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PROFILES OF POLYCHLORINATED BIPHENYL CONGENERS, ORGANOCHLORINE PESTICIDES, AND BUTYLTINS IN SOUTHERN SEA OTTERS AND THEIR PREY

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Abstract—Concentrations of organochlorine pesticides, polychlorinated biphenyl (PCB) congeners, and butyltins were measured in sea otters and selected prey species (invertebrates) collected from the California (USA) coast. Polychlorinated biphenyls, DDTs (sum of *p,p′*-dichlorodiphenyldichloroethylene [*p,p′*-DDE], *p,p′*-dichlorodiphenyldichloroethane [*p,p′*-DDD], and *p,p′*-DDT), and butyltins were the major contaminants found in sea otters and their prey. Lipid-normalized concentrations of PCBs and DDT in sea otter livers were 60- and 240-fold greater than those found in the prey. Great biomagnification of PCBs and DDT in sea otters is suggested to result from their high per-capita intake of diet compared with those of other marine mammals. Profiles of PCB congeners in sea otters and prey species suggest a great capacity of sea otters to biotransform lower-chlorinated congeners. Sea otters seem to possess a greater ability than cetaceans to metabolize PCBs. The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents of non- and mono-*ortho* PCBs in sea otters and certain prey species were at or above the theoretical threshold for toxic effects.

Keywords—Sea otters Polychlorinated biphenyls Toxic equivalents Organochlorines

INTRODUCTION

The southern sea otter (*Enhydra lutris nereis*), a California coastal species, was listed as threatened by the U.S. Fish and Wildlife Service in 1977. Although the population of southern sea otters has increased since that time, the rate of recovery has been slower than expected. Furthermore, surveys conducted since 1995 have indicated that the trend has reversed and that the population is now either stable or again decreasing [1]. The current population is estimated to be approximately 2,300 individuals. The reasons for diminished growth of the California sea otter population are unclear. The slow recovery of this sea otter population has been attributed to several factors, including habitat degradation (pollution and oil spills), fishery by-catch, predation, malnutrition, human disturbances, and diseases [1–4]. An estimated 40 to 50% of the newborn sea otters die before weaning [4]. Furthermore, increased adult mortality is considered to be an explanation for the reduced population growth [3,4]. The high rate of adult mortality has been associated with the prevalence of infectious diseases in southern sea otters [5].

The high prevalence of diseases in southern sea otters has been hypothesized to be caused by weakened immune systems resulting from exposure to toxic pollutants. Organochlorine compounds, including polychlorinated biphenyls (PCBs), and butyltin compounds, including tributyltin (TBT), are well-known immunotoxic contaminants that are distributed widely in aquatic ecosystems [6–8]. Elevated concentrations of PCBs and TBT have been reported in sea otters collected from several locations along the California coast [9,10]. Studies describing the occurrence of organochlorine and butyltin compounds in the prey species or diet of sea otters are few. In addition, isomer-specific analysis of PCBs in sea otters and their prey is needed to provide information regarding biomagnification of PCBs in sea otters.

Sea otters eat a variety of sessile and slow-moving benthic invertebrates, including sea urchins (*Strongylocentrotus* spp.), abalone (*Haliotis* spp.), clams (*Tresus nuttalli*, *T. capax*, *Mya arenaria*, *Tivela stultoreum*, and *Saxidomus nuttalli*), mussels (*Mytilus* spp.), basket cockles (*Clinocarddium nuttallii*), rock scallops (*Hinnites giganteus*), crabs (*Cancer* spp. and *Pugettia* spp.), spiny lobsters (*Panulirus interruptus*), sea stars (*Pisaster* spp.), and turban snails (*Tegula* spp.). Sea otters also feed on fat innkeeper worms (*Urechis caupo*) when they forage in mud flats, where the innkeepers are found. In general, sea urchins, abalone, and certain species of clams and crabs appear to be the preferred prey, and these are eaten when and where they are present and accessible [11]. However, individual sea otters specialize in foraging for certain invertebrate species.

The objectives of the present study were to measure concentrations of organochlorine and butyltin compounds in selected southern sea otters and their prey to elucidate food chain accumulation and biomagnification, to examine the capacity and mode of PCB biotransformation in sea otters in comparison with those in other marine mammals, and to determine 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents of toxic PCB congeners in sea otters and their prey.

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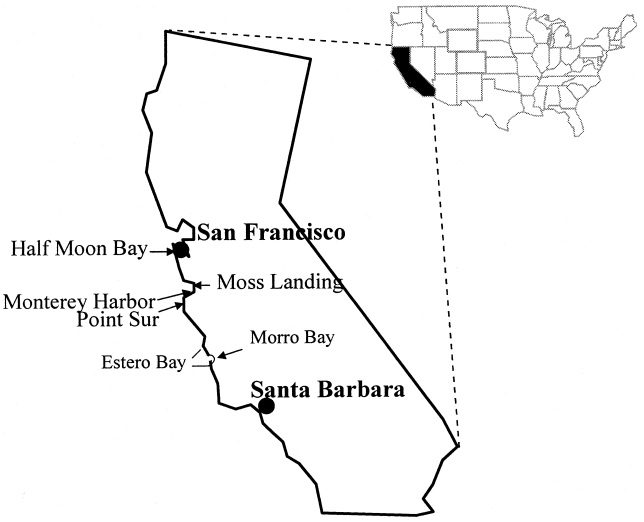

Fig. 1. Map of California (USA) showing sampling locations for sea otters and prey species.

MATERIALS AND METHODS

Samples

Deceased sea otters along the coast of California are collected through a stranding network coordinated by the U.S. Fish and Wildlife Service, U.S. Geological Survey–Western Ecological Research Center, and the California Department of Fish and Game with the cooperation of other organizations and academic as well as private institutions (Fig. 1). Otter carcasses in good postmortem condition were rapidly chilled or frozen and then shipped overnight to the National Wildlife Health Center (Madison, WI, USA) for necropsy. Location, sex, and body length of the animals analyzed are presented in Table 1. From this group, 11 sea otters that died from infectious diseases, trauma (shark bite or gunshot), unknown causes, or miscellaneous problems, such as neoplasia and emaciation, were selected. The diseased otters were fatally infected by bacteria (e.g., pneumonia), protozoa (e.g., encephalitis), acanthocephalan parasites, or fungi (e.g., coccidioidomycosis). All the animals sampled were adults, and this age designation was based on a combination of total length measurements and cementum annuli counts [12]. Generally, adults had no deciduous teeth and showed indications of at least early wear on their permanent teeth. Adult females were greater than 105 cm in total length (approximately four to five years of age or more), and adult males were greater than 115 cm in total length (approximately five years of age or more). The brain, liver, and kidneys were collected from the carcasses at the time of necropsy, wrapped in aluminum foil or Whirlpac bags (Krackeler Scientific, Albany, NY, USA), and stored frozen at −20°C until analysis. The sea otters examined in the present study were analyzed earlier for organochlorine pesticides, total PCBs, and butyltins [9,10]. Additional details about samples and sampling locations have been reported elsewhere [10]. Sampling locations are shown in Figure 1.

Black turban snails (*Tegula fumebralis, T. brannea*, and *T. monteregi*) were collected from Point Sur (south side), approximately 30 km south of Monterey (CA, USA). Red urchins (*Strongylocentrotus franciscanus*), red abalones (*Haliotis rufescens*), mussels (*Mytilus edulis*), kelp crabs (*Pugettia producta*), and fat innkeeper worms (*Urechis caupo*) were collected from Otter Point and Wharf II in Monterey Harbor. All the prey species were collected on August 25 to 27, 1999. Several individuals per species were collected by nets and pooled for analysis. Only edible, soft tissues were analyzed.

Chemical analysis

Organochlorine pesticides and PCBs were analyzed according to methods described elsewhere [13,14], with some modifications. Briefly, analysis consisted of extraction of sample tissues (2–6 g) with mixed solvents of diethyl ether (300

Table 1. Concentrations (ng/g wet wt) of polychlorinated biphenyls (PCBs), DDTs, hexachlorocyclohexanes (HCHs), chlordanes (CHLs), and butyltins (BTs) in tissues of California (USA) sea otters collected from 1992 to 1996[a]

Tissue/n	Sex	Location	Age (years)	Length (cm)	Fat (%)	PCBs	DDTs	HCHs	CHLs	BTs
Liver										
1	Male	Half Moon Bay	ND[b]	127	3.5	140	470	17	14	110
1	Male	Moss Landing	ND	ND	3	880	3,800	10	53	9,200
2	Female	Monterey Harbor	11–13 (12)[c]	118–126 (122)	2.4–3.5 (2.95)	7,000–8,700 (7,850)	2,600–5,900 (4,250)	15–50 (32.5)	370–500 (435)	2,350–4,320 (3,340)
5	Male Female	Estero Bay	2–11 (5.3)	108–126 (119)	2.6–5.2 (3.8)	360–1,400 (788)	290–2,500 (1,290)	8.7–76 (39)	10–260 (77)	61–390 (210)
2	Male	Morro Bay	10–12	123–139	3.4–8.2	280–300	1,200–1,800	5.8–68	21–28	40–5,300
Kidney										
1	Male	Half Moon Bay	ND	127	3.9	480	1,400	15	30	14
1	Male	Moss Landing	ND	ND	3.3	1,600	7,800	6.4	110	265
1	Female	Monterey Harbor	13	126	1.7	4,600	2,300	18	190	210
1	Male	Morro Bay	10	123	11	1,400	8,600	19	130	4
Brain										
1	Male	Half Moon Bay	ND	127	8.8	69	300	3.3	2.6	3.9
1	Male	Moss Landing	ND	ND	8.7	330	1,400	8.1	39	140
1	Male	Morro Bay	12	139	7.1	89	350	5.3	4.4	81

[a] DDTs = *p,p′*-DDE + *p,p′*-DDT + *p,p′*-dichlorodiphenyldichloroethane; HCHs = α- + β- + γ-isomers; CHLs = *cis*-chlordane + *trans*-chlordane + *cis*-nonachlor + *trans*-nonachlor + oxychlordane; BTs = monobutyltin = dibutyltin + tributyltin. Data are from Nakata et al. [9] and Kannan et al. [10].

[b] ND = not determined.

[c] Values in parentheses are the means.

ml) and hexane (100 ml) using a Soxhlet® apparatus (Krackeler Scientific) for 7 h. Lipid content was determined gravimetrically from concentrated aliquots of these extracts. Removal of lipids from the extracts was accomplished by elution of the extracts through a 20 g Florisil–packed glass column (Supelco, Oakville, ON, Canada) with a mixture of 150 ml of 80% acetonitrile and 20% (v/v) hexane-washed water or by gel permeation column (inner diameter, 2 cm; length, 50 cm; Bio-Beads S-X3; Bio-Rad Laboratories, Hercules, CA, USA). The extracts were then concentrated, purified with sulfuric acid, and passed through a 12 g Florisil–packed glass column for separation. The first fraction, which was eluted with hexane, contained PCBs, *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), and *trans*-nonachlor. The second fraction, which was eluted with 20% dichloromethane in hexane, contained hexachlorcyclohexane (HCH) isomers (α-, β-, and γ-HCH), chlordanes (CHLs; *trans*-chlordane, *cis*-chlordane, *cis*-nonachlor, and oxychlordane), *p,p′*-dichlorodiphenyldichloroethane (DDD), and *p,p′*-DDT. In prey species, tris(4-chlorophenyl)methane (TCPME), tris(4-chlorphenyl)methanol (TCPMOH), dieldrin, and heptachlor epoxide were also analyzed in the third fraction, which was eluted with 50% dichloromethane in hexane.

Each fraction was concentrated and injected into a Hewlett-Packard 5890 series II high-resolution gas chromatograph (Avondale, PA, USA) equipped with a ^{63}Ni electron capture detector. A fused silica capillary column (inner diameter, 0.25 mm; length, 30 m) coated with DB-1 (100% dimethyl polysiloxane; J&W Scientific, Folsom, CA, USA) at a film thickness of 0.25 μm was used for the separation of organochlorines. The column oven was programmed from an initial temperature of 60°C (1-min hold) to 160°C at a rate of 20°C/min, held for 10 min, and then ramped at a rate of 2°C/min to 260°C, with a final hold time of 20 min. The injector and detector temperatures were maintained at 260 and 280°C, respectively. Helium and nitrogen were the carrier and make-up gases, respectively. Concentrations of individually resolved peaks were summed to obtain the total PCB concentration. An equivalent mixture of Kanechlors 300, 400, 500, and 600 (Kanegafuchi, Osaka, Japan) with known PCB composition and content was used as the external quantification standard. Identification and quantification of individual PCB isomers and congeners based on Kanechlor mixtures have been reported earlier [15]. Organochlorine pesticides were quantified from the areas under individually resolved peaks compared to the corresponding peak areas of standards. Recoveries of PCB congeners and organochlorine pesticides through the analytical procedure were between 95 and 103%. Reported concentrations were not corrected for recovery. Detection limits for total PCBs and organochlorine pesticides were 0.3 and 0.03 ng/g wet weight, respectively.

Isomer-specific analysis of PCBs, including non-*ortho* coplanar PCBs, in sea otter tissues and selected prey items was based on a method described earlier [16]. Briefly, tissues were refluxed in 1 N potassium hydroxide–ethanol, re-extracted in hexane, and cleaned by passage through a silica gel column. Extracts were then passed through a glass column (inner diameter, 5 mm) packed with 125 mg of activated carbon (Wako Pure Chemical Industries, Osaka, Japan) for the separation of non-*ortho* PCB congeners, 3,3′,4,4′-T_4 chlorobiphenyl (CB; International Union of Pure and Applied Chemistry [IUPAC] 77), 3,3′,4,4′,5-P_5CB (IUPAC 126), and 3,3′,4,4′,5,5′-H_6CB (IUPAC 169) from *ortho*-chlorine–substituted PCBs. A Hewlett-Packard 5890 series II gas chromatograph interfaced with a Hewlett-Packard 5970 mass-selective detector was employed for the identification of individual PCB congeners in sea otter tissues. Selected-ion monitoring at *m/z* 290 and 292, 324 and 326, 358 and 360, 392 and 394, and 428 and 430 was used to identify tetra-, penta-, hexa-, hepta-, and octa-CBs, respectively. For the analysis of non-*ortho* PCBs, M^+, and $(M+2)^+$ cluster ions were monitored at *m/z* 290 and 292, 324 and 326, and 358 and 360 for IUPAC 77, 126, and 169, respectively. Detection limits of non-*ortho* coplanar PCBs in sea otters were 40 to 60 pg/g wet weight. A Hewlett-Packard 5890 series II gas chromatograph interfaced with an Agilent 5973N mass-selective detector (Folsom, CA, USA) was employed for the identification of individual PCB congeners in prey items. Detection limits of coplanar PCBs in prey species were 1 pg/g wet weight. The PCB congeners are represented by their IUPAC numbers. The laboratory has participated in the National Institute of Standards and Technology annual intercomparison exercises for organochlorines in marine mammal tissues. Concentrations of PCBs and organochlorine pesticides were within ±20% of the reported mean concentrations.

Butyltins, including monobutyltin, dibutyltin, and TBT were analyzed in sea otters and their prey species as described elsewhere [7]. The method involved extraction, derivatization, and clean-up followed by gas chromatography–flame photometric detection.

RESULTS AND DISCUSSION

Concentrations and bioaccumulation

The DDTs (sum of *p,p′*-DDE, *p,p′*-DDD, and *p,p′*-DDT) were the predominant compounds in sea otters from various locations, except for Monterey Harbor, where PCBs were abundant (Table 1). The maximum ΣPCB and DDT concentrations measured in sea otter livers were 8,700 and 5,900 ng/g wet weight, respectively. Concentrations of CHLs and HCHs in sea otter livers were one to two orders of magnitude lower than those of PCBs and DDTs. Concentrations of butyltins were comparable to or greater than those of DDTs. Details regarding organochlorine pesticide and organotin concentrations, including age- and gender-related variations in concentrations, and tissue-specific accumulation in sea otters have been discussed elsewhere [9,10].

In general, the concentrations of contaminants in tissues of sea otters (Table 1) showed considerable variation by individual and location—and in some cases an order of magnitude variation at the same location. Many of the animals were old (10–15 years). Sample size was limited, so these results may not be representative of the entire range of the southern sea otter population. More details on the concentration ranges of organochlorines and organotins for several other locations on the California coast are discussed elsewhere [9,10]. The concentrations of organochlorines and butyltins found in sea otter prey species may be viewed as typical for locations like Monterey Harbor [9,10].

All the locations from which sea otters were analyzed (Half Moon Bay, Moss Landing, Monterey Harbor, Estero Bay, and Morro Bay, CA, USA) were either embayments adjacent to population centers or agricultural drainage entering the ocean, where historic inputs of PCBs, DDTs, HCHs, and CHLs have occurred. A small, but significant, percentage of the southern sea otter population (~10% based on the Fall 2002 survey) is found in such areas at various times of the year. However, approximately 50 to 60% of the southern sea otter population

Table 2. Concentrations (ng/g wet wt) of polychlorinated biphenyls (PCBs), organochlorine pesticides, tris(4-chlorophenyl) methanol [TCPMOH], and tris(4-chlorophenyl)methane [TCPME] and butyltins (BTs) (monobutyltin + dibutyltin + tributyltin) in prey species of sea otters collected in August 1999 in Monterey Harbor and Point Sur, California (USA); hexachlorocyclohexanes (HCH), chlordane (CHLs)

Prey species	Lipid (%)	PCBs	DDTs	HCHs	CHLs	Hepta-chlor epoxide	Dieldrin	TCPMOH	TCPME	BTs
Fat innkeeper worms	0.87	21.8	13.9	0.26	2.09	0.03	0.38	0.33	0.05	8.1
Mussel	4.8	15.8	11.5	0.99	2.23	0.10	0.96	0.72	0.03	9.7
Red urchin	5.9	2.25	2.32	0.72	<0.03	0.09	0.12	0.05	<0.03	6.5
Red abalone	1.0	<0.3	0.08	0.12	<0.03	0.01	0.04	<0.03	<0.03	2
Kelp crab (*Pugettia producta*)	1.2	<0.3	0.27	1.04	<0.03	0.03	0.12	<0.03	<0.03	1.3
Turban snail (*Tegula fumebralis*)	1.7	<0.3	0.41	0.21	<0.03	0.02	0.09	0.02	<0.03	1.1
Turban snail (*Tegula fumebralis, T. brannea*)	0.98	<0.3	0.17	0.10	<0.03	0.03	0.18	<0.03	<0.03	1.1
Turban snail (*Tegula monteregi*)	1.3	<0.3	0.22	0.16	<0.03	0.02	0.08	0.03	0.01	1.4

is found off the Big Sur (CA, USA) coastline, a relatively pristine area with little or no history of agricultural pesticide, industrial, or urban contamination or the presence of boat harbors.

Among the various prey items of sea otters analyzed, fat innkeeper worms and mussels contained the highest concentrations of PCBs and DDTs. Concentrations of PCBs in fat innkeeper worms and mussels were 21 and 16 ng/g wet weight, respectively (Table 2). Both DDTs and butyltins were detected in all the invertebrates analyzed at concentrations ranging from 0.08 to 14 and from 1.1 to 9.7 ng/g wet weight, respectively. The HCHs, dieldrin, and heptachlor epoxide were found in all the benthic invertebrates, although at concentrations 10- to 100-fold lower than those of PCBs and DDTs. Both TCPME and TCPMOH were detected in several prey species of sea otters. An earlier study has reported the occurrence of 4 ng TCPMOH/g liver in sea otters [17]. Lipid-normalized concentrations of TCPMOH in sea otter livers (100 ng/g) were approximately ninefold greater than concentrations found in prey items (11 ng/g), suggesting biomagnification of this compound in the sea otter food chain. Earlier studies have also reported the biomagnification of TCPMOH in humans and marine wildlife [18,19]. Nevertheless, concentrations of TCPMOH in sea otter livers were lower than those found in livers of California sea lions from central California [20].

Few studies have examined the occurrence of organochlorines and butyltins in benthic invertebrates from the California coast [21–23]. Concentrations of PCBs and DDT in abalone and red sea urchin collected from the southern California coast during the early 1970s ranged from 6 to 200 ng/g wet weight [21], which were greater than those observed in the samples collected for the present study. Concentrations of PCBs and DDT have declined in mussels from several locations along the California coast since the 1980s, when restrictions were imposed on the production and use of these compounds [23].

Despite relatively low concentrations of organochlorines in prey, concentrations measured in sea otter livers from some locations were 10- to 100-fold higher than those in the prey species. Lipid-normalized concentrations of PCBs and DDTs in sea otter livers ranged from 3.7 to 363 μg/g (mean, 64 μg/g) and from 7.8 to 168 μg/g (mean, 60 μg/g), respectively. Mean concentrations of PCBs and DDTs in sea otter prey species that contained detectable concentrations of these compounds were 0.96 and 0.25 μg/g lipid weight, respectively. Thus, concentrations of PCBs and DDT in sea otter livers were, on average, 60- and 240-fold, respectively, higher than those measured in their typical prey. These values are also greater than those reported for several other marine mammal species, in which PCB and DDT biomagnification factors have ranged from 10 to 50 [24,25]. High biomagnification of PCBs and DDT may result from specific feeding habits and accumulation features of sea otters. Unlike other marine mammals, sea otters do not possess blubber [26]. Therefore, organochlorines tend to be dispersed in various body tissues, such as liver or kidney. Furthermore, sea otters consume between 23 and 33% of their body weight in food every day. This proportion is much greater than that observed in other marine mammals. For example, food consumption by sea lions and dolphins is 7% of their body weight daily [27]. A higher rate of food consumption and the lack of blubber to concentrate lipophilic pollutants may render sea otters vulnerable to accumulation of toxic contaminants in physiologically important organs, such as liver and kidney. In addition, it should be noted that prey species were collected four to five years after sea otters had been collected. Even though some time lapse occurred between the sampling of sea otters and the sampling of their prey, assessments of biomagnification and biotransformation were based on the samples collected from the same location (i.e., Monterey Harbor).

PCB congener profiles in sea otters and their prey

Isomer-specific analysis of tetra- through octa-CBs in sea otter tissues revealed the presence of 52 chromatographic peaks representing 63 isomers. Hexachlorobiphenyls were the major homologues, accounting for between 46 and 48% of the total PCB concentrations in liver and kidney, followed by hepta- (25–29%), penta- (18–21%), octa- (3.5–5.3%), and tetra-CBs (1.5–2.4%), in that order (Fig. 2). Brain tissue contained relatively higher proportions of tetra-, penta-, and hexa-CBs and lower proportions of hepta- and octa-CBs relative to liver and kidney (Fig. 2). The percentage composition of hexa-CBs

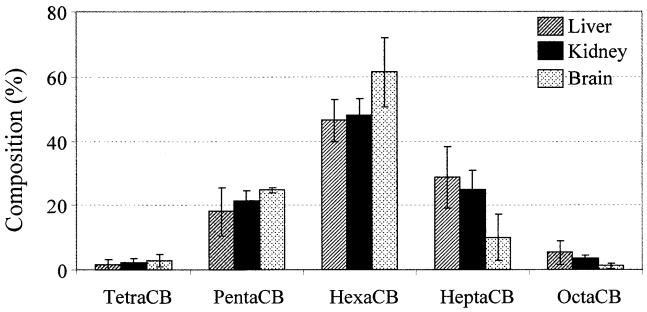

Fig. 2. Polychlorinated biphenyl homologue profiles in sea otter liver, kidney, and brain. Mean and standard deviation are presented. CB = chlorobiphenyl.

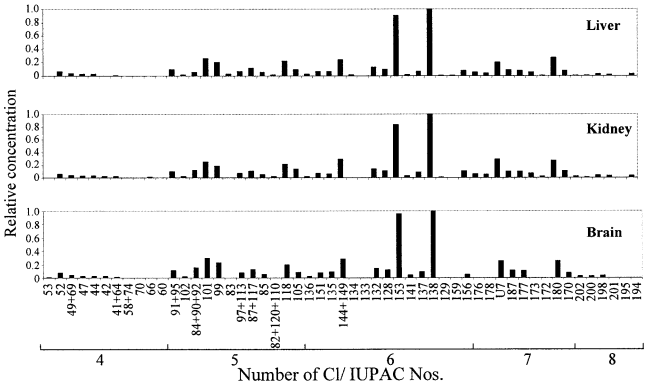

Fig. 3. Polychlorinated biphenyl (PCB) isomer and congener profiles in tissues of a male sea otter from Moss Landing (CA, USA) collected in April 1995. Concentrations of individual congeners are normalized to that of PCB 138, which is considered to be 1.0. IUPAC = International Union of Pure and Applied Chemistry; U7 = unidentified heptachlorobiphenyl.

(relative to total PCBs) in brain was significantly greater than that in liver ($p < 0.05$), whereas the percentage hepta-CBs in brain was significantly lower than that in liver ($p < 0.05$). Despite this, the relative congener concentrations among liver, kidney, and brain tissues within an individual were similar (Fig. 3). Hexachlorobiphenyls 138 (2,2′,3,4,4′,5′) and 153 (2,2′,4,4′,5,5′) were the major congeners, together accounting for 25 to 45% (mean, 35%) of the total PCB concentrations in sea otter tissues. Other major congeners in sea otter tissues were IUPAC 101, 99, 118, 144/149, and 180. This profile is comparable to that observed in marine mammals from the northern Pacific Ocean [28].

In prey species, penta- and hexa-CBs were the major homologues, accounting for, on average, 40 and 37%, respectively, of the total PCB concentration (Fig. 4). The percentage composition of tetra- and penta-CBs relative to total PCB concentration was greater in prey species than in sea otter tissues. For instance, tetra- and penta-CBs together accounted for 44 to 65% of the total PCB concentrations in prey and 20% in sea otters. The mean composition of penta-CB in sea otter liver was 19%, whereas in prey items, it was 31 to 50%. This suggests that sea otters can metabolize tetra- and penta-CB congeners. The percentage composition of hexa-CBs in sea otter liver was significantly greater than that in prey items ($p < 0.05$). Further details regarding the biotransformation of PCB congeners by sea otters are discussed below. Similar to the composition in sea otter tissues, hexa-CBs 153 and 138

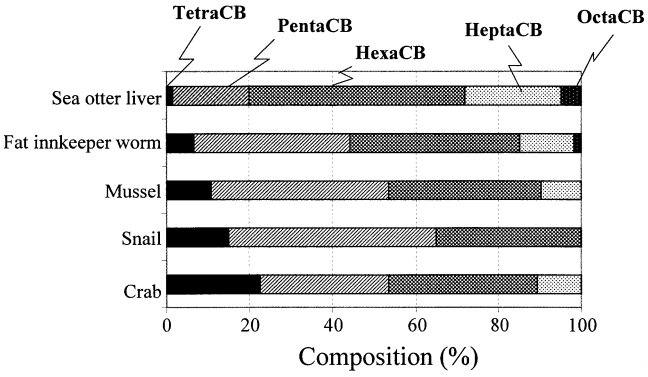

Fig. 4. Composition (%) of polychlorinated biphenyl homologues in sea otter liver and prey species. CB = chlorobiphenyl.

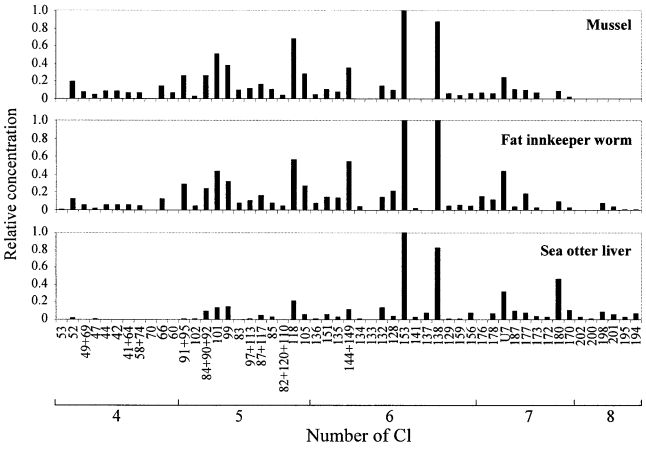

Fig. 5. Polychlorinated biphenyl (PCB) isomer and congener profiles in sea otter liver and prey species from Moss Landing/Monterey Harbor (CA, USA). Concentrations of individual congeners are normalized to that of PCB 153, which is set to be 1.0. U7 = unidentified heptachlorobiphenyl.

were the predominant congeners in mussels and fat innkeeper worms, accounting for 22 to 23% of the total PCB concentration. Relative concentrations of CB congeners 118, 144/149, 101, 99, and 105 were lower in sea otters than in prey items (Fig. 5). This provides additional evidence that certain PCB congeners are biotransformed/metabolized by sea otters. Chlorobiphenyl congener 180, which was relatively less abundant in prey species, was found to be enriched in sea otters. This implies that the highly chlorinated congeners are not efficiently metabolized by sea otters.

Metabolic capacity of sea otters relative to other marine and terrestrial mammals

The metabolism of PCB congeners is mediated by cytochrome P450–dependent, mixed-function oxygenase enzymes in liver microsomes. The group of PCB congeners with vicinal, unsubstituted carbon in the *meta-para* and the *ortho-meta* positions are metabolized by phenobarbital (PB)- and methylcholanthrene (MC)-inducible microsomal enzymes, respectively [29–31]. Certain marine mammals possess relatively active MC-type enzymes but less active PB-type enzymes [29]. Measurement of activities of hepatic cytochrome P450 enzymes, such as aryl hydrocarbon hydroxylase, ethoxyresorufin-*O*-deethylase, and pentoxyresorufin-*O*-deethylase, in fresh livers can provide information concerning their ability to transform xenobiotics. Because the sea otters were not deliberately killed for the present study, we did not have access to fresh livers for direct measurements of enzyme activity. Alternatively, however, drug-metabolizing enzyme activities can be assessed from the abundances of certain CB congeners estimated as metabolic indices. A metabolic index can be estimated as [29]:

$$MI_i = \log CR_{180} - \log CR_i$$

where MI_i is the metabolic index of congener i and CR_i and CR_{180} are the concentration ratios (concentration in predator: concentration in prey) of congeners i and 180, respectively. Polychlorinated biphenyl isomers with greater MI values are more biodegradable/excretable (including fecal egestion and urinary excretion of unmetabolized congeners) relative to CB 180 than are those with smaller, near-zero MI values. The CB 180 has been chosen as the standard against which to normalize

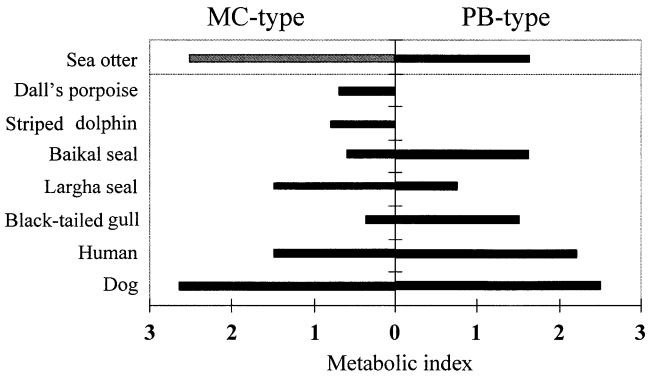

Fig. 6. Estimated activities of phenobarbital (PB)-type and methylcholanthrene (MC)-type enzymes in sea otters in comparison with those in other marine and terrestrial vertebrates. Data for species other than sea otter were taken from Nakata et al. [13] and Senthilkumar et al. [33].

MI values, because it is one of the most recalcitrant CB congeners in terms of biotransformation in aquatic and terrestrial mammals [29]. Metabolic indices were calculated for individual PCB congeners in sea otter livers. The MI values for tetra-, penta-, hexa-, hepta-, and octa-CB congeners analyzed in the present study ranged from 1.5 to 3, 1.1 to 3.2, 0.59 to 1.6, 0.08 to 1, and 0.2 to 0.8, respectively. Most of the tetra-CBs had MI values greater than two, whereas most or all of the hexa-, hepta-, and octa-CBs had MI values less than one. These results suggest that less highly chlorinated PCBs are metabolized more efficiently than hexa- and hepta-CBs. This finding is similar to what was observed for Baikal seals [13]. The MI values for PCB congeners in sea otters were greater than the indices measured for Ganges River (India) dolphins [32,33]. These results suggest that sea otters have a greater ability than Ganges River dolphins to transform/metabolize PCB congeners. The MI values for PCB isomers metabolized by MC- and PB-type enzymes were also compared with the values reported for other aquatic and terrestrial mammals (Fig. 6). Chlorobiphenyls 52 (2,2′,5,5′-tetra-CB) and 66 (2,3′,4,4′-tetra-CB) were selected to represent the congeners that are metabolized by PB- and MC-type enzymes, respectively. The MI values estimated for CB 52 and CB 66 were both greater than those values determined for certain cetaceans and pinnipeds, indicating that the activities of both the MC- and PB-type enzymes in sea otters are relatively high compared with those in other marine mammals. Therefore, it can be concluded that sea otters have a greater capacity to biotransform PCBs compared to cetaceans, such as dolphins. This aspect may be related to the high basal metabolic rate of sea otter, as indicated by their high rates of feeding and energy utilization to maintain body temperature. The basal metabolic rate of a sea otter is twice as high as that of other mammals of comparable size [11].

Toxic equivalents and toxicological implications

Non-*ortho* coplanar PCB congeners 77, 126, and 169 were not detected in several sea otters at a detection limit of 40 to 60 pg/g wet weight. A few individuals that had high concentrations of total PCBs contained coplanar PCBs. In these individuals, CB 126 was the predominant congener, followed by CBs 77 and 169 (Table 3). This profile is different from that observed in several cetaceans [6,28], in which the concentration of tetra-CB congener 77 was higher than those of CBs 126 and 169. Biotransformation of congener 77 by sea otters may explain the lower concentration of CB 77 relative to CB 126 in sea otters. Mono-*ortho* PCB congeners 118, 105, and 156 were found in several sea otters. The toxic equivalents (TEQs) were calculated for non- and mono-*ortho* coplanar PCB congeners based on the mammalian toxic equivalency factors (TEFs) reported by the World Health Organization [34]. Total TEQ concentrations of non- and mono-*ortho*-substituted congeners in sea otter livers ranged from 0.42 to 156 pg/g wet weight (mean, 33 pg/g wet wt) (Table 3). When the concentrations of nondetectable congeners were substituted by a value of half the respective detection limit, the TEQ concentrations were found to range from 2.7 to 156 pg/g wet weight (mean, 34 pg/g wet wt). Mono-*ortho* congener 156 and non-*ortho* congener 126 were the major contributors to TEQs. Although several di-*ortho*-substituted congeners were found in sea otter livers, TEFs were not available for these congeners.

The threshold concentration of TEQs in livers of aquatic mammals beyond which physiological effects are elicited has been suggested to be 520 pg/g lipid weight [35]. On a lipid-weight basis, TEQ concentrations in sea otter livers ranged from 6.7 to 6,500 pg/g (mean, 1,100 pg/g). The mean TEQ concentration in sea otter liver was greater than the suggested threshold concentration. In particular, sea otters collected from Monterey Harbor and Moss Landing and a few individuals

Table 3. Concentrations (pg/g, wet wt) of non- and mono-*ortho* polychlorinated biphenyls (PCBs) and their 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) in sea otter livers

Congener	TEF[a]	Concentration	TEQs	TEQs (at ½ detection limits)[b]
Non-*ortho* polychlorinated biphenyls				
77	0.0001	<60–110[c]	<0.006–0.011[c]	0.003–0.011 (0.004)
126	0.1	<60–350[c]	<6–35	2–35 (8.3)
169	0.01	60–110[c]	<0.6–1.1[c]	0.3–1.1 (0.4)
Mono-*ortho* polychlorinated biphenyls				
118	0.0001	2,900–404,000 (92,700)	0.29–40 (9.3)	0.29–40 (9.3)
105	0.0001	<100–107,000 (27,000)	<0.01–11 (2.7)	<0.01–11 (2.7)
156	0.0005	<100–137,000 (27,000)	<0.05–69 (14)	<0.05–69 (14)
Total TEQs			0.42–156 (33)	2.7–156 (34)

[a] TEF (toxic equivalency factor) from Van den Berg et al. [34].
[b] TEQ concentrations when nondetectable congeners were substituted by one-half of the detection limit value; values in parentheses are the means.
[c] Less than 25% detectable observations.

Table 4. Concentrations and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs[a]; pg/g on wet wt) of non- and mono-*ortho* polychlorinated biphenyls (PCBs) in sea otter diet

Congener	TEF[b]	Kelp crab	Turban snail	Mussel	Fat innkeeper worms
Non-*ortho* PCBs					
77	0.0001	1.6 (0.0002)[b]	5 (0.0005)	17.4 (0.0017)	29 (0.0029)
126	0.1	<1 (0.1)	<1 (<0.1)	3.9 (0.39)	8.9 (0.89)
169	0.01	<1 (0.01)	<1 (<0.01)	<1 (<0.01)	3.2 (0.032)
Mono-*ortho* PCBs					
118	0.0001	30 (0.003)	40 (0.004)	1,740 (0.17)	2,080 (0.208)
105	0.0001	10 (0.001)	20 (0.002)	720 (0.072)	1,020 (0.102)
156	0.0005	<1 (<0.0005)	<1	140 (0.07)	170 (0.085)
Total TEQ		0.004	0.007	0.709	1.320

[a] Values in parentheses are TEQs.
[b] TEF (toxic equivalency factors) from Van den Berg et al. [34].

from Estero Bay contained TEQ concentrations greater than the threshold value of 520 pg/g lipid weight. Although uncertainties are involved in extrapolating the effect levels recommended for several other aquatic mammals to sea otters, this comparison nevertheless can suggest the extent of PCB exposures in sea otters.

Non-*ortho* coplanar PCB congener 77 was found in all the prey items at concentrations ranging from 1.6 to 29 pg/g wet weight (Table 4). Coplanar PCB congeners 126 and 169 were found in fat innkeeper worms. The respective relative concentrations of these three non-*ortho* PCB congeners in fat innkeeper worms were on the order of 77 > 126 > 169. Toxic equivalents were calculated for non- and mono-*ortho* PCB congeners in prey species based on the mammalian TEFs [34]. Concentrations of TEQs in prey items were between 0.004 and 1.3 pg/g wet weight. A threshold concentration for TEQs of 1.06 pg/g wet weight has been suggested for the prey species of sea otter [36]. Concentrations of TEQs in fat innkeeper worms were above the suggested tolerance limits.

The estimates of TEQs in sea otters and shellfish prey may be considered conservative, because they only represent a few mono- and non-*ortho* congeners and do not consider di-*ortho* PCBs, which are present at considerable concentrations in sea otters. In addition, other dioxin-like compounds, such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans [17], may add to the existing burdens of TEQs in sea otters and their prey. Furthermore, mammalian TEFs were derived based on studies with terrestrial mammals. Aquatic mammals, including sea otters, have different toxicokinetics, which may render them more sensitive to chemical pollution. Although to our knowledge the effects of environmental contaminants on sea otters are as yet unstudied, confamilial species, such as mink and river otter, have been shown to be highly sensitive to several of these compounds [37]. Therefore, TEFs specific to aquatic mammals will be needed for an accurate risk assessment.

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