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Organochlorine Pesticides, Polychlorinated Biphenyls, and Butyltin Compounds in Blubber and Livers of Stranded California Sea Lions, Elephant Seals, and Harbor Seals from Coastal California, USA

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Abstract. Concentrations of polychlorinated biphenyls (PCBs), DDTs (*p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT), chlordanes (CHLs; *cis*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane), hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB), *tris*(4-chlorophenyl)methane (TCPMe), *tris*(4-chlorophenyl)methanol (TCPMOH), and mono- (MBT), di-(DBT), and tri-butyltin (TBT) were determined in blubber and livers of 15 California sea lions (*Zalophus californianus*), 6 northern elephant seals (*Mirounga augustirostris*), and 10 harbor seals (*Phoca vitulina*) found stranded along the coasts of California, USA, during 1991–1997. Among the organochlorines analyzed, DDTs were predominant, followed in decreasing order by PCBs, CHLs, TCPMe, TCPMOH, HCHs, and HCB. The greatest concentrations of organochlorines were found in California sea lions. The highest DDT and PCB concentrations found in the blubber of California sea lions were 2,900 and 1,300 μg/g, lipid weight, respectively. Concentrations of TCPMe and TCPMOH in California sea lions were correlated significantly with DDT concentrations. Concentration ratios of various organochlorines in harbor seal livers were different from those in California sea lions and elephant seals, which suggested that the sources of exposure of harbor seals to organochlorines were different from those in the other two species. Concentrations of butyltin compounds in livers of pinniped species ranged from 2 to 99 ng/g, wet weight, which were less than those observed in cetaceans and in California sea otters.

Chlorinated hydrocarbons, such as DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) and polychlorinated biphenyls (PCBs) are ubiquitous contaminants (Goldberg 1991). In particular, historical inputs of these organochlorine compounds in coastal waters of California have been a regional concern for more than 25 years (LeBoeuf and Bonnell 1971; Hose *et al.* 1989; Stephenson *et al.* 1995; Hothem *et al.* 1995). Earlier studies have shown that marine animals collected from coastal California accumulated elevated concentrations of DDT and its metabolites (*p,p′*-DDE and *p,p′*-DDD) (DeLong *et al.* 1973; Phillips and Spies 1988; Hose *et al.* 1989; Lieberg-Clark *et al.* 1995; Nakata *et al.* 1998). Contamination by DDTs in coastal California has been historically linked with reproductive problems in several predatory marine animals in this region. These effects include premature pupping of California sea lions (*Zalophus californianus californianus*) (DeLong *et al.* 1973; Gilmartin *et al.* 1976) and reproductive failure associated with egg shell thinning in brown pelicans (*Pelecanus occidentalis*) (Anderson *et al.* 1975) and bald eagles (*Haliaeetus leucocephalus*) (Garcelon *et al.* 1989). However, the link between DDT contamination and reproductive failure in animals is a controversial issue (O'Shea and Brownell 1998). Earlier studies measured DDTs or PCBs in selected species of marine mammals, particularly California sea lions, but reports of organochlorine concentrations in other pinniped species, such as northern elephant seals (*Mirounga augustirostris*) and harbor seals (*Phoca vitulina*) from coastal California are limited. Monitoring of toxic contaminants in harbor seals and elephant seals is important in view of the strandings and the occurrence of congenital and infectious diseases in these animals from the California coast (Beckmen *et al.* 1997; Trupkiewicz *et al.* 1997; Thornton *et al.* 1998). Little is known about contamination by hexachlorocyclohexane isomers (HCHs), chlordane compounds (CHLs), and hexachlorobenzene (HCB) of marine mammals from California.

Earlier studies have used blubber as a matrix in which to monitor DDTs or PCBs because of the lipophilic nature of these compounds, although this might not have any direct relation to toxic effects, unless the contaminants are mobilized from blubber. From a toxicological perspective, measurement

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of contaminants in the liver is more befitting than that in the blubber. In this study, the current concentrations of PCBs, DDTs, HCHs, CHLs, and HCB were examined in liver and blubber of California sea lions, northern elephant seals, and harbor seals that stranded along the coast of California. *Tris*(4-chlorophenyl)methanol (TCPMOH) and *tris*(4-chlorophenyl) methane (TCPMe), which originate possibly from DDT or other agrochemical formulations, have been identified as toxic and bioaccumulative contaminants in predatory animals (Korner *et al.* 1997; Poon *et al.* 1997; Falandysz *et al.* 1999). Butyltin compounds, such as mono-(MBT), di-(DBT), and tri-butyltin (TBT), which originate primarily from their use in antifouling paints, have been reported to accumulate in livers of sea otters (*Enhydra lutris nereis*) from California (Kannan *et al.* 1998). In this study, in addition to the organochlorines mentioned above, concentrations of TCPMe, TCPMOH, and butyltin compounds were measured in tissues of seals and sea lions.

Materials and Methods

Collection of Samples

Blubber and/or livers of 15 California sea lions, 6 northern elephant seals, and 10 harbor seals (Table 1) were acquired from The Marine Mammal Center (TMMC; a rehabilitation center for marine mammals), Golden Gate National Recreation Area, Sausalito, California. The pinnipeds that stranded alive along the coast of northern and central California in the 1990s were transported to TMMC. On admission, animals were weighed, and approximate age class was assigned on the basis of weight, length, umbilical regression, pelage, sagittal crest development, or tooth development. Diseases in rescued animals were diagnosed and treated at TMMC. Postmortem examinations were performed on animals that died during rehabilitation. Dead animals were dissected, blubber was sampled from over the sternum, and tissues were wrapped in aluminum foil, placed in air-tight plastic bags, and frozen immediately at −20°C until analysis. Sampling location, collection date, sex, age class, and cause of death (postmortem diagnosis) of pinnipeds are listed (Table 1; Figure 1). Adult California sea lions are greater than 8 years old; subadults are 4–8 years old. Harbor seal pup or calf refers to a weaning animal; elephant seal yearlings are year-old animals. Most of the California sea lions analyzed were adults, while the elephant seals were predominantly yearlings; Harbor seals were both juveniles and adults.

Chemical Analysis

Organochlorine pesticides, PCBs, TCPMe, and TCPMOH were analyzed following published methods (Tanabe *et al.* 1994; Watanabe *et al.* 1999). For chemical analysis, tissue samples were cut from the internal portion of the original thawed sample with a clean stainless steel scalpel to avoid surface contamination. The method consisted of Soxhlet extraction of sample tissues (5–15 g) for 8 h with diethyl ether (300 ml) and hexane (100 ml). Fat content was determined from K-D (Kuderna-Danish) concentrated aliquots of these extracts. The remaining extract was then transferred to a glass column packed with 20 g Florisil, followed by elution with a mixture of 150 ml of 80% acetonitrile and 20% hexane-washed water. The eluate from the Florisil column was collected in a separatory funnel containing 100 ml hexane and 600 ml hexane-washed water. Aliquots of hexane extract, after partitioning, were not treated with sulfuric acid to avoid the decomposition of TCPMOH. The extracts were passed through a 12-g activated Florisil packed glass column for fractionation. The first fraction eluted with hexane contained HCB, PCBs, *p,p′*-DDE, and *trans*-nonachlor, and the second fraction eluted with 20% dichloromethane in hexane contained chlordane compounds (oxychlordane, *cis*-chlordane, *cis*-nonachlor), *p,p′*-DDD, *p,p′*-DDT, HCHs (α-, β-, γ-isomers), and TCPMe. The third fraction was collected with 50% dichloromethane in hexane for TCPMOH.

Each fraction was concentrated and injected into a gas chromatograph with electron capture detector (GC-ECD) and a gas chromatograph with a mass selective detector (GC-MSD) for quantification. Organochlorines in the first and second fractions (except TCPMe) were quantified by GC-ECD (Hewlett Packard 6890 Series) equipped with an auto injection system. The GC column employed was DB-1 (J&W Scientific, Folsom, CA) fused silica capillary (0.25 mm × 30 m) coated with 100% dimethylpolysiloxane at 0.25 μm film thickness. The column oven temperature was programmed from 60 (1 min hold) to 160°C, held for 10 min, and then increased to 260°C at a rate of 2°C/min and held for 20 min. Injector and detector temperatures were set at 260°C and 280°C, respectively. Helium and nitrogen were used as carrier and makeup gases, respectively. Organochlorine concentrations were calculated from the peak area of the sample relative to the corresponding external standard. The PCB standard used for quantification was an equivalent mixture of Kanechlor preparations (KC-300, KC-400, KC-500, KC-600) with known PCB composition and content. Concentrations of individually resolved peaks of PCBs isomers and congeners were summed to obtain total PCB concentrations. For the quantification of TCPMe and TCPMOH, a GC-MSD (Hewlett-Packard 6890 series GC coupled with 5973 mass selective detector) was employed. Data were acquired by a Hewlett-Packard 5973C data system, in which the cluster ions were monitored at *m/z* 139, 251, 253, 362, 364 for TCPMOH and 311, 313, 346, 348 for TCPMe. Procedural blanks were analyzed simultaneously with samples to check for interferences or contamination from solvents and glassware. Recoveries of organochlorines through analytical procedure ranged from 97% to 110% for pesticides, 104–105% for TCPMe and TCPMOH, and 92–109% for PCBs. A standard reference material (SRM 1945; Gaithersburg, MD) was analyzed simultaneously for selected PCB congeners and OC pesticides and the results were in agreement with the certified values.

Butyltin compounds were analyzed following the method described elsewhere (Iwata *et al.* 1997). Briefly, about 2 g of tissue was homogenized with acidified 0.1% tropolone-acetone. The mixture was centrifuged at 3,000 rpm for 15 min and the supernatant was transferred to 100 ml of 0.1% tropolone-benzene and 500 ml of hexane-washed water in a separatory funnel. The organic layer was decanted, and the moisture was removed using 35 g anhydrous sodium sulfate. The extract was rotary evaporated and derivatized with *n*-propylmagnesium bromide as a Grignard reagent (approx. 2 mol/L in THF solution). Sulfuric acid was used to decompose the excess reagent. The derivatized extract was cleaned by passing through a wet Florisil column. For quantification of butyltin compounds, a gas chromatograph equipped with a flame photometric detector (Hewlett-Packard 5890 series II) and a tin filter at 610 nm was used. Chromatographic separation was performed on a DB-1 capillary column (30 m × 0.25 mm ID) coated at 0.25 μm film thickness. The column oven temperature was programmed from 80°C (1-min hold) to 170°C (1-min hold) at a rate of 15°C/min and then at a rate of 5°C/min to 210°C (1 min hold) followed by a second ramp at 15°C/min to 260°C with a 7-min final hold time. Injector and detector temperatures were held at 200°C and 270°C, respectively. Helium was the carrier gas; hydrogen, air, and nitrogen were passed at 160, 120, and 10 ml/min for the flame photometric detector. The recoveries of MBT, DBT, TBT, and hexyl TBT were 97 ± 20, 105 ± 8, 91 ± 11, and 109 ± 18%, respectively. Concentrations in samples were not corrected for the recoveries of internal standards. Butyltin concentrations are reported as ng of butyltin ion/g, on a wet-weight basis, unless specified otherwise.

Table 1. Details of pinnipeds collected from coastal waters of California for organochlorine and organotin analysis

Sample ID	Collection Date	Location	Sex	Age Class	Cause of Death	Tissue analyzed
California sea lion						
CSL 700	Apr 3, 93	Pacific Grove, Monterey	Female	Adult	Ovarian tumor, emaciation	Blubber, liver
CSL 1216	June 11, 91	Brisbane	Male	Adult	Transitional cell carcinoma	Blubber
CSL 1229	Jul 26, 91	San Francisco	Male	Sub-adult	Gunshot wound resulting in sepsis	Blubber
CSL 1396	Nov 7, 91	Carmel, Monterey	Male	Adult	Transitional cell carcinoma	Blubber, liver
CSL 1434	Mar 16, 92	Moss Landing	Male	Adult	Transitional cell carcinoma	Blubber, liver
CSL 2163	Aug 1, 93	Marina	Female	Adult	Bronchopneumonia, transitional cell carcinoma	Liver
CSL 2166	Aug 2, 93	Moss Landing	Female	Adult	Valvular endocraditis, adrenal tumor	Liver
CSL 2367	Jul 26, 94	Morro Bay	Male	Adult	Hydronephrosis, paralysis	Blubber, liver
CSL 2595	Jul 10, 95	Port San Luis	Male	Adult	Possible gunshot to head	Blubber
CSL 2836	Nov 5, 95	San Francisco	Male	Sub-adult	Interstitial nephritis	Blubber
CSL 2839	Oct 22, 95	San Francisco	Male	Adult	Septicemia, interstitial nephritis	Blubber, liver
CSL 3020	Jul 26, 96	Oceano	Female	Adult	Interstitial nephritis, renal embolism, sepsis	Blubber
CSL 3048	Jul 26, 96	San Francisco	Male	Adult	Cystitis, colonic impaction	Blubber, liver
CSL 3186	Jun 20, 97	Oceano	Female	Adult	Aged, septicemia, pneumonia, metritis	Liver
CSL 3448	Nov 20, 97	Pismo Beach	Female	Adult	Gunshot to head, early carcinoma	Blubber, liver
Elephant seal						
ES 772	Jul 3, 91	Pebble Beach	Female	Yearling	Septicemia, skin disease	Blubber, liver
ES 782	Sep 1, 91	Pebble Beach	Male	Yearling	Skin disease, Hemorrhagic gastrointestinal tract	Blubber, liver
ES 808	Mar 13, 92	Crescent City	Male	Yearling	Skin disease, verminous pneumonia, pyothorax	Blubber, liver
ES 819	Mar 28, 92	Bolinas	Female	Yearling	Skin disease, verminous pneumonia	Blubber, liver
ES 821	May 5, 92	Bodega Bay	Female	Yearling	Aspiration, verminous pneumonia, skin disease	Liver
ES 1183	Jul 3, 94	Pillar Point Harbor	Female	Yearling	Septicemia	Liver
Harbor seal						
HS 444	May 9, 91	Orick	Female	Pup/Calf	Esophageal obstruction/ cardiac arrest	Liver
HS 450	May 21, 91	Tomales Bay	Female	Pup/Calf	Gastritis and pneumonia	Liver
HS 454	Jun 4, 91	Eureka	Female	Pup/Calf	Cervical absceses, esophagitis, diaphragmatic hernia	Liver
HS 488	May 30, 91	Tomales Bay	Female	Pup/Calf	Liver failure, sepsis	Liver
HS 1040	May 23, 96	East Fort Baker	Female	Yearling	Pneumonia, colitis, cerebral edema	Liver
HS 1139	Nov 2, 96	San Francisco	Male	Adult	Peritonitis, perforated intestine	Liver
HS 1175	May 13, 97	San Francisco	Female	Adult	Pneumonia, adenitis, meningitis, encephalitis, hepatitis, vaginitis, enterocolitis	Liver
HS 1191	Jun 26, 97	Abbott's lagoon	Male	Pup/Calf	Pneumonia	Liver
HS 1195	Aug 20, 97	Elkhourn Slough	Female	Adult	Trauma, depression fracture	Liver
HS 1199	Oct 2, 97	Tiburon	Female	Adult	Meningocephalitis, hepatitis	Liver

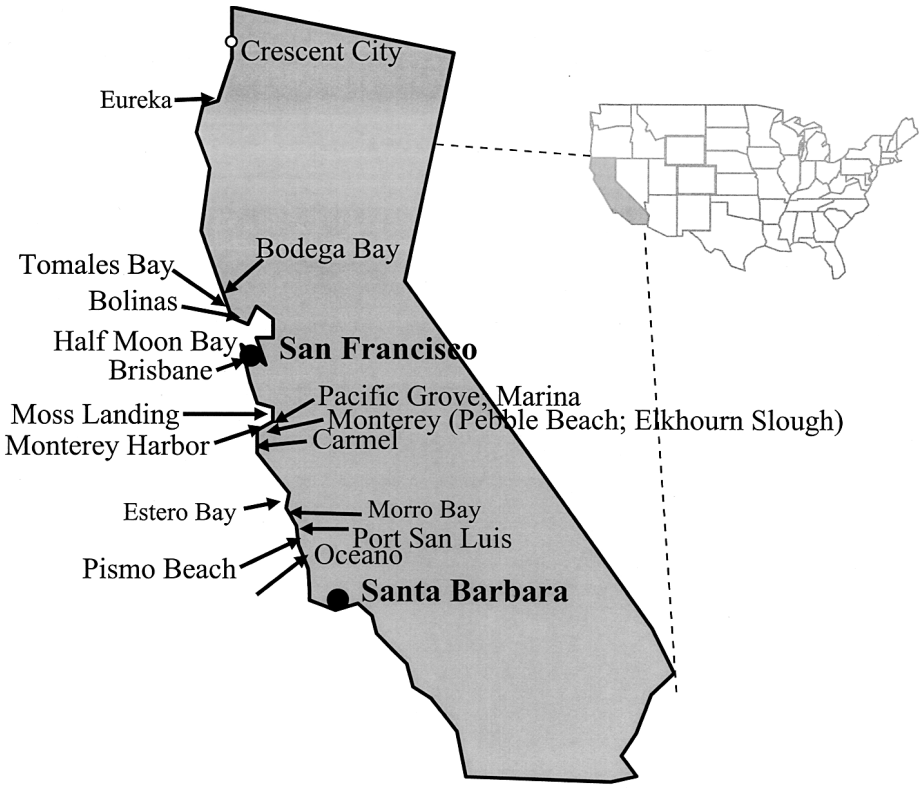

Fig. 1. Map of California showing sampling locations of California sea lions, northern elephant seals, and harbor seals

Results and Discussion

Organochlorine Concentrations

Lipid content in blubber of California sea lions and elephant seals varied widely, from 0.58% to 93% (Table 2). Most of the animals collected in this study were diseased. The wide range of lipid content indicates disturbance in the equilibration of lipid among body tissues of some animals, which could result from starvation and mobilization of fat reserves from the blubber. Concentrations of organochlorines were normalized to lipid content to facilitate comparison among individuals. The small number of samples precluded the examination of age- and location-related differences in organochlorine concentrations. Therefore, comparisons were limited to differences in concentrations among pinniped species and organochlorine compounds.

Concentrations of organochlorines in blubber and livers of California sea lions and blubber of elephant seals were on the order of DDTs > PCBs ≫ CHLs > TCPMe > TCPMOH > HCHs > HCB. Relative concentrations of PCBs and DDTs in livers of elephant seals and harbor seals were different from that in California sea lions. Concentrations of PCBs were greater than DDTs in the livers of elephant seals and harbor seals, whereas concentrations of DDTs were greater than that of PCBs in California sea lion livers.

Concentrations of DDTs in blubber and liver of California sea lions ranged from 13 to 2,900 (geometric mean 209) and from 12 to 970 (geometric mean 142) μg/g, lipid weight, respectively (Tables 2 and 3). The highest DDT concentration of 2,900 μg/g, lipid weight, was found in an adult male California sea lion from Monterey, California. However, the lipid content in the blubber of this animal was unusually low (1%), which may explain a disturbance in the equilibration of organochlorines due to the mobilization fat reserves. DDT concentrations in blubber and liver of elephant seals were 15 to 20 times less than those in California sea lions. It should be noted that elephant seals were yearlings, whereas most California sea lions were adults. Concentrations of DDTs in livers of harbor seals ranged from 2.8 to 85 μg/g, lipid weight (geometric mean 12), which was similar to those found in elephant seal livers.

The mean concentration of DDT in blubber of California sea lions that gave birth prematurely 20 years ago was 980 μg/g, lipid weight, whereas animals that gave birth after a full term had significantly lesser concentrations of 120 μg/g, lipid weight (DeLong *et al.* 1973; Gilmartin *et al.* 1976). Concentrations of DDTs in blubber of diseased and dead California sea lions collected in 1970 were between 47 and 5,077 μg/g, lipid weight (LeBoeuf and Bonnell 1971).

Concentrations of DDTs in livers of elephant seals and harbor seals were approximately 15- to 20-fold less than in California sea lions. Juvenile elephant seals that were affected by a skin disease contained significantly greater (2.73–11.8 μg/g, lipid weight) concentrations of DDT than those that were not exhibiting the skin disease (1.88–6.01 μg/g, lipid weight) (Beckmen *et al.* 1997). DDT concentrations measured in blubber of elephant seals in the present study ranged from 8.3 to 110 μg/g, lipid weight. The highest concentration of 110 μg/g, lipid weight, was eight times greater than the next highest value, which suggested a great variability in DDT concentrations in elephant seals. In general, DDT concentrations in

Table 2. Concentrations of organochlorine pesticides and PCBs (μg/g, lipid weight) in blubbers of pinnipeds collected from California coastal waters

Sample ID	Location	Sex	Age Class	Fat (%)	PCBs	ΣDDT	ΣCHL	TCPMe	TCPMOH	ΣHCH	HCB
CSL 700	Pacific Grove, Monterey	Female	Adult	41	39	130	2.7	0.58	0.47	0.80	ND
CSL 1216	Brisbane	Male	Adult	58	63	180	7.2	IF	1.3	1.2	ND
CSL 1229	San Francisco	Male	Subadult	19	1,300	540	44	2.7	8.0	4.0	ND
CSL 1396*	Carmel, Monterey	Male	Adult	0.58	1,100	2,900	71	36	12	7.0	ND
CSL 1396*	Carmel, Monterey	Male	Adult	1.7	1,300	2,900	86	IF	15	7.0	ND
CSL 1434	Moss Landing	Male	Adult	27	310	450	35	3.0	5.1	2.9	ND
CSL 2367	Morro Bay	Male	Adult	69	7.2	13	0.51	0.12	0.096	0.15	ND
CSL 2595	Port San Luis	Male	Adult	84	13	29	1.0	0.20	0.19	0.25	ND
CSL 2836	San Francisco	Male	Subadult	6.0	520	1,200	41	IF	6.0	4.7	ND
CSL 2839	San Francisco	Male	Adult	76	32	110	2.3	1.1	0.38	0.45	ND
CSL 3020	Oceano	Female	Adult	77	18	60	1.1	0.45	0.26	0.10	ND
CSL 3048	San Francisco	Male	Adult	71	24	71	2.6	0.96	0.36	0.38	ND
CSL 3448	Pismo Beach	Female	Adult	9.3	840	140	44	IF	7.2	0.89	ND
ES 772	Pebble Beach	Female	Yearling	18	58	110	8.6	0.94	0.48	0.90	0.082
ES 782	Pebble Beach	Male	Yearling	93	6.1	10	0.83	0.19	0.040	0.12	0.024
ES 808	Crescent City	Male	Yearling	91	5.0	8.3	0.83	0.033	0.035	0.20	0.045
ES 819	Bolinas	Female	Yearling	93	7.8	14	1.5	0.036	0.057	0.30	0.044

CSL = California sea lion (*Zalophus californianus*); ES = elephant seal (*Mirounga augustirostris*).
Detection limits for *p,p′*-DDT, *cis*-CA, α-HCH, β-HCH, γ-HCH, and HCB were 5.0, 10, 15, 20, 10, and 2.0 ng/g lipid weight, respectively.
ND = not detected; IF = peak could not be identified because interference existed at the same retention time.
* CSL 1396 was analyzed in duplicate because of the unusually low lipid content in the blubber.

Table 3. Concentrations of organochlorine pesticides and PCBs (μg/g, lipid weight) in livers of pinnipeds collected from California coastal waters

Sample ID	Location	Sex	Age Class	Fat (%)	PCBs	ΣDDT	ΣCHL	TCPMe	TCPMOH	ΣHCH	HCB
CSL 700	Pacific Grove, Monterey	Female	Adult	3.6	84	130	2.6	1.4	0.66	0.71	ND
CSL 1434	Moss Landing	Male	Adult	4.6	380	970	37	24	5.9	2.9	ND
CSL 2163	Marina	Female	Adult	4.8	56	100	4.7	0.68	0.45	0.69	ND
CSL 2166	Moss Landing	Female	Adult	4.2	410	860	23	50	7.5	4.5	ND
CSL 2367	Morro Bay	Male	Adult	3.8	11	12	0.44	0.13	0.044	0.17	ND
CSL 2839	San Francisco	Male	Adult	4.5	93	150	2.4	IF	1.3	0.44	ND
CSL 3048	San Francisco	Male	Adult	6.3	56	79	2.3	6.9	0.51	0.26	ND
CSL 3186	Oceano	Female	Adult	2.2	110	170	6.6	IF	2.0	0.86	ND
CSL 3448	Pismo Beach	Female	Adult	3.4	290	570	21	17	4.5	2.1	ND
ES 782	Pebble Beach	Male	Yearling	2.7	5.9	4.3	0.27	0.037	0.030	0.045	0.015
ES 821	Bodega Bay	Female	Yearling	3.0	86	59	5.1	0.19	0.38	0.43	0.043
ES 1183	Pillar Point Harbor	Female	Yearling	4.1	7.8	6.1	0.48	0.013	0.014	0.086	0.012
HS 444	Orick	Female	Pup/calf	2.3	24	7.6	1.0	0.010	0.17	0.33	ND
HS 450	Tomales Bay	Female	Pup/calf	3.1	24	21	1.3	0.025	0.81	0.12	0.008
HS 454	Eureka	Female	Pup/calf	6.1	6.8	4.3	0.52	0.0062	0.087	ND	ND
HS 488	Tomales Bay	Female	Pup/calf	16	56	41	2.4	1.0	0.29	0.23	0.012
HS 1040	East Fort Baker	Female	Yearling	3.9	140	11	1.1	0.036	0.93	ND	ND
HS 1139	San Francisco	Male	Adult	4.8	350	85	4.0	0.50	2.0	0.075	0.004
HS 1175	San Francisco	Female	Adult	5.2	240	13	1.8	0.11	0.35	ND	ND
HS 1191	Abbott's lagoon	Male	Pup/calf	1.5	11	5.2	0.56	0.030	IF	0.069	0.018
HS 1195	Elkhourn Slough	Female	Adult	4.2	5.0	9.1	0.11	0.020	0.32	0.040	0.004
HS 1199	Tiburon	Female	Adult	22	7.0	2.8	0.16	0.0081	0.045	ND	0.003

CSL = California sea lion (*Zalophus californianus*); ES = elephant seal (*Mirounga augustirostris*); HS = harbor seal (*Phoca vitulina*).
Detection limits for *p,p′*-DDT, *cis*-CA, α-HCH, β-HCH, γ-HCH, and HCB were 5.0, 10, 15, 20, 10, and 2.0 ng/g lipid weight, respectively.
ND = not detected; IF = peak could not be identified because interference existed at the same retention time.

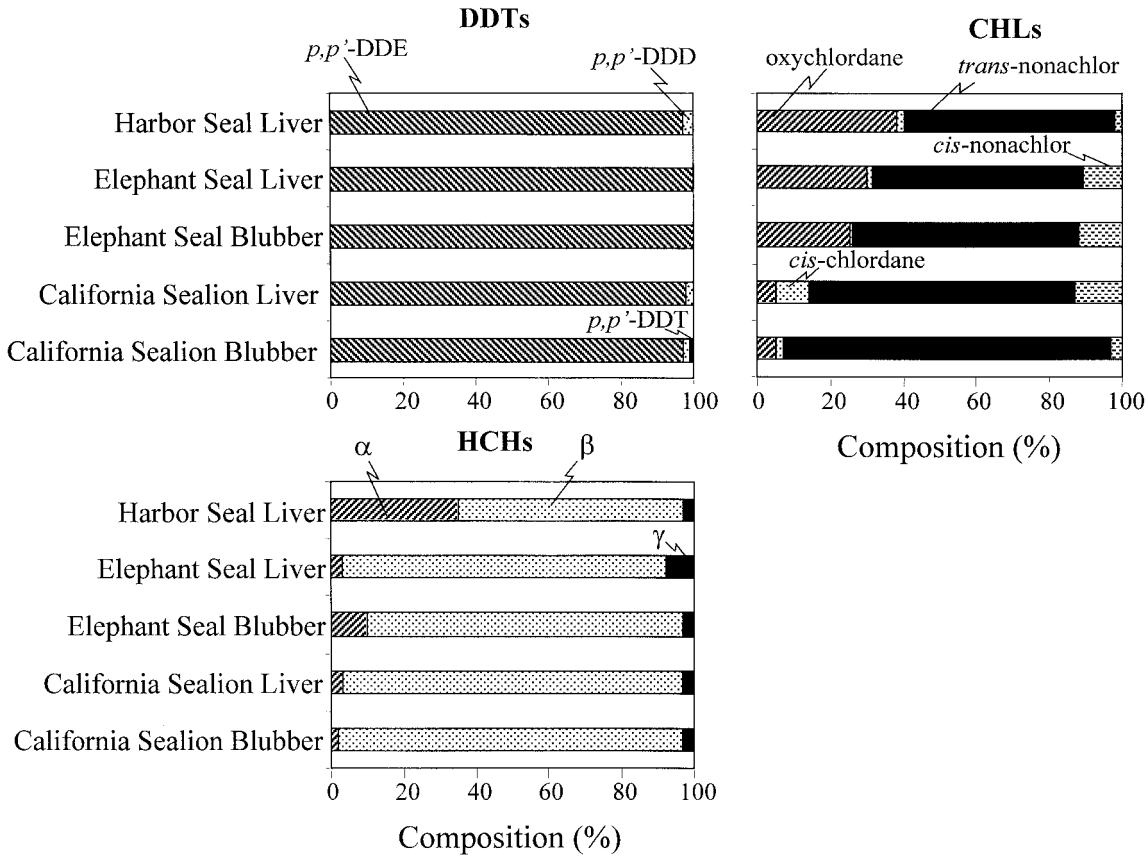

Fig. 2. Composition (%) of DDT, CHL, and HCH compounds in liver and blubber of California sea lions, northern elephant seals, and harbor seals

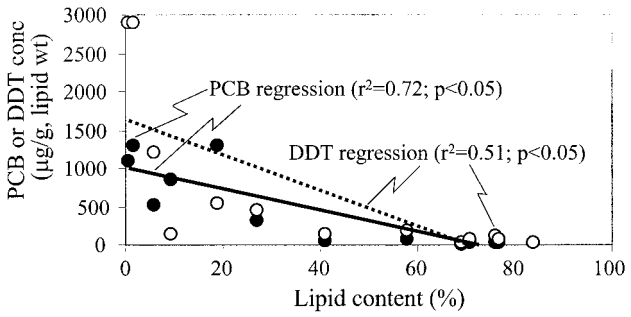

Fig. 3. Relationship between PCB or DDT concentrations and blubber lipid content in California sea lions

blubber of elephant seal were comparable to those reported earlier for diseased seals collected in the 1990s (Beckmen *et al.* 1997), with the exception of the highest value.

Concentrations of DDT in harbor seal livers collected in 1970 ranged from 2.4 to 3 μg/g, wet weight (Shaw 1971). On a wet-weight basis, DDT concentrations in harbor seal livers analyzed in this study ranged from 0.08 to 6.6 μg/g. On average, DDT concentrations were twofold less than those reported in 1970 for harbor seal livers. However, concentrations varied greatly among individuals.

Several earlier studies have reported DDT as a major contaminant in marine biota from the coastal waters of California, particularly those from southern California (O'Shea *et al.* 1980; Beckmen *et al.* 1997; Nakata *et al.* 1998; Bacon *et al.* 1999; Schiff and Allen 2000). Great concentrations of DDT have been explained by historical discharges of DDT-contaminated waste water in the Southern California Bight (SCB). The California sea lions analyzed in this study stranded in central and northern California, but probably spent a period of their lives feeding in the SCB as they migrate annually along the California coast.

p,p′-DDE accounted for 97–100% of the total DDT concentrations in pinnipeds (Figure 2). This composition is indicative of the lack of fresh inputs of DDT. Relatively great concentrations of DDT in California sea lions compared to those in elephant seals and harbor seals may suggest that sea lions have been exposed to DDT in the SCB, whereas the latter species feed in relatively less contaminated areas. Particularly, sea lions breed on islands within the SCB and are found throughout the year in the SCB, although males make seasonal northern migrations to feeding areas off northern California. In addition to foraging characteristics, diet and migratory behavior can also influence differences in the concentrations observed among pinniped species. Age and sex are also important determinants of contaminant burden, and these differed among the three species sampled.

PCBs were the second most abundant organochlorines in elephant seals and sea lions. Concentrations of PCBs in blubber

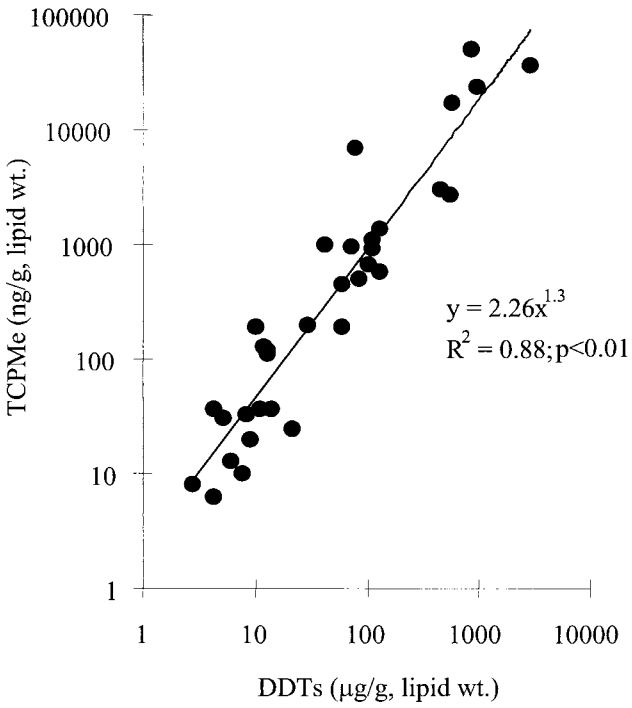

Fig. 4. Relationship between DDT and TCPMe concentrations in pinniped species collected from coastal waters of California

and liver of sea lions and blubber of elephant seals were twofold less than those of DDTs. However, PCB concentrations in livers of harbor seals were fourfold greater than DDT concentrations. Although blubber samples were not available for harbor seals, greater concentrations of PCBs than DDTs in harbor seals may suggest that their exposure sources or areas are different from those of sea lions. Earlier studies have shown that concentrations of PCBs in harbor seals from the San Francisco coast were two- to fivefold greater than those of DDTs, whereas seals from the Los Angeles coast contained greater concentrations of DDTs than PCBs (Risebrough 1978). Greater concentrations of PCBs than DDTs in harbor seals may imply that these animals have received exposures primarily north of the SCB. The greatest concentrations of PCBs were found in blubber of adult and subadult male California sea lions from Monterey and San Francisco.

It is noteworthy that a significant ($p < 0.05$) negative correlation between DDT and PCB concentrations and fat content in the blubber of California sea lions was observed (Figure 3). The decrease in lipid content may be associated with fat mobilization due to a state of negative energy balance in diseased animals. Although organochlorines are expected to be mobilized along with lipids, it appears that the extent of mobilization of organochlorines is less than that of the lipids. An inverse relationship between blubber thickness or blubber mass and organochlorine concentrations in blubber has been observed in diseased marine mammals (Aguilar 1985; Addison 1989; Corsolini *et al.* 1995; Beckmen *et al.* 1997).

Concentrations of PCBs in blubber of adult female sea lions collected in 1970 were as great as 133 μg/g, lipid weight (De Long *et al.* 1973). In the present study, PCB concentrations in blubbers of two adult females were 18 and 39 μg/g, lipid weight, respectively, whereas a female from Pismo Beach contained 840 μg/g, lipid weight. Due to the small number of samples and great variations in concentrations, it was not possible to discern temporal trends. PCBs concentrations in blubber of elephant seals in the present study were greater (5–58 μg/g, lipid weight) than those reported earlier (2.1 ± 0.41 μg/g, lipid weight) (Beckmen *et al.* 1997). The difference in PCB concentrations could be due to the differences in the method of PCB quantification. In the current study, all the PCB congeners present in elephant seal were identified and quantified; the earlier study (Beckmen *et al.* 1997) had quantified only 36 congeners.

Other studies have examined the association between elevated accumulation of organochlorines and impairment of immune function, disease development, or adverse health effects in marine mammals (Reijnders 1986; Kannan *et al.* 1993, 1998; Ross *et al.* 1995; Corsolini *et al.* 1995; Nakata *et al.* 1998). Impairment of immune function and reproductive failure in harbor seals feeding on a PCB-contaminated diet has been reported (Reijnders 1986; Ross *et al.* 1995). Threshold concentrations for PCBs in blubber and liver of marine mammals to elicit effects at physiological levels were estimated to be 13–22 and 6.6–11 μg/g, lipid weight, respectively (Kannan *et al.* 2000). Concentrations of PCBs in blubber or livers of diseased sea lions, elephant seals, and harbor seals analyzed in this study were greater than the reported threshold concentrations.

Concentrations of CHLs in blubber and liver of sea lions were approximately 15-fold less than those of PCBs. Similarly, CHL concentrations in livers of elephant seals were 17 times less than those of PCB concentrations. CHL concentrations in harbor seals were 67-fold less than those of PCBs. The difference in the patterns of relative CHL concentrations in harbor seals further implies that their exposure to organochlorine sources were different from those of sea lions and elephant seals. Few studies have reported the occurrence of CHLs in marine mammals from the coastal waters of California. Concentrations of CHLs in livers of sea lions and seals from coastal California were within the range of values found in livers of sea otters (0.05–21 μg/g, lipid weight) (Nakata *et al.* 1998). *Trans*-nonachlor accounted for a major percentage (58–90%) of total CHL concentrations in pinnipeds (Figure 2). The ratio of oxychlordane to total CHL concentrations in elephant seals and harbor seals were greater than those in sea lions. This may suggest differences in metabolic potential to CHL compounds between sea lions and seals. The greater proportion of oxychlordane in seals may suggest their greater ability to metabolize chlordane compounds.

TCPMe concentrations of up to 36 μg/g, lipid weight, in blubber and 50 μg/g, lipid weight, in liver were observed in sea lions. Concentrations of TCPMe and TCPMOH in sea lions were greater than those in elephant seals and harbor seals. Concentrations of TCPMe and TCPMOH were one to three orders of magnitude greater than those found in various pinniped species from several other locations (Watanabe *et al.* 2000). Possible sources of TCPMe and TCPMOH are considered to be technical DDT, dicofol and other agrochemicals, and large synthetic polymers and light-fast dyes for acrylic fibers (Jarman *et al.* 1992; Buser 1995). Great concentrations of TCPMe and TCPMOH in pinnipeds, particularly California sea lions, are in agreement with elevated concentrations of DDTs

Table 4. Comparison of organochlorine concentrations (μg/g, lipid weight) between blubber and liver of pinnipeds collected from California coastal waters

Sample ID	Location	Sex	Age Class	Tissue	Fat (%)	PCBs	ΣDDT	ΣCHL	TCPMe	TCPMOH	ΣHCH
CSL 700	Pacific Grove, Monterey	Female	Adult	blubber	41	39	130	2.7	0.58	0.47	0.80
				liver	3.6	84	130	2.6	1.4	0.66	0.71
CSL 1434	Moss Landing	Male	Adult	blubber	27	310	450	35	3.0	5.1	2.90
				liver	4.6	380	970	37	24	5.9	2.90
CSL 2367	Morro Bay	Male	Adult	blubber	69	7.2	13	0.51	0.12	0.096	0.15
				liver	3.8	11	12	0.44	0.13	0.044	0.17
CSL 2839	San Francisco	Male	Adult	blubber	76	32	110	2.3	1.1	0.38	0.45
				liver	4.5	93	150	2.4	IF	1.3	0.44
CSL 3048	San Francisco	Male	Adult	blubber	71	24	71	2.6	0.96	0.36	0.38
				liver	6.3	56	79	2.3	6.9	0.51	0.26
CSL 3448	Pismo Beach	Female	Adult	blubber	9.3	840	140	44	IF	7.2	0.89
				liver	3.4	290	570	21	17	4.5	2.10
ES 782	Pebble Beach	Male	Yearling	blubber	93	6.1	10	0.83	0.19	0.040	0.12
				liver	2.7	5.9	4.3	0.27	0.037	0.030	0.045

CSL = California sea lion (*Zalophus californianus*); ES = elephant seal (*Mirounga augustirostris*).
Detection limits for *p,p′*-DDT, *cis*-CA, α-HCH, β-HCH, and γ-HCH were 5.0, 10, 15, 20, and 10 ng/g lipid weight, respectively.
ND = not detected; IF = peak could not be identified because interference existed at the same retention time.

Table 5. Concentrations of butyltins (ng/g, wet weight) in the livers of pinnipeds from California coastal waters

Sample ID	Location	Sex	Age Class	MBT	DBT	TBT	ΣBTs
CSL 700	Pacific Grove, Monterey	Female	Adult	35	24	1.0	60
CSL 1396	Carmel	Male	Adult	17	20	2.5	40
CSL 1434	Moss Landing	Male	Adult	21	14	2.8	38
CSL 2163	Marina	Female	Adult	16	7.3	1.0	24
CSL 2166	Moss Landing	Female	Adult	30	36	1.7	68
CSL 2367	Morro Bay	Male	Adult	12	25	2.2	39
CSL 2839	San Francisco	Male	Adult	11	11	1.6	24
CSL 3048	San Francisco	Male	Adult	9.2	17	0.80	27
CSL 3186	Oceano	Female	Adult	21	18	0.56	40
CSL 3448	Pismo Beach	Female	Adult	46	40	1.3	87
ES 782	Pebble Beach	Male	Yearling	9.5	50	0.55	60
ES 821	Bodega Bay	Female	Yearling	19	78	2.1	99
HS 450	Tomales Bay	Female	Pup/calf	5.0	6.5	0.69	12
HS 1040	East Fort Baker	Female	Yearling	54	27	1.2	82
HS 1175	San Francisco	Female	Adult	6.2	4.2	2.2	13
HS 1191	Abbott's lagoon	Male	Pup/calf	< 2.3	< 2.4	2.0	2.0
HS 1195	Elkhourn Slough	Female	Adult	3.2	< 2.4	1.6	4.8
HS 1199	Tiburon	Female	Adult	77	12	2.0	91

CSL = California sea lion (*Zalophus californianus*); ES = elephant seal (*Mirounga augustirostris*); HS = harbor seal (*Phoca vitulina*).

in pinnipeds. A significant ($p < 0.01$) positive correlation between total DDT and TCPMe concentrations in pinniped tissues was found (Figure 4), suggesting that DDT is a possible source of TCPMe and TCPMOH found in pinniped tissues. Concentrations of TCPMe in technical DDT preparations were less than 0.01% (Buser 1995). In blubber of pinnipeds, TCPMe accounted for 0.25–1.25% of the total DDT concentrations. Similarly in livers, TCPMe accounted for 0.21–8.5% of the total DDT concentrations. This indicates the presence of sources other than DDT mixtures and/or preferential enrichment and biomagnification of TCPMe in pinniped tissues.

TCPMOH concentrations were generally lower than those of TCPMe (1–13-fold) in California sea lions and in some elephant seals. However, TCPMOH concentrations in harbor seals were 3 to 32 times greater than TCPMe concentrations. The differences in the ratios of TCPMe to TCPMOH among pinniped species suggest differences in exposure sources and/or metabolism. In particular, the differences in ratios suggest that harbor seal populations have been exposed to different sources from those to which sea lions and elephant seals are exposed. TCPMOH is likely to originate partly as a metabolite of TCPMe or *tris*(4-chlorophenyl)methyl chloride (Jarman *et al.* 1992; Falandysz *et al*. 1999).

Mean concentrations of HCHs in blubber and livers of sea lions were 2.3 and 1.4 μg/g, lipid weight, respectively (Tables 2 and 3). HCH concentrations in elephant seals were 6–7-fold

less than those in sea lions. To our knowledge, no reports of HCHs and HCB concentrations in pinnipeds from California coastal waters are available. This study establishes current concentrations for these pesticides in pinnipeds from coastal California. β-HCH accounted for 87–95% of the total HCH concentrations in sea lions and elephant seals. Occurrence of a considerable proportion of α-HCH in harbor seal livers indicates exposure to recent sources. This, in addition to the ratios of PCBs/DDT and TCPMe/TCPMOH in harbor seals, reinforces the conclusion that harbor seal populations experience different exposures compared to sea lions and elephant seals.

Blubber versus Liver

Residue concentrations of organochlorines in those animals for which liver and blubber concentrations were available were compared (Table 4). Lipid-normalized concentrations of DDTs and PCBs in liver were, on average, 1.6 times greater than those in blubber. While the ratios of liver to blubber concentrations in California sea lions were between 1 and 2.9, one individual (CSL 3448) with low blubber lipid content showed a wide variation in organochlorine concentration ratios between blubber and liver. Liver-to-blubber concentration ratios of TCPMe were, on average, 3.8, whereas the ratios for TCPMOH, CHLs, and HCHs were near unity. In a sea lion from Monterey (CSL 1396), lipid content of the blubber was unusually low (0.58%). This sample was analyzed in duplicate to confirm the residue levels. As mentioned before, low lipid content in blubber could be explained by the diseased condition of the animals analyzed, which resulted in the mobilization of fat reserves from the blubber. In general, it is found that the blubber to liver ratios of organochlorines vary depending on the health status of the animal.

Butyltin Concentrations

In contrast to organochlorines, butyltin compounds tend to accumulate preferentially in liver rather than blubber. Therefore, butyltins were analyzed only in livers of California sea lions, elephant seals, and harbor seals (Table 5). Butyltins were found in livers of all of the animals analyzed, and their concentrations varied from 2 to 99 ng/g, wet weight. No significant difference existed in butyltin concentrations among species ($p > 0.05$). These concentrations are less than those reported for sea otters from California (Kannan *et al.* 1998). Similarly, concentrations of butyltin compounds in pinnipeds were less than those in cetaceans and mustelids from coastal waters of the United States (Kannan *et al.* 1997, 1999). Lesser concentrations of butyltins in pinnipeds have been explained by the excretion of butyltins via molting and effective metabolism (Kim *et al.* 1996). This is the first report of butyltin concentrations in pinniped species from coastal waters of California.

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