

PCBs and DDT in the serum of juvenile California sea lions: associations with vitamins A and E and thyroid hormones

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Results show high levels of organochlorine contaminants in juvenile California sea lions and a link between vitamin A, thyroid hormones and PCB exposure.

Abstract

Top-trophic predators like California sea lions bioaccumulate high levels of persistent fat-soluble pollutants that may provoke physiological impairments such as endocrine or vitamins A and E disruption. We measured circulating levels of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) in 12 healthy juvenile California sea lions captured on Año Nuevo Island, California, in 2002. We investigated the relationship between the contamination by PCBs and DDT and the circulating levels of vitamins A and E and thyroid hormones (thyroxine, T4 and triiodothyronine, T3). Serum concentrations of total PCBs (\sum PCBs) and total DDT were 14 ± 9 mg/kg and 28 ± 19 mg/kg lipid weight, respectively. PCB toxic equivalents (\sum PCB TEQs) were 320 ± 170 ng/kg lipid weight. Concentrations of \sum PCBs and \sum PCB TEQs in serum lipids were negatively correlated ($p < 0.05$) with serum vitamin A and T3, potentially reflecting PCB-related toxicity. A slight but not significant negative correlation ($p < 0.1$) was observed between serum T4 and the levels of \sum PCBs and \sum PCB TEQs. Conversely, no relationship was evident between the contaminant concentrations and vitamin E ($p > 0.1$). As juvenile California sea lions are useful sentinels of coastal contamination, the high levels encountered in their serum is cause for concern about the ecosystem health of the area.

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1. Introduction

The marine ecosystem of California is highly contaminated by organochlorine pollutants (OCs) like PCBs and DDT that have been historically discharged

to the Palos Verdes Shelf in southern California (Stull et al., 1996; Zeng and Venkatesan, 1999; Lee and Wiberg, 2002). In the early 1970s, California sea lions, *Zalophus californianus*, contained very high tissue levels of DDT and PCBs (blubber levels of up to 2700 and 145 mg/kg, respectively; Le Boeuf and Bonnell, 1971; DeLong et al., 1973). Since the cessation of DDT manufacture in the United States and the dumping of its

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by-products in southern California waters in 1970 (Zeng and Venkatesan, 1999), levels in sea lions have decreased by over one order of magnitude between 1970 and 2000 (Kajiwara et al., 2001; Le Boeuf et al., 2002). The trend for PCBs during the last 30 years is more difficult to assess, due to a paucity of data and analytical variability. It is, however, clear that current levels of these OCs remain higher (up to 2 orders of magnitude) in California sea lions than in many other marine mammals of the world (Le Boeuf et al., 2002).

OCs have been linked to reproductive impairment, immunotoxicity, skeletal abnormalities, endocrine disruption and disease outbreaks in several marine mammal populations (Helle et al., 1976; Reijnders, 1986; Martineau et al., 1988; Brouwer et al., 1989; Zakharov and Yablokov, 1990; Jenssen et al., 1995; Ross et al., 1996; Jepson et al., 1999; Hall et al., 2003). In recent years, various biomarkers have been used to measure exposure, as well as potential effects, of environmental contaminants on wildlife populations. Among them, serum levels of vitamin A (retinol) and thyroid hormones (thyroxine, T4 and triiodothyronine, T3) appear to be modulated by several OCs such as PCBs and may therefore represent useful biomarkers (Brouwer and Van Den Berg, 1986; Brouwer, 1989; Zile, 1992). Vitamin A is essential for normal vision, reproduction, immunity, growth and development (Blomhoff, 1994; Blomhoff and Smeland, 1994; Napoli, 1999) and thyroid hormones play an important role in metabolism, growth and development (Legrand, 1986; Van Hardeveld, 1986; St Aubin, 2001). T4 is secreted by the thyroid gland but has little biological activity unless deiodinated to T3. Serum T3 originates from thyroid gland secretion as well as from peripheral monodeiodination of T4 (Hard, 1998).

There are several levels of OC interaction with thyroid hormones and vitamin A. One mechanism of OC action has been well elucidated and concerns an interference with the transport binding proteins. Several PCB congeners are metabolised by the cytochrome P-450 enzymes and form hydroxymetabolites that can interfere with the blood transport system of vitamin A and T4 (the thyroxine–transthyretin (TTR)–retinol–retinol binding protein (RBP) complex) (Brouwer and Van Den Berg, 1986; Brouwer, 1989; Brouwer et al., 1998). By exhibiting structural similarities with T4, some OC hydroxymetabolites compete for the binding site of T4 on its transport protein (TTR), resulting in a loss of free T4 from the circulation. The binding of hydroxymetabolites of OCs to TTR also causes a conformational change in the transport protein that reduces its affinity for RBP–retinol. This results in the dissociation of the RBP–TTR complex and the increase of glomerular filtration of the relatively small RBP–retinol (Brouwer and Van Den Berg, 1986; Brouwer, 1989; Brouwer et al., 1998).

The other levels of OC interaction involve a disruption of vitamin A and thyroid hormone metabolism. OCs can enhance the catabolism of vitamin A and its clearance from the liver through the induction of cytochrome P-450 and UDP-glucuronosyl transferase (UDP-GT) hepatic enzyme systems (Bank et al., 1989; Zile, 1992; Roberts et al., 1992; Rolland, 2000). As for circulating thyroid hormones, their levels can be altered by a disruption in their peripheral metabolism (glucuronidation, deiodination or sulfation), following OC exposure (Sepkovic and Byrne, 1984; Beetstra et al., 1991; Hard, 1998; Brouwer et al., 1998; Schuur et al., 1998; Osius et al., 1999; Rolland, 2000). Effects of OC contamination on vitamin A and thyroid hormones have been shown in laboratory animals as well as in several wildlife species, including seals (Brouwer et al., 1989; Jenssen et al., 1995; Simms et al., 2000; Chiba et al., 2001; Skaare et al., 2001; Hall et al., 2003; Jenssen et al., 2003; Braathen et al., 2004).

Another potential biomarker that plays an important role in marine mammals is the antioxidant vitamin E. Marine mammal adipose stores are very rich in polyunsaturated fatty acids (Iverson, 1993), which, in turn, are particularly sensitive to oxidation. Contamination by OCs usually causes oxidative stress through the activation of the cytochrome P-450 system and may affect the vitamin E status of these animals. Laboratory experiments have reported in vitro and in vivo effects of PCBs and related compounds on vitamin E status in several bird, mammal and fish species (Kato et al., 1989; Saito, 1990; Halouzka et al., 1994; Toborek et al., 1995; Palace et al., 1996; Kakela et al., 1999). In the liver, PCB contamination results in an increase in lipid peroxide levels and a decrease in vitamin E concentrations (Saito, 1990; Palace et al., 1996; Kakela et al., 1999). In serum, exposure to PCBs leads to increased vitamin E levels that may result from an enhanced tocopherol intestinal absorption as well as an increase of some lipoprotein fractions induced by the xenobiotics (Kato et al., 1989; Katayama et al., 1991). However, the exact mechanism remains to be elucidated. The potential impact of OCs on vitamin E status in marine mammals has been reported twice, to the best of our knowledge. A positive association between plasma and blubber vitamin E and PCBs/DDT contamination was observed in Baltic ringed and grey seals, suggesting a potential involvement of vitamin E in combating contaminant-related oxygen radical production (Nyman et al., 2003). On the other hand, no clear relationship was detected between blubber or serum PCB concentrations and serum vitamin E levels in lactating grey seals, *Halichoerus grypus*, in the United Kingdom (Debier, 2001).

Few studies have investigated the potential toxic effects of PCBs and DDT in California sea lions (DeLong et al., 1973; Gilmartin et al., 1976). Although the California sea lion population is increasing, toxic

effects may still be evident at the individual level (Le Boeuf et al., 2002). Recent studies on the contamination of California sea lions by PCBs and DDT have focused on dead, stranded animals (Lieberg-Clark et al., 1995; Kajiwara et al., 2001; Le Boeuf et al., 2002). This kind of sampling, however, has limitations since stranded individuals probably fast prior to death, resulting in the concentration of contaminant levels in the remaining amount of blubber (Aguilar, 1985). Lipid profiles, and hence contaminant levels, may also be altered in carcasses that are not fresh (Borrell and Aguilar, 1990).

We investigated for the first time PCBs and DDT levels in the serum of apparently healthy (Weise, unpublished data), juvenile California sea lions captured on Año Nuevo Island, off the coast of central California. We tested the relationship between OC contamination and the circulating levels of vitamin A, vitamin E, T3 and T4 and the applicability of these parameters as biomarkers of effect in juvenile California sea lions. Because they range less distance than sub-adult and adult males (Weise, unpublished data), juveniles can be considered as useful sentinels for monitoring the contamination of the area where they have been sampled. This study provides baseline data for comparison of OC levels in juvenile California sea lions with those sampled in potentially more contaminated ecosystems such as southern California.

2. Materials and methods

2.1. Sample collection

Blood samples were collected from 12 free-ranging juvenile California sea lions (9 males and 3 females—estimated age: 1–3 years old) from Año Nuevo Island, CA, USA (37°06′30″N, 122°20′10″W), between September 19 and October 10, 2002, as part of another study. Sea lions were captured using a specially designed hoop net constructed from soft, strong, knotless mesh (Fuhrman Diversified, Texas, USA). The headend of the net was multilayered to reduce the vision of the animal (but still allow free breathing), with a hole at the apex that allowed the animal's nose to protrude. Once the animals were in the net, the net handle was removed and the sea lions were physically restrained and sedated with Midazolam (Henry Schein, New York, USA) intramuscularly at 0.20 mg/kg (mixed with atropine (The Butler Company, Ohio, USA) at 0.02 mg/kg) alone or in conjunction with isoflurane gas (Abbott Laboratories, Illinois, USA) (0.5–2.5% w/O₂) or with isoflurane alone. Blood was collected from the caudal gluteal vein in plain Vacutainer tubes (Becton Dickinson, New Jersey, USA) using an 18 gauge × 1.5 inches needle. Animals were weighed to the nearest 1.0 kg using a digital scale suspended from a tripod. Average body mass was

45 ± 12 kg (range: 33–62 kg). Blood samples were stored on ice until transportation to the lab where Vacutainer tubes were centrifuged at 2000 × g for 15 min within 4 h of collection. Serum was aliquoted into 1.8 ml polypropylene cryotubes (Nunc cryotube™, Nalge Nunc International, Roskilde, Denmark) and stored at –80 °C until analyses were performed.

2.2. Chemical analyses

2.2.1. OC analyses

Sea lion serum samples were analyzed for selected OCs using a rapid high-performance liquid chromatography photodiode array (HPLC/PDA) method (Krahn et al., 1994). Serum (~2.0 g) was mixed with hexane/pentane (1:1 v/v), sodium sulfate (10 g) and a surrogate standard (1,2,3,4-tetrachlorodibenzo-*p*-dioxin; 250 ng). The OCs were separated from interfering compounds (e.g., lipids, aromatic compounds) on a gravity flow cleanup column (DJ Glass Factory, San Jose, California) that contained neutral, basic and acidic silica gels. The OCs were eluted from the cleanup column with hexane/methylene chloride (1:1 v/v). Prior to the cleanup step, a 1-ml aliquot of the sample extract was removed for lipid quantization by thin-layer chromatography with flame ionization detection (TLC/FID) (see description below). The dioxin-like PCB congeners (PCBs 77, 105, 118, 126, 156, 157, 169, 189) were resolved from other selected PCBs (PCBs 101, 128, 138, 153, 170/194, 180) and the different forms of DDT (*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. The recovery of the surrogate standard ranged from 76 to 100%.

2.2.2. Lipid determination

Sea lion serum samples were analyzed for lipid content using thin-layer chromatography with flame ionization detection (TLC/FID) (Shantha, 1992) as described by Krahn et al. (2001). The lipid sample extract was spotted on a Chromarod (Type SIII) and developed in a solvent system containing hexane:diethyl ether:formic acid (60:10:0.02, v/v/v). Various classes of lipids (i.e., wax/steryl esters, triglycerides, free fatty acids, cholesterol and phospholipids) were separated on the basis of their different polarity. Total lipid concentrations were calculated by adding the concentrations of the five lipid classes for each sample and were reported as weight percent (total lipids weight in relation to wet weight). Duplicate TLC/FID analyses were performed

for each sample extract. The mean value is reported. It is important to notice that the extraction solvent mixture (hexane/pentane) used here does not extract polar lipids (e.g., phospholipids) as readily as neutral lipids (e.g., wax esters, steryl esters, triglycerides). For this reason, the total lipid concentration may be underestimated.

2.2.3. Biochemical analyses

Retinol and α -tocopherol were extracted from serum and analyzed by HPLC as described in Debier et al. (2002a,b). Total T3 (tT3) and total T4 (tT4) (free and bound to transport proteins) were analyzed by radioimmunoassay (RIA) using commercially available kits (Diagnostic Products Corporation, Los Angeles, CA). We validated the method for California sea lion serum by spiking serum samples with known concentrations of tT3 and tT4. Percentages of recovery were 97.5 for tT4 and 98.9 for tT3.

2.3. Data analyses

2.3.1. PCB TEQ calculation

Toxic equivalents (TEQs) of dioxin-like PCBs were calculated by multiplying the molar concentrations of these PCB congeners by their respective toxic equivalent factors (TEFs), as described by Van den Berg et al. (1998). \sum PCB TEQs are the sum of individual PCB TEQs. The TEQ values calculated for the serum samples of sea lions in the current study are conservative values because only concentrations of dioxin-like PCBs were included in the summed TEQ values.

2.3.2. Statistical tests

Relationships between variables were tested using correlation coefficients. We calculated correlations on log transformed data and raw data and there was no major difference between the two. For the sake of simplicity, only raw data correlations are presented here. The other statistical analyses were conducted using the GLM procedure (SYSTAT 10.0). The effects of sex and weight were examined with a two-way fixed-model analysis of covariance (with weight as a continuous variable).

3. Results

3.1. Serum lipids

Serum lipid content was $0.20 \pm 0.12\%$ (range: 0.07–0.44%) and mainly consisted of wax and steryl esters (82%), triglycerides (4%) and cholesterol (14%). Serum lipids were not significantly correlated with body mass ($r = -0.36$; $p = 0.28$).

3.2. Serum OCs

Mean total serum PCB (\sum PCBs) concentrations were $26 \pm 24 \mu\text{g/kg}$ of wet weight (ww) (range: 8.1–99 $\mu\text{g/kg}$ ww) and $14 \pm 8.8 \text{ mg/kg}$ of lipid weight (lw) (range: 1.8–30 mg/kg lw). Mean \sum PCB TEQs were $0.57 \pm 0.49 \text{ ng/kg}$ ww (range: 0.17–2 ng/kg ww) and $320 \pm 170 \text{ ng/kg}$ lw (range: 38–600 ng/kg lw).

Mean total DDT (*o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT) (tDDT) concentrations in serum were $47 \pm 31 \mu\text{g/kg}$ ww (range: 19–130 $\mu\text{g/kg}$ ww) and $28 \pm 19 \text{ mg/kg}$ lw (range: 4.4–74 mg/kg lw). *p,p'*-DDE accounted for $96.4 \pm 2.1\%$ of tDDT. The tDDT/ \sum PCBs ratio averaged 2.0 ± 0.4 .

There was a significant positive correlation between serum \sum PCBs and tDDT concentrations ($r = 0.90$, $p < 0.001$) (lw basis). \sum PCBs and tDDT concentrations per ww were not correlated with serum lipids ($r = 0.36$, $p = 0.25$ and $r = 0.25$, $p = 0.43$, respectively).

There was no effect of body mass on the serum concentrations of \sum PCBs and tDDT, expressed as lw or ww ($F_{1,8} = 0.07$, $p = 0.80$ for \sum PCBs per lw; $F_{1,8} = 0.00$, $p = 0.99$ for \sum PCBs per ww; $F_{1,8} = 0.42$, $p = 0.54$ for tDDT per lw; $F_{1,8} = 0.15$, $p = 0.71$ for tDDT per ww). Similarly, no effect of sex on the levels of serum \sum PCBs and tDDT levels was detected ($F_{1,8} = 0.18$, $p = 0.68$ for \sum PCBs per lw; $F_{1,8} = 0.00$, $p = 0.99$ for \sum PCBs per ww; $F_{1,8} = 0.72$, $p = 0.42$ for tDDT per lw; $F_{1,8} = 0.16$, $p = 0.70$ for tDDT per ww). There was no significant interaction between sex and body mass ($F_{1,8} = 0.19$, $p = 0.68$ for \sum PCBs per lw; $F_{1,8} = 0.00$, $p = 0.99$ for \sum PCBs per ww; $F_{1,8} = 0.70$, $p = 0.43$ for tDDT per lw; $F_{1,8} = 0.16$, $p = 0.70$ for tDDT per ww).

3.3. Serum vitamins and thyroid hormones

Serum vitamin A and vitamin E concentrations were $330 \pm 180 \mu\text{g/l}$ (range: 82–550.5 $\mu\text{g/l}$ ww) and $10.5 \pm 6.5 \text{ mg/l}$ (range: 2.2–19.5 mg/l ww), respectively. There was a positive relationship between vitamins A and E and serum lipids ($r = 0.66$, $p = 0.03$ and $r = 0.59$, $p = 0.06$, respectively). There was no effect of body mass on the vitamin A and vitamin E concentrations in serum ($F_{1,7} = 0.19$, $p = 0.67$ for vitamin A; $F_{1,7} = 0.66$, $p = 0.44$ for vitamin E). Similarly, there were no significant differences in vitamin A and vitamin E concentrations between males and females ($F_{1,7} = 0.40$, $p = 0.55$ for vitamin A; $F_{1,7} = 1.06$, $p = 0.34$ for vitamin E). There was no significant interaction between sex and body mass ($F_{1,7} = 0.44$, $p = 0.53$ for vitamin A; $F_{1,7} = 1.03$, $p = 0.34$ for vitamin E).

Serum tT4 and tT3 concentrations were $24.7 \pm 10.5 \text{ ng/ml}$ (range: 9.0–44.5 ng/ml) and $0.52 \pm 0.20 \text{ ng/ml}$ (range: 0.28–0.84 ng/ml), respectively. The two thyroid hormones were correlated ($r = 0.87$, $p < 0.001$).

There was no effect of body mass on tT4 and tT3 levels ($F_{1,8} = 0.30$, $p = 0.60$ for tT4; $F_{1,8} = 0.23$, $p = 0.65$ for tT3). Similarly, there were no significant differences in tT4 and tT3 concentrations between males and females ($F_{1,8} = 0.48$, $p = 0.51$ for tT4; $F_{1,8} = 0.18$, $p = 0.68$ for tT3). There was no significant interaction between sex and body mass ($F_{1,8} = 0.40$, $p = 0.55$ for tT4; $F_{1,8} = 0.24$, $p = 0.64$ for tT3).

3.4. Associations of OCs with vitamins and thyroid hormones

Vitamin A negatively correlated with \sum PCB, \sum PCB TEQ and tDDT concentrations per lw (Table 1 and Fig. 1). The correlation was non-significant when these values were expressed per ww. There was no significant correlation between \sum PCBs, \sum PCB TEQ, and tDDT (expressed per serum ww or lw) and vitamin E (expressed per serum ww, lw or cholesterol) concentrations (Table 1).

tT3 levels negatively correlated with \sum PCB and \sum PCB TEQ concentration per lw (Table 1 and Fig. 2). A borderline significant correlation ($p < 0.1$) was observed between \sum PCB, \sum PCB TEQ concentrations per ww or lw and tT4 (Table 1 and Fig. 2).

4. Discussion

This is the first study to measure OC concentrations in the serum of free-ranging California sea lions and to examine the relationship with biochemical and endocrine biomarkers. Working with healthy animals avoids some confounding factors such as variations in physiological status due to malnutrition, prolonged fasting, or disease. Moreover, sex is not an issue for comparisons between groups of juveniles as differences in OC concentrations between males and females usually appear after sexual maturity, when females eliminate fat-soluble contaminants in the fetus and during

lactation (Addison and Brodie, 1987; Aguilar and Borrell, 1994; Beckmen et al., 1999; Debier et al., 2003).

4.1. Serum OCs

Reporting OC levels in blood may be problematic in terms of choosing the most appropriate basis (ww or lw) to express concentrations. In humans, postprandial increase in serum lipids leads to similar increases in circulating PCB and DDE levels, suggesting that serum OC concentrations should be expressed on a lw basis to avoid variations related to nutritional status (Phillips et al., 1989). On that basis, the fact that we do not know the nutritional status of the animals at the time of sampling in our study would thus favour the use of the lw basis to express the data. However, our results did not show any correlation between serum OC levels and serum lipid levels. It is possible that other factors such as differences in diet contamination may affect OC levels and interfere with this relationship. On the other hand, it is important to keep in mind that the methods used to determine serum lipid content may differ among studies, mainly because of variation in the extraction solvent used. The uncertainty associated with lipid determination favours the use of the ww basis to express the results and facilitate comparisons with the literature. Because each basis (ww or lw) for reporting serum OC levels presents potential advantages, we decided to keep both forms of expression for further discussion.

The mean OC concentrations in juvenile California sea lions from Año Nuevo Island were lower than those measured in 2001 in whole blood of juvenile California sea lions from San Miguel Island, southern California (Ylitalo, unpublished data). For example, the mean level of tDDT in juvenile San Miguel animals (98 ± 94 mg/kg whole blood, lw), which was determined by the same analytical procedures as in the present study, was more than three times higher than the mean concentration measured in the serum of the Año Nuevo animals. This profile is in accordance with the historical DDT contamination of the southern California Bight (Zeng

Table 1

Correlations between serum contaminant levels (\sum PCB, \sum PCB TEQ and tDDT per ww or lw) and serum biomarkers (vitamin E, vitamin E/cholesterol, vitamin E/total lipids, vitamin A and thyroid hormones—thyroxine (tT4) and triiodothyronine (tT3))

	Vitamin E	Vitamin E/cholesterol	Vitamin E/total lipids	Vitamin A	tT4	tT3
\sum PCBs (ww)	0.39 (0.24)	0.04 (0.91)	-0.06 (0.86)	-0.10 (0.77)	-0.54 (0.07)	-0.44 (0.15)
\sum PCBs (lw)	-0.31 (0.35)	-0.28 (0.40)	-0.12 (0.73)	-0.74 (0.01*)	-0.54 (0.07)	-0.66 (0.02*)
\sum TEQs (ww)	0.38 (0.25)	0.02 (0.95)	-0.09 (0.79)	-0.10 (0.77)	-0.55 (0.06)	-0.45 (0.14)
\sum TEQs (lw)	-0.41 (0.21)	-0.28 (0.40)	-0.16 (0.64)	-0.80 (0.003*)	-0.55 (0.06)	-0.70 (0.01*)
tDDT (ww)	0.20 (0.56)	-0.07 (0.84)	-0.18 (0.60)	-0.20 (0.56)	-0.50 (0.10)	-0.42 (0.17)
tDDT (lw)	-0.54 (0.08)	-0.39 (0.24)	-0.18 (0.60)	-0.73 (0.01*)	-0.36 (0.25)	-0.50 (0.10)

The correlation coefficient is given with the associated p value in parenthesis. The sample size was 12.

*Significant correlation ($p < 0.05$).

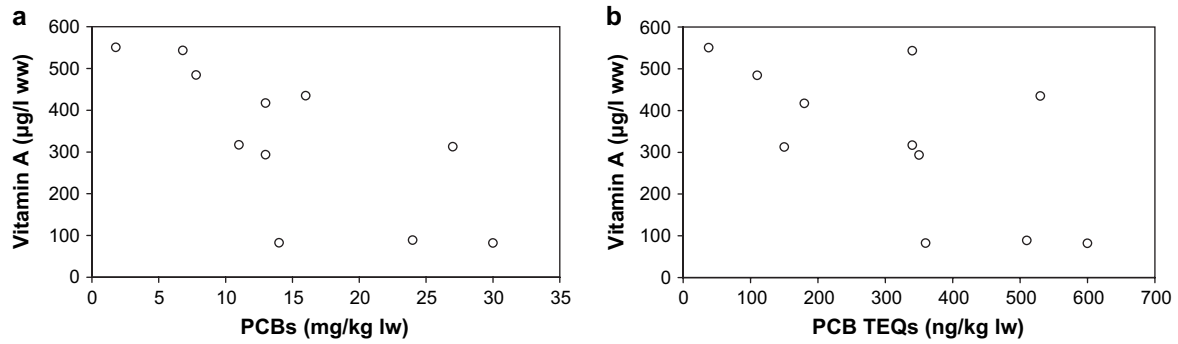


Fig. 1. Relationships between serum vitamin A and \sum PCBs (a) and \sum PCB TEQs (b) (expressed per lipid weight) in California sea lions. The vitamin A levels decreased as a function of increasing \sum PCB ($r = -0.74$; $p = 0.01$) and \sum PCB TEQ ($r = -0.80$; $p = 0.003$) concentrations.

and Venkatesan, 1999). Similar trends were found for serum \sum PCBs and \sum PCB TEQs between Año Nuevo and San Miguel juveniles.

Data concerning levels of OCs in the blood or serum of marine mammals from the west coast of North America are relatively scarce. One study reports PCB levels in whole blood samples of harbour seals captured in San Francisco Bay and on the Monterey coast in 1991–1992 (Young et al., 1998). Whole blood OC concentrations are usually lower than serum concentrations (Gomez-Catalan et al., 1991; Fujimine et al., 2000; Jenssen et al., 2003), even though OC partitioning among the different blood fractions depends on the species as well as on the type of residue that is considered (Gomez-Catalan et al., 1991). In order to express the PCB concentrations of the harbour seal study (Young et al., 1998) per unit of serum and to allow a comparison with our study, we multiplied the published blood PCB concentrations by a factor of 1.2, which was estimated from data on blood fraction percentage and PCB partitioning among fractions in harbour seals (Boon et al., 1987; Young et al., 1998). The ranges of concentrations of \sum PCBs in the serum of juvenile California sea lions were in the range of \sum PCB levels (15–400 µg/kg serum ww) calculated for harbour

seals. Another study reports serum levels of PCBs and DDT in healthy northern elephant seal (*Mirounga angustirostris*) yearlings from Año Nuevo (Beckmen et al., 1997). Circulating levels of these OCs were around 10 times lower than in the juvenile California sea lions. The lower contamination of northern elephant seals may be explained by the long-distance migrating habits and the different diet of this species.

Concentrations of tDDT, in our sample, were approximately twice as high as \sum PCB levels, which is in accordance with the literature on marine mammals and marine biota in California (Froescheis et al., 2000; Le Boeuf et al., 2002). This pattern is in contrast to other areas of the world such as the North Atlantic, the Arctic, the Gulf of St Lawrence or the Mediterranean Sea where DDT levels are comparable or lower than the concentrations of PCBs (Le Boeuf et al., 2002).

4.2. Serum vitamins and thyroid hormones

Circulating vitamin A and vitamin E levels were within the range of concentrations reported for other pinnipeds such as grey seals *Halichoerus grypus*, Weddell seals *Leptonychotes weddelli*, South American fur seals *Arctocephalus australis* and South American sea lions

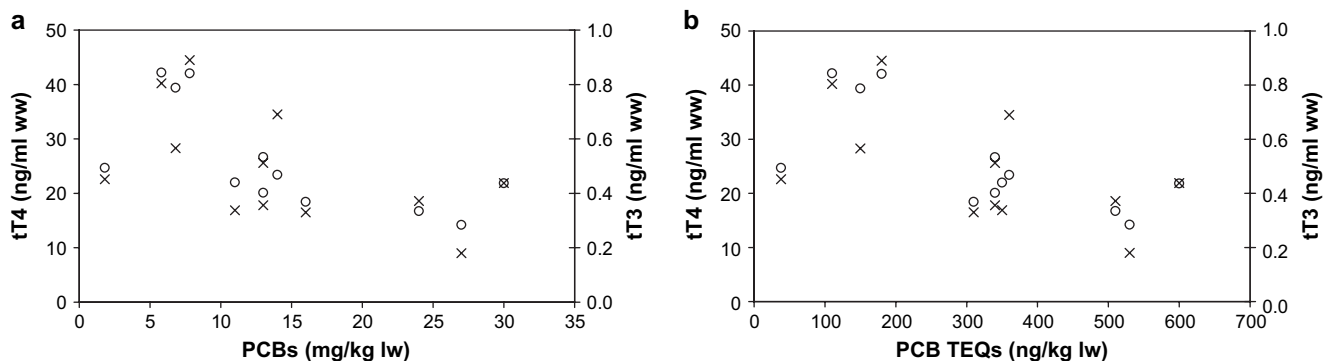


Fig. 2. Relationship between serum thyroxine (tT4, cross symbols), triiodothyronine (tT3, open circles) and \sum PCBs (a) and \sum PCB TEQs (b) (expressed per lipid weight) in California sea lions. The thyroid hormone levels decreased as a function of increasing \sum PCBs ($r = -0.54$; $p = 0.07$ for tT4; $r = -0.66$; $p = 0.02$ for tT3) and \sum PCB TEQs ($r = -0.55$; $p = 0.06$ for tT4; $r = -0.70$; $p = 0.01$ for tT3).

Otaria byronia (Karesh et al., 1997; Gelatt et al., 1999; Debier et al., 2002a,b).

Vitamin A correlated positively with serum lipid levels. At first glance, this correlation is surprising because most of this fat-soluble vitamin is usually found as retinol bound to its transport protein (RBP), which circulates associated with TTR-T4 (Blomhoff, 1994; Green and Green, 1994) and is therefore not expected to depend on serum lipid content. Further investigations should, however, be undertaken to describe the transport system of vitamin A in the blood of juvenile California sea lions and determine if circulating retinol is carried bound to its transport protein or if a significant part circulates as free retinol like in harbour seal pups (Simms and Ross, 2000). Interestingly, Ulukaya and Tokullug (1999) reported a positive correlation between blood vitamin A and triglycerides in humans and suggested an inhibitory effect of vitamin A on very low density lipoprotein catabolism, apparently through a decrease of lipoprotein lipase activity. A similar mechanism might occur in juvenile California sea lions, which could explain the positive relationship observed between vitamin A and lipids. In contrast to vitamin A, vitamin E is not transported by any specific protein and circulates in serum in association with lipoproteins. Its positive relationship with serum lipids is therefore not surprising.

Ranges of thyroid hormones, tT3 and tT4, were within those reported in the literature for other pinniped species such as northern elephant seals, grey seals, Weddell seals and harbour seals (St Aubin, 2001; Ortiz et al., 2001). Circulating levels of thyroid hormones may be subject to seasonal changes associated with moulting (St Aubin, 2001). However, in our study, all sampling occurred within a narrow one-month interval (mid-September to mid-October), and thus eliminated the potential for variation in thyroid hormone associated with moult.

4.3. Associations of OCs with vitamins and thyroid hormones

Vitamin A and tT3 levels were negatively correlated with \sum PCBs and \sum PCB TEQs, expressed per lipid weight. A trend towards a negative relationship was also observed with tT4. These negative correlations are in accordance with previous reports in several species including marine mammals (Brouwer et al., 1989; Jenssen et al., 1995; Simms et al., 2000; Chiba et al., 2001; Skaare et al., 2001; Jenssen et al., 2003; Braathen et al., 2004). They might be due to the interference of some PCB hydroxymetabolites with vitamin A and T4 blood transport, resulting in their loss from the circulation by glomerular filtration (Brouwer and Van Den Berg, 1986; Brouwer et al., 1989, 1998). The OC contamination may also induce an enhanced catabolism

and biliary excretion of vitamin A and thyroid hormones (Zile, 1992; Brouwer et al., 1998; Rolland, 2000). The stronger relationship between PCBs and T3 as compared to T4 has already been reported in several other studies with terrestrial and marine mammals (Osius et al., 1999; Chiba et al., 2001; Hagmar et al., 2001; Hsu et al., 2003; Braathen et al., 2004). A disruption of peripheral T4 deiodination activity could be responsible for this phenomenon (Osius et al., 1999).

It is important to notice that the circulating PCBs levels which have elicited decreases in plasma retinol, tT3 and tT4 in other pinniped species were lower than those reported in the juvenile California sea lions from the present study. For example, PCB concentrations (up to 42 congeners) in captive harbour seals fed fish from the PCB-contaminated Dutch Wadden sea, averaged 15 $\mu\text{g}/\text{kg}$ blood cells (Boon et al., 1987), which corresponds to 22 $\mu\text{g}/\text{kg}$ serum, when applying the conversion factors mentioned above and in Young et al. (1998). These levels have elicited decreases in plasma retinol, tT3 and tT4 levels resulting in reproductive failure in captive harbour seals (Brouwer et al., 1989; Reijnders, 1986). A strong negative impact on plasma vitamin A levels has also been reported in free-ranging grey seal pups from Norway, which exhibited serum PCB levels of 11.4 $\mu\text{g}/\text{kg}$ ww and 846 $\mu\text{g}/\text{kg}$ lw (Jenssen et al., 2003).

There was no relationship between \sum PCBs, \sum PCB TEQs and tDDT contamination and circulating vitamin E levels, even when vitamin E levels were expressed per unit of cholesterol or per unit of total lipids, the usual biochemical indices of serum vitamin E status (Mino and Nagamatu, 1986; Lachili et al., 1999; Böhles, 1997). This suggests that circulating vitamin E levels are not a sensitive biomarker of OC contamination in California sea lions.

5. Conclusions

Although OC concentrations in juvenile California sea lions from central California were lower than those measured in sea lions from southern California, the observed levels still exceeded levels that affected reproductive success, vitamin A and thyroid hormone levels in captive harbour seals (Reijnders, 1986; Boon et al., 1987; Brouwer et al., 1989). The negative relationships between OCs and circulating serum levels of vitamin A and thyroid hormones support their use as biomarkers of contaminant load in juvenile California sea lions. Further studies are, however, required to determine if the relation still holds for other age groups and times of year and to investigate its significance on the health status of the animals.

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