bioaccumulate and biomagnify up the food chain; they are absorbed by organisms more rapidly than they are metabolized and/or excreted and increase in concentration as they pass up each trophic hierarchy [7]. Because of linkages with risk of cancer and infections in animals and humans and the lack of information on effects of complex environmental mixtures of persistent OCs, studying sentinel species like California sea lions (*Zalophus californianus*), who share a mammalian physiology, similar trophic position, and seafood diet with humans, could be useful for assessing the potential exposure and health effects of OCs, especially in marine environments and coastal communities [7–12].

* Corresponding author. Tel.: +1 530 752 7526.

E-mail address: wildlifehealth@ucdavis.edu (J.A.K. Mazet).

Being long-lived apex predators, California sea lions are particularly sensitive to bioaccumulation and biomagnification; their lipid-rich blubber serves as a major storage tissue for these contaminants [13]. While organochlorines have been linked with adverse effects in marine mammals, including California sea lions [14–20], long-term studies encompassing large sample sizes are few and haven't accounted for the animal biology associated with lipid storage (e.g. age and sex) (Table S.2). It is thus a challenge to make rigorous conclusions regarding health outcomes or to extrapolate across studies to ascertain temporal trends.

We investigated blubber concentrations of OCs and associated health problems in California sea lions that stranded along the central California coast from 1992 to 2007. Our specific aims were to determine if there were temporal trends in OC concentrations in the California sea lions or associations between blubber OC levels and cancer or infectious diseases. We also sought to investigate differences between stranded and healthy sea lions to further understand the potential impacts of environmental contamination. Because observations from this study are applicable to other marine mammals and humans, it can help identify concerns for environmental management, wildlife conservation, and public health with respect to environmental OCs.

Materials and Methods

Sample and data collection

Blubber samples were collected from 204 California sea lions that stranded along the central California coast (USA) between 1992 and 2007 and were brought to The Marine Mammal Center (TMMC; Golden Gate National Recreation Area, Sausalito, California, USA) for examination and treatment (previous manuscripts using subsets of these data are presented in Table S.1). For sea lions that died before a blubber biopsy could be conducted, samples were collected at necropsy. Records from TMMC were reviewed to obtain age class, sex, and stranding location and cause, which were determined according to Greig et al. (2005) [21]. Based on the stranding cause, the animals were categorized as animals with cancer or infectious disease, or other conditions (e.g. trauma and domoic acid toxicity). Free-ranging, healthy sea lions from Puget Sound (adult males, $n = 60$) and San Miguel Island (adult females, $n = 46$) were live caught and biopsy sampled between 2001 and 2003. All samples were wrapped in Teflon sheets and stored at $-40\text{ }^{\circ}\text{C}$ before transfer to and subsequent analyses at the NOAA Fisheries' Northwest Fisheries Science Center in Seattle, Washington, USA.

OC and lipid analyses

Lipid-corrected blubber values of PCBs and DDTs were obtained for each sample by dividing the observed PCB and DDT values by blubber percent lipid. Lipid content in the sea lion blubber samples was determined gravimetrically [22] or by using thin-layer chromatography/flame ionization detection (TLC/FID), as previously described [23]. Results from the gravimetric method were converted to approximate TLC/FID values according to the following equation:

$$\text{TLC/FID value} = -0.132608 + 0.8093865 * \text{Gravimetric lipid value}$$

PCBs and DDTs in sea lion blubber samples were determined by one of three analytical methods: gas chromatography with electron capture detection (GC/ECD), high-performance liquid chromatography with photodiode array detection (HPLC/PDA), or gas chromatography/mass spectrometry (GC/MS). The choice of the method employed varied across the study period and was not mutually exclusive to any year (see below). Values below the limit of quantitation (LOQ) were set at $\text{LOQ}/2$ [24].

Sixty-seven blubber samples collected between 1992 and 1999 were analyzed by GC/ECD [25,26]. Identification of selected individual PCBs and DDTs was confirmed using GC/MS. The summed PCBs were calculated by summing the concentrations of PCBs 18, 28, 44, 52, 66, 101, 105, 118, 128, 138, 153, 170, 180, 187, 195, 206, and 209. The concentrations of p,p' -DDT, p,p' -DDE, p,p' -DDD, o,p' -DDD, o,p' -DDE and o,p' -DDT were combined to obtain the summed DDT values. The GC/MS method [22] was used to analyze 65 blubber samples collected between 1997 and 2007. The summed PCBs and DDTs were obtained by summing the concentrations of the congeners and DDTs detailed above.

Blubber PCBs and DDTs from 178 samples collected between 1998 and 2003 were analyzed using a rapid HPLC/PDA method [23,27]. Summed PCBs were calculated by summing the concentrations of PCBs 77, 101, 105, 118, 126, 128, 138, 153, 156, 157, 169, 170, 180, 189, 190, and 200; and the concentrations of other PCB congeners were calculated by summing the areas of peaks identified as PCBs and using an average PCB response factor. The concentrations of five DDTs (p,p' -DDT, p,p' -DDE, p,p' -DDD, o,p' -DDD, o,p' -DDT) were added to calculate the summed DDT concentrations.

Because it has been observed that OC values for a wide range of marine organisms obtained by HPLC/PDA and GC/ECD methods have been consistent [27], data from these analytical techniques were considered to be comparable. Similarly, summed PCBs and DDTs obtained by HPLC/PDA and GC/MS have been found to be in good agreement with each other [28]. As part of a performance-based quality assurance program [29], a method blank and a National Institute of Standards and Technology (NIST) Standard Reference Material (SRM 1945 whale blubber) were analyzed with each batch of California sea lion blubber samples. Concentrations of individual OC analytes measured in SRM 1945 were in excellent agreement with the reference values published by NIST.

Statistical analyses

Lipid-corrected OC values were analyzed to control for variations in blubber lipid concentrations [30]. Normality of OC levels was assessed, and natural log transformation was used to normalize data. Outliers were removed if their values exceeded mean levels ± 2.5 standard deviations. A one-way ANOVA was used to determine the association between OC levels, age group, and sex in stranded animals [31]. Subsequently, Tukey's multiple comparison tests were performed to compare the levels among adult males, adult females, subadults, and pups at a 0.05 level of significance [31]. For the purpose of this analysis, pups and subadults were not classified by sex due to their low numbers in the study and lack of differences in sexually dimorphic features due to life stage.

The lipid-corrected OC values obtained during the 16-year study (1992 to 2007) were divided into four periods, which balanced sample sizes within strata: 1992–95 ($n = 34$), 1996–99 ($n = 44$), 2000–03 ($n = 73$), and 2004–07 ($n = 49$). The temporal trend of contaminant levels in adult animals in the four time periods was evaluated by multiple linear regression, with time period and sex as independent variables (organochlorine levels have been associated with sex in other adult marine mammals [32], hence sex was considered a potential confounder and controlled for in the analysis). Pups and subadults were not included in this and subsequent analyses due to their low numbers.

The association between OC levels and either cancer or infectious disease in stranded adult animals was evaluated by multivariable logistic regression analyses. The presence of cancer or infectious disease was the dependent variable; with \ln summed PCBs/DDTs and sex as independent variables (thereby controlling for sex in the analyses). The comparisons were made against animals without either cancer or infectious disease. Finally, contaminant levels in healthy males from Puget Sound and in stranded males from TMMC, as well as in healthy females from San Miguel Island and in stranded females, were compared with the GLM procedure. Because the healthy sea lions were sampled

between 2001 and 2003, they were compared with the sea lions that stranded in the third temporal period, i.e. 2000–2003.

Additional analyses on important congeners common across the OC analytical methods (PCB 105, 118, 138, and 153), as well as analyses including blubber lipid percent were performed, the results of which are presented in the supplementary information.

All statistical analyses were performed using R version 3.1.2.

Results

General characteristics and association between OCs, age group, and sex

A total of 310 blubber OC samples collected from sea lions in California were analyzed. Four outliers were identified and removed from statistical analyses. The general characteristics and mean OC levels of the remaining 306 animals are presented in Table 1. Male California sea lions had the highest mean summed PCB and DDT levels, significantly higher than those of adult females (Table 1). The lowest contaminant levels were observed in stranded subadults. Summed DDTs dominated the contaminant profiles in all animals except adult males from Puget Sound.

Temporal analysis (stranded adults)

A decreasing temporal trend in blubber OC levels was observed during the study period (In summed PCBs: coefficient of time period = -0.42 , $p = 0.001$; In summed DDTs: coefficient of time period = -0.67 , $p < 0.001$). For common PCB congeners, the coefficient was -0.58 ($p < 0.001$). Adult females had lower mean OC levels than adult males throughout the study period (Fig. 1).

Organochlorines and disease association (stranded adults)

Of the adult stranded animals 87 (51%) of 164 were classified as diseased with cancer or infectious conditions. Cancer and infectious disease were significantly associated with the log transformed blubber

lipid concentrations of PCBs and DDTs (Table 2). As an example of extrapolation to real values (Table 3), cancer odds were almost eight and six times greater in animals with higher summed PCBs and DDTs of 100 $\mu\text{g/g}$, compared to lower levels of 10 $\mu\text{g/g}$ (95% CI 5.55–10.51 and 4.54–7.99, respectively), independent of sex. Similarly, fatal infectious diseases odds were also seven and five times higher in animals with 10-fold higher OC levels compared to lower contaminant burdens (PCBs 95% CI 4.20–10.89 and DDTs 3.27–7.86). Because blubber percent lipid could affect contaminant levels, further analyses controlling for lipid percent (by including it as an independent variable in the model with OCs on a lipid weight or wet weight basis), as well as congener specific associations are presented in Tables S.4 and S.5. Controlling for lipid percent still yielded significant association between cancer and OCs, and borderline significant (PCBs) to non-significant (DDTs) associations for infectious conditions.

Comparison with apparently healthy sea lions (adults)

Adult female sea lions that stranded along the central California coast (2000–03) and were brought to TMMC had higher mean OC levels than healthy adult females sampled from San Miguel Island (PCB: $p < 0.001$, DDT: $p < 0.001$, Fig. 2). Likewise, adult stranded males brought to TMMC in this time period had higher OC levels than healthy males sampled from Puget Sound (PCB: $p = 0.010$, DDT: $p < 0.001$, Fig. 2).

Discussion

Stranded sea lions with higher blubber PCBs and DDTs were more likely to have cancer. While it is not possible to establish a causal link from this study, the observed increased odds of cancer in sea lions with higher contaminant loads might well be due to their carcinogenic and tumor promoting activities [33], possibly by enhancing the effects of other carcinogenic agents by induction of the oxidative stress mechanism [34,35]. It is also likely that OCs facilitate the development of cancer in sea lions by affecting their immune systems, making them

Table 1

Mean \pm SD summed PCBs and DDTs ($\mu\text{g/g}$, lipid weight) and percent lipid (%) in the blubber of stranded and apparently healthy California sea lions in the study ($n = 306$).

	N	Summed PCB mean \pm SD (Minimum, maximum)	Summed DDT mean \pm SD (Minimum, maximum)	Percent lipid mean \pm SD (Minimum, maximum)
Sex and age group (stranded sea lions)*				
Adult male	43	126.2 ^a \pm 218.9 (3.4, 1333.3)	523.3 ^a \pm 878.3 (13.7, 5096.7)	29.2 \pm 22.7 (0.1, 67.8)
Adult female	121	50.23 ^b \pm 120.91 (0.31, 863.7)	161.74 ^b \pm 342.26 (0.78, 2290.5)	31.2 \pm 19.1 (0.9, 71.0)
Subadult	25	26.3 ^b \pm 29.9 (0.90, 125.0)	105.3 ^{ab} \pm 104.1 (8.0, 458.3)	38.3 \pm 17.7 (3.1, 67.0)
Pup	11	75.4 ^{ab} \pm 112.8 (3.4, 350.0)	368.7 ^{ab} \pm 524.7 (11.4, 1400.0)	30.7 \pm 23.1 (0.1, 62.9)
Adult stranded sea lions				
Males				
Cancer	24	189.1 \pm 273.9 (3.4, 1333.3)	787.0 \pm 1087.4 (18.0, 5096.7)	22.0 \pm 20.3 (0.1, 66.6)
Infectious disease	10	65.1 \pm 81.2 (11.4, 221.2)	267.3 \pm 360.7 (73.8, 1203.0)	28.2 \pm 22.0 (3.3, 67.7)
Other	9	26.2 \pm 23.3 (5.9, 68.6)	104.4 \pm 125.4 (13.6, 369.2)	49.5 \pm 18.8 (5.7, 67.8)
Females				
Cancer	44	93.1 \pm 176.4 (0.8, 863.6)	303.6 \pm 491.9 (2.3, 2290.5)	22.7 \pm 16.2 (0.9, 71)
Infectious disease	9	63.8 \pm 57.1 (2.5, 160.0)	163.5 \pm 163.1 (8.5, 506.4)	19.6 \pm 20.4 (2.0, 49.0)
Other	68	20.7 \pm 60.8 (0.31, 390.0)	69.7 \pm 171.8 (0.8, 1040.1)	38.2 \pm 17.9 (0.9, 71)
Apparently healthy sea lions				
Puget sound				
Adult male	60	41.0 \pm 49.2 (4.6, 228.1)	26.15 \pm 19.9 (6.6, 102.5)	36.3 \pm 19.4 (1.2, 65.0)
San Miguel Island				
Adult female	46	3.9 \pm 4.1 (0.5, 21.7)	10.8 \pm 14.3 (1.0, 72.3)	38.2 \pm 13.7 (15.0, 72.0)

^{a,b}Means with different letters in superscript are significantly different (Tukey's HSD, $p < 0.05$).

* Analyzed for association between OC levels, age group, and sex in stranded animals.

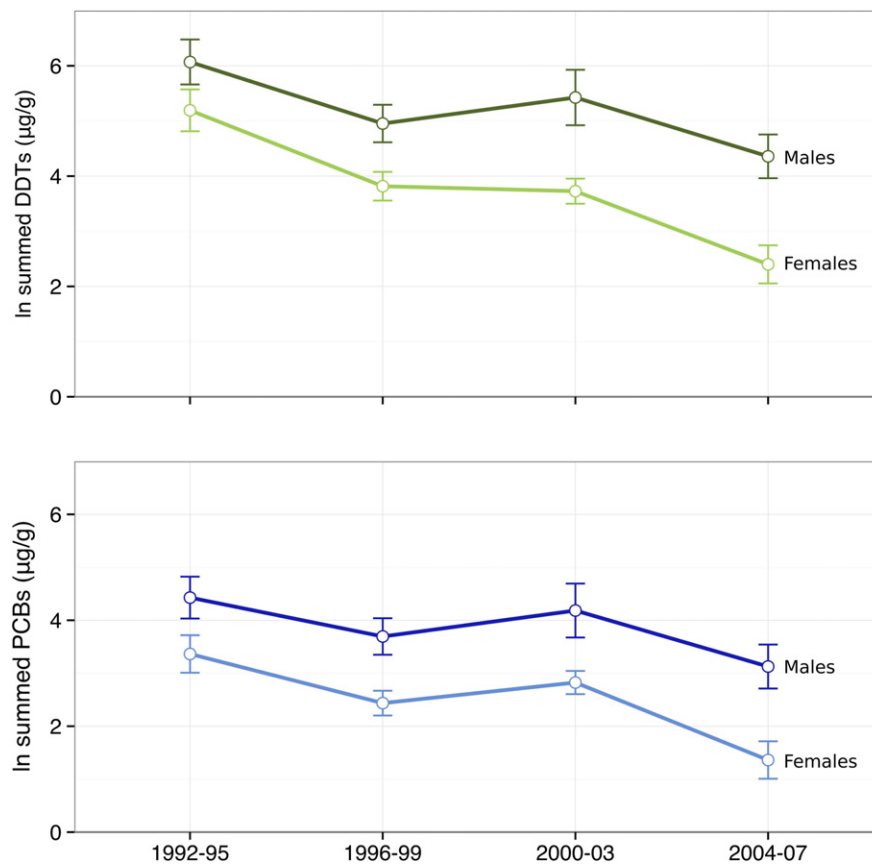


Fig. 1. Mean (\pm SE) In summed PCB and DDT levels in the blubber of adult stranded California sea lions across time periods 1992–95 (males = 13; females = 13), 1996–99 (11:27), 2000–03 (10:60), and 2004–07 (9:21).

susceptible to carcinoma-inducing viruses, such as herpesviruses [36]. Although an earlier study conducted on a smaller number of sea lions linked carcinoma and PCB exposure only [20], ours also demonstrated an association between DDT levels and risk of carcinoma.

The mean levels of PCBs in our study were much greater than the immunotoxic eliciting threshold blubber concentration of 17 $\mu\text{g/g}$ (lipid wt.) extrapolated for other aquatic mammals [37]. Considering a similar threshold for California sea lions, the observed association between PCBs and infectious disease could result from compromises in their immune responses, possibly via lower T-cell function [38] and natural killer cell function [39]. In juvenile California sea lions, PCBs have been negatively correlated with serum Vitamin A and thyroid (T3) hormones [19]. Vitamin A, T3, T-cells, and natural killer cell functions all play important roles in mammalian immune systems, the disruption of which could increase susceptibility to infections.

Stranded animals are commonly underweight and in a state of negative energy balance due to illness or malnutrition, which is accompanied by decreases in blubber lipid proportions and the animal's total blubber mass [40]. Because the blubber of sea lions is a major storage tissue for highly lipophilic persistent organic pollutants [13], and

therefore, weight changes influence the concentration and fluctuation of these contaminants in the animal's blubber and distribution to other tissues [30], we studied the relationship between OCs and disease conditions within the stranded population only. Using lipid-normalized values also facilitates comparability among animals within the study and is strongly encouraged for future monitoring [41]. While adjusting for lipid content and comparing among groups in similar physiological states (e.g. health status and sex) may not completely account for the complex processes associated with loss and gain of OCs from the lipid pool during weight loss, finding a significant relationship between cancer/infectious diseases and contaminant loads in a population with existing higher OC levels increases the weight of evidence for the association between OCs and poor health.

Comparing OC levels between apparently healthy and stranded animals provides a contrast against the backdrop of different blubber dynamics among these animals. The apparently healthy adult Puget Sound males and San Miguel Island females had lower contaminant loads than their stranded male and female counterparts. This is not surprising given that sick animals tend to concentrate OCs in their blubber layers (because of weight loss), though it points at the

Table 2
Associations of cancer and infectious diseases with blubber OC levels in adult stranded sea lions (odds ratios calculated for as per unit increase in the log transformed summed contaminant levels)*.

		Coefficient	S.E.	Pr > χ^2	OR	95% CI
Cancer	In summed PCBs	0.883	0.163	<0.001**	2.4	1.76–3.33
	In summed DDTs	0.780	0.144	<0.001**	2.2	1.65–2.89
Infectious disease	In summed PCBs	0.830	0.243	<0.001**	2.3	1.43–3.69
	In summed DDTs	0.705	0.224	0.002**	2.0	1.30–3.14

* Adjusted for sex.

** Significant at $p < 0.05$.

Table 3
Calculation of odds ratios using sample OC values and coefficient information derived from Table 2*.

	Blubber OCs (µg/g)	ln blubber OCs	PCBs		DDTs	
			Coefficient	Odds ratio	Coefficient	Odds ratio
Cancer	100	4.605	0.883	Exp(4.605*0.883)/	0.780	Exp(4.605*0.780)/
	10	2.303		Exp(2.303*0.883) = 7.63		Exp(2.303*0.780) = 6.02
Infectious disease	100	4.605	0.830	Exp(.830*4.605)/	0.705	Exp(.705*4.605)/
	10	2.303		Exp(.830*2.303) = 6.76		Exp(.705*2.303) = 5.07

* 95% CI of OR is calculated in a similar manner, using coefficient ± 1.96*S.E.

usefulness of continuous monitoring of healthy animals to study levels of persistent contaminants over time in the marine biosphere. The resultant observations may be very useful for assessing temporal trends in exposure, with stranded animal evaluations being more useful for linking illnesses with blubber contaminant concentrations.

While male sea lions had higher OC levels than those of sub-adults and adult females, they were not significantly higher than those determined in pups, likely because pups receive a large contaminant load from their mothers during gestation and lactation [32,42]. Sea lions are exposed early in life during gestation and through lactation and subsequently via the consumption of contaminated prey [42,43]. Amplifying exposure in the young and vulnerable, adult female marine mammals transfer much of their contaminant load to their offspring during nursing [44]. The observation of different congener specific PCB levels in males and females (Table S.3), particularly PCB 153, is noteworthy, possibly due to their dissimilar mobilization dynamics during lactation and gestation. OC concentrations in males, on the other hand, continue to accumulate with increasing age [45], emphasizing the justification for studies that control for age and sex.

Being extremely persistent, DDT is slowly degraded into metabolites which differ in biological activities [46]. Environmental PCBs also have

compositions different from those originally released [47,48] and are preferentially bioaccumulated across the food web [49]. Bioaccumulated PCBs tend to be more resistant to metabolism and elimination [50] and are thus more persistent in the body. Some PCB degradation products and metabolites are more toxic than their original chemical formulations [51]; hence, the complex routes of exposure, degradation, and health effects of congener mixtures may be better observed through a sentinel species rather than through laboratory studies alone.

We examined OC levels in sea lions over 16 years, and although different analytical techniques were employed, we had comparable PCB and DDT results based on analyses of NIST SRMs and field samples using these methods [27,28]. After controlling for the effect of sex, blubber OCs in adult animals appeared to decline across the study period, with DDTs decreasing more than PCBs probably reflecting a decrease in exposure. There have been few long-term data sets rigorously examining contaminants in marine mammals. To date, this study used the largest sample size of California sea lions of the same age group (n = 164 adult animals), while controlling for the effects of age and sex, as well as lipid percent—these factors having a significant effect on contaminant levels in animal tissues. Further, assessing temporal trends in healthy sea lions instead of stranded sea

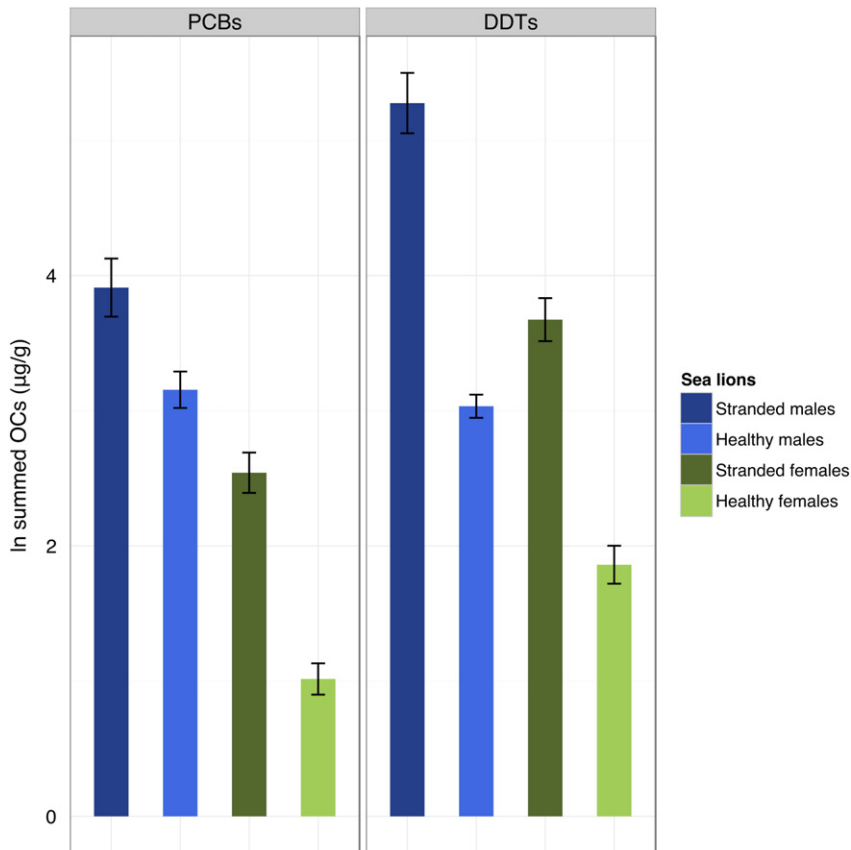


Fig. 2. Mean (±SE) of the In summed PCBs and DDTs in the blubber of adult stranded sea lions presenting to TMMC (males = 10; females = 60) between 2000 and 2003; healthy males from Puget Sound (n = 60) and healthy females from San Miguel Island (n = 46).

lions may provide a better picture of changing levels in the marine environment.

Despite the decrease in blubber OCs over time, concentrations of these compounds remain high, raising concerns about the organochlorine contaminants circulating in the marine environment. From a public health point of view, PCBs are classified as Group 1 human carcinogens [12], and DDTs have also been linked to breast cancer in humans [10,11]. Both may affect the human immune system via alterations in the thyroid hormone and T cells [52,53]. Humans are exposed to OCs mainly via consumption of contaminated food, especially fish [54,55], and since marine mammals like California sea lions share a physiology and diet similar to humans, regular and systematic monitoring of sea lions may provide an early indication of exposure changes and potential adverse health effects from bioaccumulated contaminants [7].

In conclusion, environmental OCs have significant health implications for California sea lions, as they are associated with an increased risk of cancer and infectious disease in these animals. While OC levels appear to be decreasing at the top of the food web in this ecosystem, their presence in the environment warrants concern for other animals, including humans consuming contaminated seafood. California sea lions, as sentinels, could help monitor future OC trends in the marine environment, as well as provide information about the effect of exposure to these contaminants.

Supplemental data to this article can be found online at <http://dx.doi.org/10.1016/j.onehlt.2015.08.003>.

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