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PERSISTENT ORGANIC POLLUTANTS IN THE BLOOD OF FREE-RANGING SEA OTTERS (*ENHYDRA LUTRIS* SSP.) IN ALASKA AND CALIFORNIA

David A. Jessup,[1,6] Christine K. Johnson,[2] James Estes,[3,4] Daphne Carlson-Bremer,[2] Walter M. Jarman,[4,5] Stacey Reese,[3] Erin Dodd,[1] M. Tim Tinker,[3] and Michael H. Ziccardi[2]

[1] California Department of Fish and Game, Marine Wildlife Veterinary Care and Research Center, 1451 Shaffer Road, Santa Cruz, California 95060, USA
[2] Wildlife Health Center, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, California 95616, USA
[3] US Geological Survey, Western Ecological Research Center, 100 Shaffer Road, Long Marine Laboratory, Santa Cruz, California 95060, USA
[4] Long Marine Laboratory, University of California, 100 Shaffer Road, Santa Cruz, California 95060, USA
[5] Current address: 230 South 5th West, Suite 240, Salt Lake City, Utah 84101, USA
[6] Corresponding author (email: djessup@ospr.dfg.ca.gov)

ABSTRACT: As part of tagging and ecologic research efforts in 1997 and 1998, apparently healthy sea otters of four age-sex classes in six locations in Alaska and three in California were sampled for persistent organic pollutants (POPs) and other chemicals of ecologic or environmental concern (COECs). Published techniques for the detection of POPs (specifically Σpolychlorinated biphenyls [PCBs], ΣDDTs, Σhexachlorocyclohexanes [HCHs], Σpolycyclic aromatic hydrocarbons [PAHs], Σchlordanes [CHLs], hexachlorobenzene [HCB], dieldrin, and mirex) in the tissue of dead otters were modified for use with serum from live sea otters. Toxic equivalencies (TEQs) were calculated for POPs with proven bioactivity. Strong location effects were seen for most POPs and COECs; sea otters in California generally showed higher mean concentrations than those in Alaska. Differences in contaminant concentrations were detected among age and sex classes, with high levels frequently observed in subadults. Very high levels of ΣDDT were detected in male sea otters in Elkhorn Slough, California, where strong freshwater outflow from agricultural areas occurs seasonally. All contaminants except mirex differed among Alaskan locations; only ΣDDT, HCB, and chlorpyrifos differed within California. High levels of ΣPCB (particularly larger, more persistent congeners) were detected at two locations in Alaska where associations between elevated PCBs and military activity have been established, while higher PCB levels were found at all three locations in California where no point source of PCBs has been identified. Although POP and COEC concentrations in blood may be less likely to reflect total body burden, concentrations in blood of healthy animals may be more biologically relevant and less influenced by state of nutrition or perimortem factors than other tissues routinely sampled.

Key words: Blood, DDT, *Enhydra lutris*, PCB, persistent organic pollutants, sea otter, TEQ, toxicology.

INTRODUCTION

Prior to excessive fur hunting during the eighteenth and nineteenth centuries, sea otters (*Enhydra lutris*) ranged continuously across the North Pacific Ocean. Two subspecies resulted from the remaining scattered populations, the northern sea otter (*Enhydra lutris kenyoni*) and the southern sea otter (*Enhydra lutris nereis*). The northern sea otter recovered from the fur trade of the late 1700s and was quite abundant prior to the mid-1990s. The western subpopulation, however, was listed as "threatened" by the US Fish and Wildlife Service (USFWS) in 2006 under the Endangered Species Act (ESA) following significant population declines (70% or more) in some areas (Doroff et al., 2003; Estes et al., 2005). The causes for these population declines are not completely understood (due primarily to the difficulties recovering carcasses in the relatively sparsely populated and rugged coastline), but increased predation pressure from killer whales has been implicated as a cause (Estes et al., 1998). The southern sea otter population is found only off the central California coast from Half Moon Bay south to Point Conception. This population has been listed as "threatened" under the ESA since 1977,

and yearly counts show that the population numbered approximately 2,654 animals in spring 2009 (USGS, 2009). The southern sea otter population has increased only slightly since experiencing a period of decline and stasis from 1994 to 2004. The primary cause of this stasis appears to be high mortality in adult animals, resulting in a population that fails to reach full life expectancy and reproductive potential (Estes et al., 2003). It has been proposed that exposure of sea otters to persistent organic pollutants (POPs) could influence sea otter disease susceptibility (Kannan et al., 2006a; Jessup et al., 2007). Approximately half of southern sea otter deaths are due to infectious diseases (Kreuder et al., 2003); however, it is unclear whether the increased mortality from disease is due to increased exposure to infectious agents, increased susceptibility to endemic pathogens, or both.

Sea otters are the only mustelid marine mammal, and, although they must deal with the same thermal challenges confronting cetaceans and pinnipeds, the anatomy and physiology of their fat storage is that of a terrestrial mammal. They use air trapped in their coat instead of blubber for insulation and have a high metabolic rate, requiring consumption of 25–30% of their body weight per day in shellfish to maintain body weight (Costa and Kooyman, 1982). Sea otters use fat for energy, generally do not build large fat stores, and likely more rapidly mobilize and metabolize lipophilic contaminant burdens (Mulcahy and Ballachey, 1994).

Global pollution, particularly in aquatic ecosystems, is a serious problem (Moller, 2003). Large-scale population declines or increased impacts of disease-causing agents in the upper marine trophic levels may be potentiated by elevated exposure to xenobiotics or POPs, such as organochlorines, polychlorinated biphenyls, dioxins, or dioxin-like compounds, and other legacy pesticides (Kannan et al., 1993). Other, more recently developed herbicides and pesticides (e.g., chlorpyrifos, dacthal, and oxadiazon), while not as persistent in the environment and thus not classified by the Stockholm Convention as POPs, can also be considered "chemicals of ecologic and environmental concern" (COECs) due to their potential for adverse health effects and presence at high levels in many environments. Different risk factors and routes of exposure govern the accumulation of organophosphates and other hydrophilic pesticides in wildlife.

Health risks to marine mammals from exposure to POPs can be high due to bioaccumulation of lipophilic POPs in large body fat reserves (necessary for insulation) and mobilization of such compounds during stress, illness, or fasting. Striped dolphins (*Stenella coeruleoalba*) in the western North Pacific accumulated contaminants at a concentration 10 million times higher than the concentration in the water (Tanabe et al., 1983). Blubber biopsy samples from southern resident killer whales (*Orcinus orca*) have shown polychlorinated biphenyl (PCB) levels ranging from 4600 to 120,000 ng/g lipid weight (Krahn et al., 2009). Pinniped species shown to have high levels of POPs include California sea lions (*Zalophus californianus*), harbor seals (*Phoca vitulina*), and northern elephant seal pups (*Mirounga angustirostris*; Ross et al., 2004; Debier et al., 2005a, b). High levels of POPs in marine mammals have been associated with premature birth (DeLong et al., 1973) and mass mortalities (Troisi et al., 2001; Jepson et al., 2005).

Postmortem levels of organochlorine pesticides (e.g., DDTs, hexachlorocyclohexanes [HCHs], and chlordanes [CHLs]) and PCBs have been measured in sea otter organ tissue, primarily liver (Estes et al., 1997; Norstrom et al., 1997; Nakata et al., 1998; Bacon et al., 1999; Kajiwara et al., 2001; Kannan et al., 2004b, 2008). Lipid-normalized concentrations of PCBs and DDTs in liver samples were 60- and 240-fold higher, respectively, than levels in prey items from Monterey Bay, suggesting

that otters have a great capacity to bioaccumulate POPs in organ tissue (Kannan et al., 2004b). In the same study, levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalents (or TEQs) for bioactive PCBs in tissue and prey samples were at or above a theoretical threshold concentration for toxic effects. The overall "toxic equivalency" of the sample (TEQ) can be estimated for each bioactive contaminant within a biologic sample by comparing its ability to induce the aryl hydrocarbon metabolic pathway as compared to TCDD (Safe, 1990; Van den Berg et al., 2006). Additional investigators have quantified significant burdens of contaminants, including butyltins, polychlorinated diphenyl ethers (PBDEs), perfluorinated compounds, heavy metals, and polycyclic aromatic hydrocarbons (PAHs), in sea otter populations in California and throughout the Pacific Rim (Kannan et al., 1998, 2004b, 2006a, 2007, 2008; Kannan and Perrotta, 2008; Murata et al., 2008).

Levels of anthropogenic contaminants found in sea otters can illuminate marine health concerns at an ecosystem level because otters are relatively site fidelic, consume large quantities of shellfish, and are a sentinel species in nearshore kelp forest systems (Jessup et al., 2004, 2007). A factor of concern at both the individual animal and population levels is the associations between high contaminant burdens in sea otter tissue and infectious disease as a cause of death (Kannan et al., 2004b, 2006b, 2007). While these results are striking, several important confounding factors make it difficult to identify causal relationships. These referenced studies used tissues from dead, stranded animals. Because stranded otters are often debilitated due to the identified causes of death, antemortem fasting can alter contaminant levels in the tissues by increasing mobilization of lipophilic POPs from body stores. This, in concert with decreased metabolic capacity and excretion, can significantly increase POP levels in lipophilic organ tissues. Assertions that overall body burdens of contaminants in these animals are elevated may be correct, but linking these levels with increased incidence of disease is tenuous and difficult to prove.

Here, we report for the first time contaminant levels in the serum from healthy northern and southern sea otters and provide baseline data for prospective longitudinal monitoring of sea otter contaminant burdens. Blood contaminant levels reported here not only reflect the distribution of contaminant burdens in threatened sea otter populations, but also identify locations with high levels of contamination, where pollution of marine ecosystems has occurred.

MATERIALS AND METHODS

Study populations and sample collection

Sea otters were captured in California and Alaska as part of large-scale monitoring programs conducted by the US Geological Survey (USGS) Biological Resources Division and the California Department of Fish and Game (CDFG), Office of Spill Prevention and Response (OSPR), under permit from the USFWS (permits MA 672724-9 and MA 766818). As shown in Figure 1 (inset 4), free-ranging southern sea otters were captured July 1995–March 2000 at three locations in California: Monterey Bay (36°52′–36°62′N, 121°91′–121°94′W; $n=24$), Elkhorn Slough (36°48′N, 121°38′W; $n=8$), and Santa Cruz (36°58′N, 122°01′W; $n=8$). As shown in Figure 1 (insets 1, 2, and 3), captures of free-ranging northern sea otters occurred July–September 1997 at six locations in Alaska, five in the central-west Aleutian archipelago and one in southeast Alaska. The Aleutian locations were: Adak–Bay of Islands (51°50′N, 176°50′W; $n=20$), Adak–Kuluk Bay/Clam Lagoon Island (51°47′N, 176°38′W; $n=9$), Amchitka Island (51°33′N, 178°59′W; $n=8$), Kanaga Island (51°42′N, 177°27′W; $n=7$), and Kiska Island (51°58′N, 177°8′W; $n=14$). The southeast Alaska location was Cross Sound/Port Althorp (58°12′N, 136°21′W; $n=20$). Sea otters in California were captured with Wilson traps by divers using rebreathers; otters in Alaska were captured primarily by drift nets (Ames et al., 1986). All captured animals were anesthetized and sampled as previously described (Monson et al., 2001). Otter age was estimated by tooth condition and wear, and

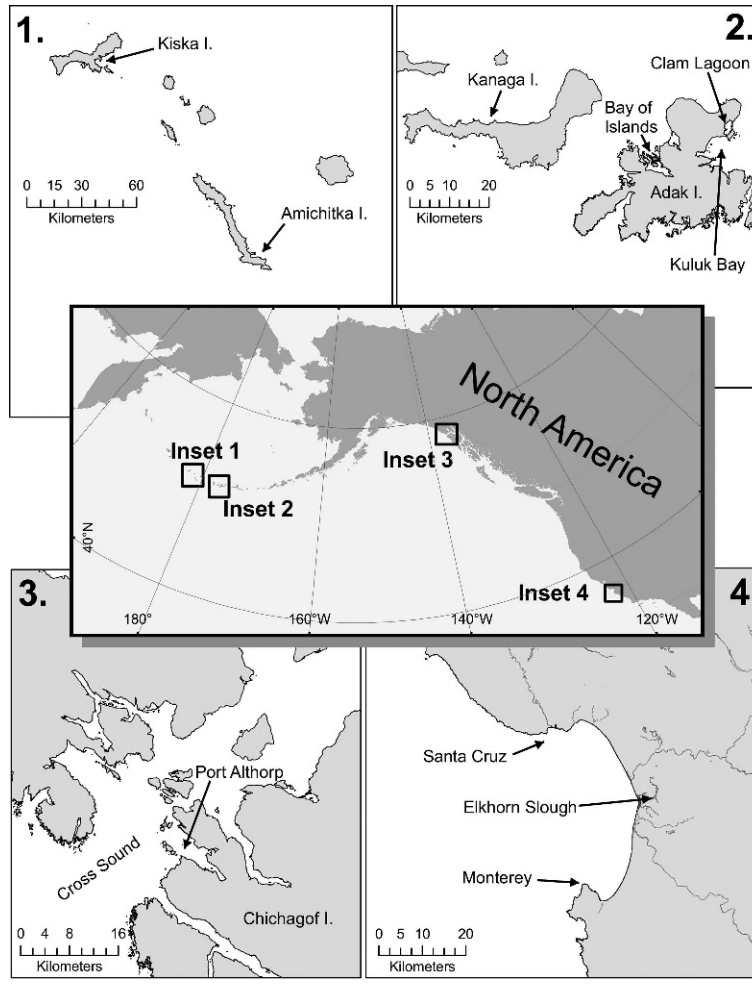

FIGURE 1. Sea otter sampling locations. As part of ongoing ecologic studies, blood samples were taken at seven locations in Alaska (insets 1–3) and three locations in California (inset 4) and tested for persistent organic pollutants and chemicals of ecologic concern.

otters were classified as pups (all milk teeth present), subadults (adult teeth present but with little to no wear), or adults (teeth showed wear). A vestigial premolar tooth was extracted from many otters and examined to determine exact age, and these data (not shown) confirmed age classes determined by eruption and wear. Examination of each animal by a veterinarian for signs of serious, preexisting health problems or injuries, retrospective analysis of complete blood counts, blood chemistry panels, and pathogen exposure assays were used to determine state of health (Hanni et al., 2003). Blood samples were obtained by jugular venipuncture, collected in serum clot tubes, and centrifuged at 1,100 × G for 15 min within 4 hr of collection. Serum was aliquoted into acid-washed, brown glass vials (Fisher Scientific, Pittsburgh, Pennsylvania, USA) and stored at −70 C until analysis.

Chemical analyses

Previously described laboratory techniques for analysis of tissues (Jarman et al., 1996b) were adapted for serum (Newman et al., 1994). Serum samples were evaluated for concentration (ng/g wet weight) of the individual PCB congeners, DDT metabolites, HCH compounds, CHL pesticides (heptachlor, heptachlor epoxide, oxychlordane, methoxychlor, cis-nonachlor, trans-nonachlor, cis-chlordane, and trans-chlordane), other insec-

ticides (chloropyrifos, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, and mirex), herbicides and fungicides (hexachlorobenzene (HCB), dacthal, and oxadiazon), and polycyclic aromatic hydrocarbons (PAHs). Serum was extracted using a C18 vacuum method. Briefly, the serum was thawed, mixed, spiked with a mixture of internal standards, and 2 ml were placed in a 10-ml culture tube (Chang et al., 1993). Two milliliters of formic acid was added (to denature proteins) and mixed on a vortex mixer. C18 columns (1 g) were conditioned with methanol and water, and the sample was loaded on the column and eluted with 2 × 3 ml portions of 1:1 hexane/diethyl ether. Eluates were separated into three fractions using gravity Florsil column chromatography. Lipids were determined using a colorimetric method using sulfo-phospho-vanillin (Frings et al., 1972).

QA/QC and detection limits

A Standard Reference Material (SRM) was analyzed with each set of samples to determine analytical accuracy. An SRM for blood could not be obtained from the National Institute of Standards and Technology, so an in-house, spiked calf serum matrix was used; this contained 22 individual PCB congeners and 14 pesticides (and metabolites). Greater than 90% of the surrogate recoveries met the Environmental Protection Agency recovery criterion of 30–150%. Marginal recoveries, which met this criterion but exceeded the range of 50–130%, were closely inspected. Samples analyzed for PAHs (California) were spiked with a mixture of 26 isotopically labeled compounds. No true sample duplicates were analyzed; however, instrument duplicates were analyzed to indicate instrumental precision. All residues detected had good relative percent differences indicating acceptable analytical precision for these compound classes. No organochlorine contamination was detected in the method blanks.

Instrument analysis

PCBs and organochlorine pesticide in two of the analytical fractions (F1, F2/F3) were analyzed on a Hewlett Packard 6890 Series II capillary gas chromatograph utilizing electron capture detectors (GC/ECD) and two phases of columns to provide two-dimensional confirmation of each analyte. PAHs were quantified in the F2 fraction by analysis on a Hewlett Packard 6890 Series II capillary gas chromatograph equipped with a 5971A mass spectral detector (GC/MS). The samples were analyzed in a selected ion monitoring (SIM) mode and corrected for recoveries. The detection limits were determined using the instrumental detection limits, and then the detection limits for each sample were calculated (using sample weight and injection volume). Approximate detection limits were 4 ng/g lipid weight for pesticides and PCBs, and approximately 200 ng/g lipid weight for PAHs.

Statistical analyses

Values for congeners and metabolites of PCBs, DDTs, HCHs, CHLs, and PAHs were summed for statistical analyses and are reported as ΣPCB, ΣDDT, ΣHCH, ΣCHL, and ΣPAH concentrations, respectively. Sums of concentrations for each PCB homolog (based on the number of chlorine substituents) were also determined for further assessment. Contaminant values that were under instrument detection limits or not detected were converted to zero (the most conservative estimate) for the analyses. For cases with duplicate samples, only the last sample collected was included in the analyses. Toxic equivalencies (TEQs) for bioactive PCBs were calculated for each sample as previously discussed (Safe, 1990; Van den Berg et al., 2006). Briefly, this method quantifies the contaminants as follows:

$$\mathrm{TEQ} = \sum \{([\mathrm{PCB}_1] \times \mathrm{TEF}_1) + ([\mathrm{PCB}_2] \times \mathrm{TEF}_2) + \ldots + ([\mathrm{PCB}_x] \times \mathrm{TEF}_x)\},$$

where $[\mathrm{PCB}_x]$ is the concentration of PCB_x, and TEF_x is the toxic equivalency factor for that specific PCB, arrived at by international consensus evaluating a number of species and toxic end points (Ahlborg et al., 1994; Van den Berg et al., 2006).

Medians and ranges were calculated for all contaminants in each demographic and location category. Stratification of the data by age-sex and location resulted in most categories having too few samples; therefore, multivariate age-sex and location analyses could only be conducted for Alaskan adult males and females and Californian adult males. Contaminant concentrations measured in sea otters were not normally distributed, and samples were sparsely distributed among the strata of interest. The log 10(1 + value) transformation was calculated, but the distribution of the transformed variable still did not meet the homogeneity of variance assumption necessary for analysis of variance (ANOVA). Therefore, the nonparametric equivalent, Kruskal-Wallis ANOVA (KWANOVA), was used to compare independent contaminant concentrations

TABLE 1. Distribution of age and sex class of northern and southern sea otters by capture location in California and Alaska.

State/locality	Pups	Subadults	Adult females	Adult males	Total
Alaska					
Adak–Bay of Islands	1	1	14	3	19
Adak–Kuluk Bay/Clam Lagoon	0	2	5	2	9
Amchitka	1	1	1	1	4
Kanaga	0	0	4	3	7
Kiska	1	1	9	3	14
Cross Sound/Port Althorp	0	9	5	4	18
Subtotal	3	14	38	16	71
California					
Monterey	5	4	14	1	24
Elkhorn Slough	0	0	0	8	8
Santa Cruz	0	2	4	2	8
Subtotal	5	6	18	11	40
Total	8	20	56	27	111

among demographic groups and locations. Pairwise comparisons were calculated between classes for significant variables using Mann-Whitney *U*-tests and used the significance level for interpreting differences between groups. Specifically, concentrations among age and sex classes (adult males, adult females, subadults, and pups) were compared separately for California and Alaska. Contaminant concentrations were also compared for capture locations in California and Alaska. In California, six of the adult females were caught with pups, and values from these females were included in the adult female category. Repeated contaminant levels for a young adult female caught before primiparity and recaptured during lactation with her first pup are reported. Correlations between TEQ values and PCB burdens were evaluated using Spearman's rho. All statistical analyses were conducted using statistical software (STATA 9.2, StataCorp, College Station, Texas; SPSS 17.0, SPSS Inc., Chicago, Illinois), and significant differences were reported if $P \leq 0.05$.

RESULTS

Age and sex classes were not distributed evenly among most capture locations, particularly in California (Table 1). In California, only adult males were captured at Elkhorn Slough, while all pups, females with pups, and the majority of subadults (4/6) were captured along the Monterey Peninsula. In Alaska, age classes were more evenly distributed among locations, although most subadults (9/14) were captured at Cross Sound/Port Althorp, and a majority of adult females (23/38) were captured at Adak–Bay of Islands and Kiska. The medians and ranges of serum lipid percentage are provided in Tables 2–5 and did not vary widely (0.6–1.0%) by age, sex, or location. Median and range values for contaminant concentrations (ng/g lipid weight) in sea otter blood for age-sex classes in Alaska and California are reported in Tables 2–3. Endosulfan I and II were not detected in any otter enrolled in the study, endosulfan sulfate and oxadiazon were not detected in Alaskan otters, and endrin and dacthal were not detected in California animals. In Alaska, all contaminants with detectable levels, excluding mirex and PCB-associated TEQs, differed significantly among combined age and sex classes (KWANOVA, $P<0.05$), with most of the differences being driven by higher subadult concentrations (Mann-Whitney *U*, $P<0.05$). In California, only ΣDDT, ΣHCH, and ΣPAH concentrations differed significantly among combined age-sex classes for contaminants with detectable levels (KWANOVA, $P<0.05$). In the ΣHCH and ΣPAH concentrations, subadults

TABLE 2. Median and range serum lipid percentage, contaminant concentrations (ng/g lipid weight), and toxic equivalencies (TEQs) (pg/g lipid weight) for age-sex classes of northern sea otters in Alaska. Different letters indicate statistically significant differences between the values for a given contaminant (KWANOVA, $P<0.05$); letters in common between classes did not differ significantly by Mann-Whitney *U*-test ($P>0.05$).

Contaminant	Pups ($n=3$)	Subadults ($n=14$)	Adult females ($n=38$)[a]	Adult males ($n=16$)
Serum lipid (%)	0.61 (0.60–0.87)	0.67 (0.49–0.86)	0.73 (0.34–1.00)	0.64 (0.26–0.95)
ΣPCBs	490 (310–3,800) AB	880 (410–8,000) A	390 (140–2,500) B	705 (160–8,600) AB
ΣDDTs	84 (63–110) AB	210 (47–730) C	59 (4.5–460) A	145 (29–730) BC
ΣHCHs	74 (46–280) AB	180 (82–770) B	65 (3.7–470) AB	125 (10–430) A
ΣCHLs	120 (73–130) A	195 (110–410) B	61 (4.2–280)A	135 (17–240) A
Hexachlorobenzene	58 (34–64) AC	89 (64–160) B	51 (11–100) C	75 (11–130) AB
Chlorpyrifos				
Dieldrin	28 (15–44) ABC	29 (15–95) B	16 (0.00–57) AC	15 (0.00–27) C
Mirex	2.8 (0.00–6.9)	0.0 (0.00–29)	0.0 (0.00–33)	6.1 (0.00–120)
TEQs (pg/g)	19 (17–77)	8.1 (1.7–161)	20 (0.0–80)	11 (1.1–81)

[a] Sample size for Σpolychlorinated biphenyls (PCBs), hexachlorocyclohexanes (HCHs), hexachlorobenzene, and mirex, $n=36$; Σchlordanes (CHLs), $n=37$.

TABLE 3. Median and range serum lipid percentage, contaminant concentrations (ng/g lipid weight), and toxic equivalencies (TEQs) (pg/g lipid weight) for age-sex classes of southern sea otters in California. Different letters indicate statistically significant differences between the values for a given contaminant (KWANOVA, $P<0.05$); letters in common between classes did not differ significantly by Mann-Whitney *U*-test ($P>0.05$).

Contaminant[a]	Pups ($n=5$)	Subadults ($n=6$)	Adult females ($n=18$)	Adult males ($n=11$)
Serum lipid (%)	0.93 (0.73–1.10)	0.72 (0.35–1.23)	0.97 (0.28–1.25)	1.06 (0.27–1.42)
ΣPCBs	670 (140–820)	1,400 (730–2,700)	645 (72–4,500)	650 (99–8,500)
ΣDDT	1,300 (130–3,000) A	2,950 (890–21,000) AB	1,080 (100–9,400) A	8,000 (1,300–35,000) B
ΣHCHs	70.4 (36.2–370) BC	135 (75.5–1,800) B	93.0 (0–1,600) B	31.0 (0–130) AC
ΣCHLs	150 (60.7–190)	345 (170.1–930)	180 (7.2–540)	170 (41.4–460)
Hexachlorobenzene	15.5 (0–38.6)	11.4 (0–85.5)	8.7 (0–90.8)	4.9 (0–89.1)
Chloropyrifos	53.6 (26.1–98.6)	39.2 (19.9–342.6)	43.8 (0.3–201.7)	26.7 (5.7–88.4)
Dieldrin	78.8 (0–82.2)	77.5 (23.3–311.1)	36.3 (0.00–212.6)	59.7 (0.00–554.8)
Endosulfan sulfate	10.6 (0.00–17.3)	0.00 (0.00–43.6)	7.2 (0.00–86.5)	15.5 (0.00–63.1)
Mirex	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–13.2)	0.00 (0.00–52.0)
Oxadiazon	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–37.4)	0.00 (0.00–6.2)
ΣPAHs	4,718 (1,817–5,794) A	9,886 (4,772–13,044) B	4,745 (60.3–11,836) A	3,389 (715–8,996) A
TEQs (pg/g)	8.8 (3.9–11)	13 (6.0–47)	5.0 (0.0–51)	13 (1.3–232)

[a] Abbreviations for contaminates listed: PCBs = polychlorinated biphenyls, HCH = hexachlorocyclohexanes, CHLs = chlordanes, PAH = polycyclic aromatic hydrocarbons.

TABLE 4. Median and range serum lipid percentage, contaminant concentrations (ng/g lipid wt), and toxic equivalencies (TEQs) (pg/g lipid wt) for each capture location of northern sea otters in Alaska. Different letters indicate statistically significant differences between the values for a given contaminant (KWANOVA, $P<0.05$); letters in common between classes did not differ significantly by Mann-Whitney U-test ($P>0.05$).

Contaminant[a]	Adak–Bay of Islands ($n=20$)[b]	Adak–Kuluk Bay/Clam Lagoon Island ($n=9$)	Amchitka ($n=8$)	Kanaga ($n=7$)	Kiska ($n=14$)	Cross Sound/Port Althorp ($n=20$)
Serum lipid (%)	0.68 (0.73–1.10)	0.59 (0.11–0.94)	0.68 (0.49–0.76)	0.78 (0.66–1.00)	0.63 (0.26–0.90)	0.74 (0.47–0.95)
ΣPCBs	400 AB (140–1,100)	2,200 D (740–8,600)	1095 CD (270–8000)	220 A (170–680)	445 BC (170–1,900)	530 C (150–4,900)
ΣDDTs	58 (4.5–270)	96.5 (40–730)	150 (34–690)	110 (57–710)	90.5 (21–730)	115 (33–340)
ΣHCHs	62 A (3.7–470)	270 B (60–480)	205 B (150–350)	11 C (3.8–39)	69.5 A (4.7–120)	180 B (60–770)
ΣCHLs	60 A (4.2–290)	220 B (30–410)	165 B (81–310)	62 A (23–130)	80 A (19–390)	155 B (32–290)
Hexachlorobenzene	41 AC (11–81)	85 BD (33–160)	74B D (39–98)	24 A (16–45)	57.5 CD (21–130)	83.5 B (54–140)
Dieldrin	27 (6.2–95)	18 (0–45)	22.5 (0–50)	11 (6.5–19)	15 (6.5–54)	16.5 (0–60)
Mirex	0.00 A (0.00–11)	0.00 B (0.00–0.00)	0.00 B (0.00–0.00)	10 C (10–66)	7.6 C (0–120)	0.00 B (0.00–0.00)
TEQs (pg/g)	26 AB (23–64)	28 AB (7.3–77)	17 AB (1.3–161)	11 A (8.8–25)	35 B (9.4–81)	3 C (0.0–79)

[a] Sample size for Σpolychlorinated biphenyls (PCBs), hexachlorocyclohexanes (HCHs), Σchlordanes (CHLs), hexachlorobenzene, and mirex, $n=19$.

again had significantly higher levels, but for ΣDDT, adult males were found to have almost 2.5 times greater levels than subadults.

All detectable contaminant levels differed among all nine capture locations (data not shown; KWANOVA, $P<0.05$). When all Alaskan otters were compared with all California otters regardless of specific location, ΣDDT, ΣCHL, HCB, chlorpyrifos, dieldrin, endosulfan sulfate, and oxadiazon differed significantly (data not shown; Mann-Whitney U, $P<0.05$), and all contaminants (with the exception of HCB) were at higher concentrations in California compared to Alaska locations (Tables 4–5). When location data were further analyzed within each state, chlorpyrifos and mirex were the only analytes that were nonsignificant by location among Alaskan otters (Table 4), while only ΣDDT, ΣHCH, and ΣPAH differed significantly among California locations (Table 5). Pairwise comparisons showed numerous differences among areas in Alaska, where most analytes were greater at the Adak-Kuluk Bay/Clam Lagoon location, followed by Amchitka and Cross Sound/Port Althorp.

Due to small sample sizes for many of the age-sex classifications, only adult females in Alaska and adult males in California could be further stratified to determine any interactions or confounding factors between age-sex and location categories. For Alaskan females, ΣPCB, ΣHCH, HCB, and TEQs remained significantly different between locations in a similar pattern to the nonstratified results (data not shown). However, for California adult males, only dieldrin was found to differ significantly among the three locations, with Elkhorn Slough having higher median concentrations (data not shown).

In addition to ΣPCB concentrations, PCB homologs were also evaluated between locations (Tables 6 and 7). Overall, higher chlorinated compounds (hexachlorobiphenyls to nonachlorobiphenlys) were found to be at greater concentrations (and

TABLE 5. Median and range serum lipid percentage, contaminant concentrations (ng/g lipid wt), and toxic equivalencies (TEQs) (pg/g lipid wt) for each capture location of southern sea otters in California. Different letters indicate statistically significant differences between the values for a given contaminant (KWANOVA, $P<0.05$); letters in common between classes did not differ significantly by Mann-Whitney U-test ($P>0.05$).

Contaminant[a]	Monterey ($n=24$)	Elkhorn Slough ($n=8$)	Santa Cruz ($n=8$)
Serum lipid (%)	0.99 (0.35–1.28)	1.01 (0.27–1.42)	0.74 (0.28–1.28)
ΣPCBs	670.0 (72–3,300)	460 (98.7–8,500)	1,650 (649.8–4,500)
ΣDDTs	1,100 (100–23,000) A	7,950 (1,300–35,000) B	7,100 (1,300–21,000) B
ΣHCHs	84.5 (0.0–1,800)	40.1 (0.0–130)	65.8 (0.0–480)
ΣCHLs	170 (7.2–460)	210 (41.4–370)	330 (70.5–930)
Hexachlorobenzene	9.4 (0.0–90.8)	3.0 (0.0–89.1)	11.4 (0.0–50.4)
Chloropyrifos	45.0 (0.3–342.6) A	22.6 (5.7–43.8) B	56.8 (16.7–159.3) AB
Dieldrin	55.6 (0.0–554.8)	83.7 (32.6–132.1)	82.6 (0.0–311.1)
Endosulfan sulfate	6.0 (0.0–18.8) A	15.7 (0.0–63.1) B	20.4 (0.0–86.5) B
Mirex	0.0 (0.0–13.2)	0.0 (0.0–52.0)	0.0 (0.0–0.0)
Oxadiazon	0.0 (0.0–37.5)	0.0 (0.0–6.3)	0.0 (0.0–0.0)
ΣPAHs	4,925 (60.3–13,044)	3,133 (715.0–8,996)	4,993 (2,623–11,836)
TEQs (pg/g)	5.6 (0.0–57) A	12 (1.3–232) AB	17 (7.2–47) B

[a] Abbreviations for contaminates listed: PCBs = polychlorinated biphenyls, HCHs = hexachlorocyclohexanes, CHLs = chlordanes, PAH = polycyclic aromatic hydrocarbons.

proportion of ΣPCB levels) in Alaskan sea otters captured at Adak–Kuluk Bay/Clam Lagoon and Amchitka. Of the 59 PCB congeners evaluated, six (PCBs 105, 118, 156, 157, 167, and 189) were considered bioactive (Van den Berg et al., 2006) and detected in these samples. The primary active congener detected (and that contributing the most to TEQ estimates) was PCB 118, making up on average 61% of all TEQ values, followed by PCB 156, which made up 18% of TEQs estimates (data not shown); PCB 118 was the third greatest PCB congener, constituting 7.0% of ΣPCB levels.

DISCUSSION

Previous work on concentrations of organochlorine pesticides, polychlorinated biphenyls, and other POPs in sea otters has measured postmortem levels most often in liver tissue (Estes et al., 1997; Nakata et al., 1998; Bacon et al., 1999; Kannan et al., 2004b, 2007, 2008; Kannan and Perrotta, 2008; Murata et al., 2008). Liver is often selected due to high lipid content and its role in the metabolism and excretion of contaminants, as well as availability in postmortem sampling. Liver can provide a good overall assessment of body burdens of specific chemicals; however, most of the contaminants of concern are lipophilic in nature, and rapid sequestration of contaminants in fat stores (when the animal is healthy) occurs upon exposure. When experiencing disease or food stress shortly before death, these fat stores are mobilized, elevating the apparent contaminant levels in lipophilic tissues. In marine mammals, body condition may be a serious confounder of contaminant levels measured in lipophilic tissues. POPs, specifically ΣDDTs, Σpolybrominated diphenyl ethers (PDBEs), ΣCHLs, and ΣHCHs, in the blubber of living California sea lions that suffered starvation followed by feeding to repletion more than doubled from baseline during starvation before falling back to near baseline when body condition was restored (Hall et al., 2008). Contaminants studies on other marine mammals have generally measured blubber levels as a better estimator of overall exposure to lipophilic POPs (Jarman et al., 1996b; Kajiwara et al., 2001; Troisi et al., 2001; Kannan et al., 2004a; Debier et al., 2005a); however, sea otters rely on their thick fur to combat hypothermia and have no blubber and minimal

TABLE 6. Median (ng/g lipid wt), percentage of Σpolychlorinated biphenyls (PCB), and range serum concentrations (ng/g lipid wt) of PCB homologs for each capture location of northern sea otters in Alaska. Different letters indicate statistically significant differences between the values for a given contaminant (KWANOVA, $P<0.05$); letters in common between classes did not differ significantly by Mann-Whitney U-test ($P>0.05$).

Contaminant[a]	Adak–Bay of Islands ($n=20$)	Adak–Kuluk Bay/Clam Lagoon Island ($n=9$)	Amchitka ($n=8$)	Kanaga ($n=7$)	Kiska ($n=14$)	Cross Sound/Port Althorp ($n=20$)
Di-CB	35 AD/9.3% (0.0–73)	98 BE/5.6% (0.0–270)	30 AD/2.8% (18–60)	0.0 C/0.0% (0.0–0.0)	19.4D/2.0% (0.0–120)	150^{E}/31.1% (49–650)
Tri-CB	28 A/8.2% (0.0–64.3)	39.5 A/2.1% (0.0–84)	38.6 A/3.5% (21–61.4)	18 B/6.8% (12.8–30)	26.5A/6.5% (17.5–67)	57.5^{C}/10.7% (4.9–160)
Tetra-CB	96.3 A/25.0% (0–183.9)	126 AB/6.5% (0–216)	161.0 B/12.9% (44.8–445)	68.7 A/25.8% (45.4–131)	125.5B/27.2% (63–284)	91^{A}/15.1% (58.3–200)
Penta-CB	60.8 A/17.1% (0–193.1)	308.7 BC/16.2% (0–1,343)	375.8 B/35.1% (60.8–3,119)	61.3 AD/28.0% (43.3–196.4)	104.6CD/20.5% (32.3–619)	115.2^{C}/23.5% (24–1783)
Hexa-CB	95.8 AC/25.5% (0–363.0)	710.2 B/35.8% (0–3,661)	360.7 BD/30.2% (54.9–2,639)	51.5 A/23.9% (36.6–237.8)	126CD/26.8% (31.1–746.6)	70.7^{A}/15.3% (0–1,890)
Hepta-CB	17.2 A/4.5% (0–162.7)	500 B/27.2% (0–2,562.3)	118.2 B/10.9% (28.7–1,318)	8.8 A/3.3% (0–51.3)	12.9A/2.0% (0–156.9)	2.1^{A}/0.5% (0–484)
Octa-CB	22.6 AC/5.1% (0.0–53)	91 B/5.0% (0.0–597)	29.3 ABC/4.0% (0.0–356.4)	4.2 A/1.6% (0.0–10)	26.9C/5.7% (4.9–59)	0.0^{D}/0.0% (0.0–53.3)
Nona-CB	0.0 A/0.0% (0.0–8.5)	23 B/0.9% (0.0–63)	11 B/1.0% (0.0–110)	0.0 A/0/0% (0.0–0.0)	0.0A/0/0% (0.0–0.0)	0.0^{A}/0.0% (0.0–10)
Deca-CB	21 ACD/5.6% (16–42)	0.0 BC/0.0% (0.0–52)	0.0 BE/0.0% (0.0–0.0)	21 CD/9.3% (17–24)	24.5D/5.2% (16–59)	0.0^{E}/0.0% (0.0–0.0)

[a] Abbreviations for contaminates listed: CB = chlorinated biphenyls.

TABLE 7. Median (ng/g lipid wt), percentage of Σpolychlorinated biphenyls (PCB), and range serum concentrations (ng/g lipid wt) of PCB homologs for each capture location of southern sea otters in California.

	Monterey (n=24)			Elkhorn Slough (n=8)			Santa Cruz (n=8)		
Contaminant[a]	Median	%	Range	Median	%	Range	Median	%	Range
Di-CB	0.0	0.0	0.0–217.8	0.0	0.0	0.0–0.0	0.0	0.0	0.0–37.2
Tri-CB	36.6	6.4	0.0–1,229.4	43.5	11.3	23.8–204.2	68.0	5.2	31.1–2,205.7
Tetra-CB	66.2	9.7	0.0–326.7	37.5	9.0	19.7–142.9	68.0	6.1	14.4–534.9
Penta-CB	133.1	22.9	0.0–1,148.7	91.0	21.8	12.7–3,073.5	368.4	19.9	36.6–1,035.6
Hexa-CB	171.0	24.1	0.0–1,246.4	162.1	36.7	16.8–3,247.9	477.6	34.8	166.9–1,078.5
Hepta-CB	71.7	15.0	0.0–708.7	41.1	9.0	0.0–1,358.6	296.2	20.8	24.3–661.6
Octa-CB	16.7	2.7	0.0–215.7	28.4	5.1	5.0–408.3	72.0	4.0	0.0–169.0
Nona-CB	0.0	0.0	0.0–65.7	0.0	0.0	0.0–78.0	10.5	0.6	0.0–39.4
Deca-CB	18.2	2.6	0.4–67.5	17.0	3.3	6.0–181.9	19.9	1.3	15.7–61.3

[a] Abbreviations for contaminates listed: CB = chlorinated biphenyls.

fat stores to sample. They have a high metabolic rate, and the anatomy and physiology of their fat stores are like those of terrestrial mammals. High rates of biomagnification of PCBs and DDTs (60- and 240-fold greater than prey) have been shown for sea otters in Monterey Bay, California, but profiles of PCB congeners suggest a great capacity to biotransform lower-chlorinated congeners and a greater ability to metabolize PCBs than cetaceans (Kannan et al., 2004b). Therefore, comparison between POP levels in lipophilic tissues of sea otters and those of pinnipeds and cetaceans may be confounded by differences in anatomy and physiology of fat stores, as well as metabolic capacity.

Peripheral blood samples are relatively simple to collect from live-captured otters, a comparatively large sample can be acquired, and longitudinal sampling can be performed on individual animals upon recapture. While some work has been done both on evaluating blood-based bioassays of exposure to contaminants in various otter species (Duffy et al., 1994; Ballachey, 1995; Duffy et al., 1995; Bickham et al., 1998; Murk et al., 1998; Ziccardi et al., 2002; Brancato et al., 2009) as well as directly assessing contaminant levels in blood of marine mammals (Newman et al., 1994; Lahvis et al., 1995; Derocher et al., 2001; Neale et al., 2005), little information is available on the dynamics and storage of chemical pollutants in peripheral blood of sea otters. In terrestrial mammals such as dogs, the primary soluble lipids in blood are cholesterol, triglycerides, and small energy-rich fatty acids. Elevations in triglyceride levels are grossly recognized as lipemic (cloudy) serum, but even highly elevated levels of cholesterol do not affect serum clarity because the molecules are too small to diffract light (Johnson, 1989). None of the serum in this study appeared lipemic, and cholesterol levels were within normal limits (Hanni et al., 2003). Absent evidence of hyperlipidemia, we assume that the lipid levels in the blood of the sea otters tested in this research were at equilibrium levels with other body compartments, and lipophilic POPs detected in serum reflected normal homeostatic relationships. Percentage of lipid in serum, if the lipids were derived from mobilization of body fat, could significantly influence the levels of lipophilic contaminants detected. Although there was variation between otters (approximately 0.3–1.4%), the median percentages of all age, sex, and locations were 0.6–1.0%. These low, narrow ranges do not suggest that variation in serum lipid content was a significant confounder.

All sea otters sampled for this project

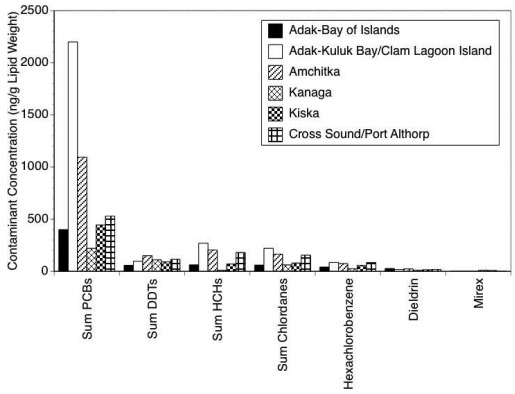

FIGURE 2. Variation in the sum totals of several major classes of persistent organic pollutants (POPs) and other chemicals of ecologic or environmental concern (COECs) in blood of live healthy northern sea otter in six locations in Alaska. Polychlorinated biphenyl (PCB) levels in otters from Adak–Kuluk Bay and Amchitka were statistically significantly greater than at other locations.

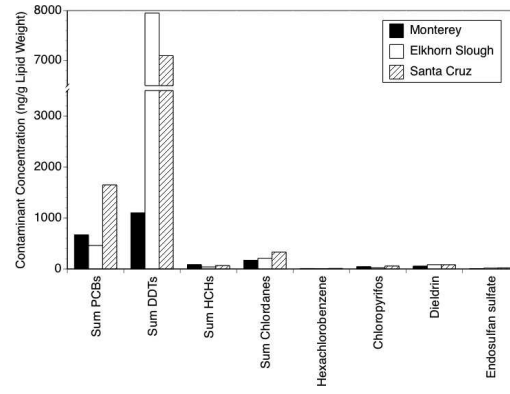

FIGURE 3. Variation in the sum totals of several major classes of persistent organic pollutants (POPs) and other chemicals of ecologic or environmental concern (COECs) in blood of live healthy southern sea otter in three locations in California. Levels of most POPs and COECs in Alaska were 10–20 times lower than any California locations.

were healthy at the time of capture, and hematology and blood chemistry parameters in samples collected at capture did not indicate organ system dysfunction or other general health problems (Hanni et al., 2003). In general, California sea otters had significantly higher mean POP levels (5–20 times) than otters in most Alaska locations (Figs. 2, 3). Median values for all contaminants with detectable levels differed by otter age class within Alaska with the exception of mirex and TEQ levels (Table 2). Conversely, otters in California only differed between age-sex classes for ΣDDTs, ΣHCHs, and ΣPAHs (Table 3). These results may reflect true differences in contaminant accumulation in sea otters classes; however, the age distribution varied among locations, and small sample size prevented stratified analysis to control for age and sex class other than Alaskan adult females and Californian adult males. Therefore, differences in blood contaminant levels due to differential accumulation by age, or by location, cannot be ruled out. For Alaskan females, ΣPCB, ΣHCH, ΣHCB, and TEQs remained significantly different; however, for California adult males, only dieldrin differed significantly. In general, subadults had higher contaminant levels than the other age-sex classes (Fig. 4), followed by (but often not statistically distinct from) pups. These relatively high POP burdens in young otters suggest that bloodborne contaminants accumulate quickly, probably as a result of maternal transfer in milk. Sea otters have very high metabolic requirements, and young growing animals seldom have any body fat stores in which POP burdens might be sequestered. Recently weaned subadults may have both the maximal POP burden transferred from their mothers and may also be metabolizing stored fats as they learn to forage efficiently while still growing.

Adult females, for the most part, had significantly lower POP burdens, presumably from the transfer of chemical pollutants to pups through either gestation and/or lactation. Additional evidence for lactational transfer was provided by one young female southern sea otter that had been orphaned, rehabilitated, and released and was sampled when pregnant with her first pup and again after giving birth (data not shown). Various POP concentrations de-

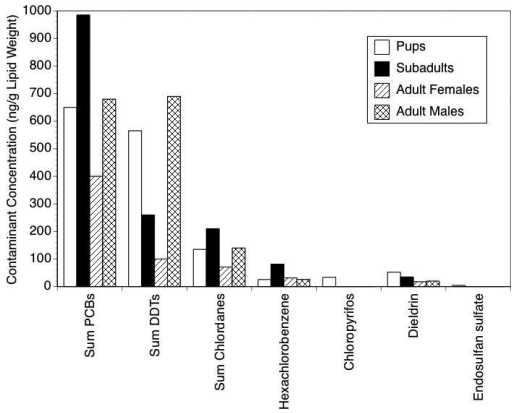

FIGURE 4. Variation in the median values of several major classes of persistent organic pollutants (POPs) and other chemicals of ecologic or environmental concern (COECs) by age and age/sex in blood of live healthy adult southern (California) and northern (Alaska) sea otters. Pups and subadults had statistically significantly higher levels than adults, with exception of polychlorinated biphenyls (PCBs) and DDTs in adult males. Females had significantly lower levels of all contaminants.

clined dramatically, ranging from 9.5% to 79% reductions, indicating a rapid postpartum decline.

One notable exception to the observation that subadults had greater POP burdens was in California, where adult males were found to have higher (though statistically indistinct) median ΣDDT concentrations, which may be an artifact of location, since most adult males (8/11) were captured in Elkhorn Slough (Table 1). The sea otter population in Elkhorn Slough feeds primarily on clams, fat innkeeper worms, mussels, and an assortment of other benthic invertebrates. Kannan et al. (2004b) showed that fat innkeeper worms, which are pseudo–filter feeders, accumulate levels of POPs in the same order of magnitude as true filter feeders such as blue mussels (Kannan et al., 2004b). Water samples taken from Tembledero Slough, one of the seasonal wetlands feeding into Elkhorn Slough, contained the highest levels of DDT, DDD, and DDE detected in California waters (Hartwell, 2004). Hundreds of acres of irrigated farmlands north of Castroville and south of Moss Landing (lands in intense agricultural production since the turn of the century) drain via subsurface collection tiles to Tembledero, and then to Elkhorn Slough, which may help to explain the high levels of ΣDDTs in male sea otters at this location. Although not explored in this paper, the high ratios of DDE and DDD to DDT in these animals and waters strongly suggest that the high ΣDDT levels are a legacy of historic use and that environmental breakdown is occurring. However, the median ΣDDT level was 4,700 ng/g lipid weight for two adult males captured in Santa Cruz and 2,300 ng/g lipid weight for one adult male captured in Monterey, suggesting that adult males may generally have higher ΣDDT levels than other age and sex classes, regardless of their capture location in California. Only one of 30 live-captured sea otters in Washington State had any detectable DDT-related compounds in whole blood, a 3-yr-old male with 180 ppb (ng/g) wet weight p,p′-DDE (Brancato et al., 2009).

The relatively high levels of ΣPCBs in blood samples from Adak–Kuluk Bay/Clam Lagoon and Amchitka sea otters (approximately 5 times levels in other Alaska locations) mirror findings from previous investigations of fish, eagles, and sea otters at these locations (Jarman et al., 1996a; Estes et al., 1997; Anthony et al., 1999, 2007; Miles et al., 2009). These are locations where intense military activity and ocean dumping of equipment have occurred. In examining individual PCB congeners, most of this difference within Alaska can be attributed to differences in the higher chlorinated congeners and homologs (Table 6), with particular emphasis on several pentachlorobiphenyls (PCBs 110, 118), hexachlorobiphenyls (PCBs 138, 153), and heptachlorobiphenlys (PCBs 170, 180, 183, and 187; data not shown). Most of these congeners are those known to bioaccumulate in the marine environment (Ricca et al., 2008),

and thus were found in greater concentrations at those locations known to have historical evidence of PCB contamination associated with military activities, namely Adak and Amchitka. There were also some notable differences between Alaskan locations for lighter, less chlorinated PCB congeners. These were seen at greater levels in the other regions throughout the Aleutians and southwest Alaska, likely due to more distant environmental sources and transportation by global distillation. Similar differential congener distributions have been found previously in studies involving seabird species (Ricca et al., 2008) and sea otter (Bacon et al., 1999) in Alaska, and a similar cause has been hypothesized.

Sea otters at the three locations in California had relatively high levels of PCBs. Moderate to high levels of exposure to many different congeners in all the three areas of California sampled suggest different exposure and accumulative processes compared to Alaska. While exposures of sea otters from Adak and Amchitka may be characteristic of sites with intensive historic military activity, and exposures at other Alaskan locations reflect low levels of environmental fallout, the pattern observed in otters at the three California locations may reflect a variety of PCB inputs from electrical insulator, runoff, waste disposal, incineration, and agricultural solvent uses over the last century. Levels of some PCBs were as high, or higher, than those detected in Adak and Amchitka otters, despite the fact that no point source or disposal site is known to exist in the greater Monterey Bay area. The mean ΣPCB levels (5.57 ppb wet weight) in blood from 30 northern sea otters (*Enhydra lutris kenyoni*) recently reported from the Olympic Peninsula of Washington State (Brancato et al., 2009) were higher than we found in more pristine areas of Alaska, but lower than seen in all three California locations, and less than half the levels seen at Adak and Amchitka.

Previous studies evaluating contaminant burdens in California otters have shown higher levels of PCBs in liver samples (Nakata et al., 1998) compared to southeastern Alaskan otters (Bacon et al., 1999; Kannan et al., 2008). In Kannan et al. (2008), levels of POPs in livers from dead, stranded sea otters in California and the Aleutian Islands were compared to levels in livers from otherwise healthy otters shot during native harvest in southeast Alaska; the difference in perimortem nutrition was a potentially significant confounding variable. Recent studies evaluating PCB levels in sediment throughout the San Francisco–Monterey areas show discrete areas of higher concentrations in Monterey, though these are thought to be derived primarily from the San Francisco Bay region (Hartwell, 2008). Wildlife captured in the San Francisco area possess massive PCB burdens (Hothem et al., 1995; She et al., 2008), including blood levels in excess of 300 ng/g for harbor seals (Young et al., 1998), which is consistent with our finding of elevated PCB levels in otters in Monterey Bay just south of San Francisco.

While overall tissue levels of POPs and COECs could suggest health consequences, to better understand the synergistic effects that multiple contaminants might have in animal systems, as well as to attempt to associate body burdens with potential adverse effects, the use of toxic equivalents (or TEQs) can be valuable. Through comparing the concentrations of compounds having similar mechanisms of action to 2,3,7,8-TCDD and utilizing in vivo and in vitro results that have previously established comparative activation of the aryl hydrocarbon (Ah) receptor, the overall "bioactivity" of different samples may be compared. Several assumptions are inherent in this approach, including that associations between congeners are additive and, more importantly, that activation of the Ah receptor relates directly to potential harmful effects in animal systems. With these limitations considered, the TEQ method allows for better

estimation of potential toxic risk than simply comparing sum totals. A significant correlation between ΣPCB levels and TEQ concentrations in the overall data set (Spearman's rho=0.500, $P<0.001$) and for most age-sex and location categories was seen, with a few notable exceptions. Adak-Kuluk Bay/Clam Lagoon and Amchitka had very high ΣPCB levels, but they had comparatively similar TEQ activity to Adak–Bay of Islands, Kanaga, and Kiska. These differences were driven by large concentrations of PCB congeners in both Adak–Kuluk Bay/Clam Lagoon and Amchitka that have not been shown to possess Ah receptor activity (PCB 138, 153, 180, and 187) and yet are persistent in the marine environment as discussed herein. As to age-sex differences, Alaskan pups had low median PCB burdens, yet relatively high median TEQs; this result was likely a function of low sample size. Alaskan males also showed little correlation between PCBs and TEQs, with many animals having high PCB burdens but low TEQ estimates. This disparity was primarily driven by several males captured in Cross Sound/Port Althorp (an area with very low TEQ estimates and greater concentrations of lower chlorinated, nonbioactive congeners such as PCB 5/8, 18, and 97) as well as on Kiska and Kanaga Islands (which had much higher TEQ estimates). Therefore, while understanding the overall ΣPCB burden in wildlife is important, an evaluation of the underlying congeners present (and their potential for adverse health effects) is critical to understanding the overall toxicologic impact of such contamination.

Another important consideration in better understanding the impact of such potentially deleterious compounds is evaluating the TEQ values in light of the tissue examined and effects seen in similar species. There is little information on TEQ estimates versus physical effects for sea otters, but previous work in mink, a closely related species used as laboratory models for otter, estimated a lipid-normalized liver TEQ no observable adverse effects level (NOAEL) at 11 pg/g, a threshold dose for reproductive effects at 60 pg/g, and a lowest observable adverse effects level (LOAEL) at 324 pg/g (Tillitt et al., 1995). Kannan et al. (2004b) suggested that the threshold concentration in liver of TEQs in aquatic mammals beyond which physiologic effects can be seen was 520 pg/g lipid weight. Based on these cutoff values, no otters in this study exceeded the LOAEL or aquatic mammal threshold, but two California otters (both from Elkhorn Slough) and 11 Alaskan otters (Amchitka n=3, Kiska $n=4$, Adak–Kuluk Bay/Clam Lagoon Island $n=2$, Cross Sound/Port Althorp $n=1$, Adak–Bay of Islands $n=1$) exceeded the 60 pg/g reproductive TEQ threshold suggested for mink. However, another consideration must be that liver tends to accumulate and magnify contaminant burdens, and serum concentrations of live sea otters may be significantly lower than liver levels of dead ones. Abraham et al. (1988) showed that tissue distribution of 2,3,7,8-TCDD in Wistar rats was approximately 30 ng/g in liver versus 0.17 ng/g in serum, a 176-fold increase. Applying this distribution to the aquatic mammal threshold suggested by Kannan et al. (2004b), threshold TEQ concentrations for serum in aquatic mammals can be estimated at 2.95 pg TCDD equivalents/g serum lipid weight. In this study, 90 of the 116 otters had TEQ levels exceeding this estimated level. Similarly, Kannan et al. (2004b) found that a number of otter livers they evaluated significantly exceeded this cutoff level. While this extrapolation from serum to liver is extremely crude and does not necessarily directly relate to adverse effects associated with such exposure, additional work should be undertaken to further interpret these findings, especially considering that all captured otters were assessed to be physically healthy with no evidence of disease.

One interpretation of our data is that a relationship may exist among moderate to

high levels of exposure of southern sea otters to many POPs and COECs, their high adult mortality level, and failure to grow. However, it is difficult to conclusively determine the nature of that relationship because of the wide range of contaminants present in the nearshore marine ecosystem, their unknown synergistic or antagonistic effects, and the poorly understood effects of these compounds on immune function. Levin et al. (2007) used an in vitro system to compare several white blood cell immune function assays in whole blood from healthy sea otters spiked with several PCBs, 2,3,7,8-TCDD, and mixtures of these compounds to the same animal controls. They found evidence of both up-regulation and down-regulation of various immune functions and more significant and consistent differences between captive nonstressed otters and stressed wild caught otters when compared with spiked and nonspiked samples of the same animal.

Recently, researchers have suggested a correlation between levels of POPs of dead sea otters and the likelihood of dying from infectious diseases (Nakata et al., 1998; Kannan et al., 2006a, b). Indeed, the level of mortality in prime-age adult southern sea otters from a variety of causes has been high for 12–14 yr, with infectious diseases and parasites causing 40–50% of mortality (Thomas and Cole, 1996; Kreuder et al., 2003; USGS, 2009). Our study does not refute potential associations between POP concentrations from postmortem liver samples of sea otters and cause of death, but we show that similar POP accumulation patterns can be found in the blood of healthy sea otters. Our findings show that live pups and subadults rapidly accumulate the highest levels of most POPs, with the possible exception of DDTs in adult males, similar to previous studies using liver from dead stranded otters. Because high mortality rates in prime-age adults (particularly females) are believed to cause population stasis and periodic declines in southern sea otters, and adult females have been shown to possess the lowest POP levels in blood and liver, if POPs are having negative effects at the population level, they must be delayed or cumulative and take years to manifest. Furthermore, most sampled Alaskan sea otters in this study, except those from Clam Lagoon and Cross Sound/Port Althorp, came from populations that had recently experienced or were experiencing a significant population decline. Patterns of exposure varied widely in Alaska, but no correlation between high or low POP levels and populations in decline was noted. The decline of sea otter populations in locations with relatively low POP exposure levels would seem to suggest that these anthropogenic chemicals are not associated with recent Alaskan sea otter declines.

While the population-level contributions of POP burdens to sea otter population stasis or decline may be limited or subtle, we provide some evidence that body burdens of POPs (particularly the bioactive PCB congeners) may be at levels of concern in certain locations and age-sex classes. Blood, due to its relatively low lipid content, may not be the ideal tissue for analysis of total POP body burden; nevertheless, it is a means of determining biologically relevant POP levels in living animals without the confounding influence resulting from use of dead animals undergoing terminal life processes such as starvation and fat mobilization. Both methods in use for determining POP exposure in sea otters may have inherent biases. Continued collection and analysis of easily accessed samples in healthy and unhealthy otters will allow longitudinal analyses, more detailed analyses by location and food habits of specific compounds, congeners, or metabolites with known toxicity, the establishment of "normal" ranges of POPs and COECs, and the further differentiation of age, gender, and reproductive status impacts.

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LITERATURE CITED

ABRAHAM, K., R. KROWKE, AND D. NEUBERT. 1988. Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Archives of Toxicology 62: 359–368.

AHLBORG, U. G., G. C. BECKING, L. S. BIRNBAUM, A. BROUWER, H. DERKS, M. FEELEY, G. GOLOR, A. HANBERG, J. C. LARSEN, A. K. D. LIEM, S. H. SAFE, C. SCHLATTER, F. WAERN, M. YOUNES, AND E. YRJANHEIKKI. 1994. Toxic equivalency factors for dioxin-like PCBs. Report on a WHO-ECEH and IPCS consultation, December 1993. Chemosphere 28: 1049–1067.

AMES, J. A., R. A. HARDY, AND F. E. WENDELL. 1986. A simulated translocation of sea otters (*Enhydra lutris*) with review of capture, transport, and holding techniques. Marine Resources Technical Report, California Department of Fish and Game, Sacramento, California, 17 pp.

ANTHONY, R. G., A. K. MILES, J. A. ESTES, AND F. B. ISAACS. 1999. Productivity, diets, and environmental contaminants in nesting bald eagles from the Aleutian Archipelago. Environmental Toxicology and Chemistry 18: 2054–2062.

———, ———, M. A. RICCA, AND J. A. ESTES. 2007. Environmental contaminants in bald eagle eggs from the Aleutian Archipelago. Environmental Toxicology and Chemistry 26: 1843–1855.

BACON, C. E., W. M. JARMAN, J. A. ESTES, M. SIMON, AND R. J. NORSTROM. 1999. Comparison of organochlorine contaminants among sea otter (*Enhydra lutris*) populations in California and Alaska. Environmental Toxicology and Chemistry 18: 452–458.

BALLACHEY, B. E. 1995. Biomarkers of damage to sea otters in Prince William Sound, Alaska, following potential exposure to oil spilled from the Exxon Valdez. Marine Mammal Study 6-1. Exxon Valdez Oil Spill State/Federal Natural Resource Damage Assessment Final Report, United States, US Fish and Wildlife Service, Anchorage, Alaska, 20 pp.

BICKHAM, J. W., J. A. MAZET, J. BLAKE, M. J. SMOLEN, Y. LOU, AND B. E. BALLACHEY. 1998. Flow cytometric determination of genotoxic effects of exposure to petroleum in mink and sea otters. Ecotoxicology 7: 191–199.

BRANCATO, M. S., L. MILONAS, C. E. BOWLBY, R. JAMESON, AND J. W. DAVIS. 2009. Chemical contaminants, pathogen exposure and general health status of live and beach-cast Washington sea otters (*Enhydra lutris kenyoni*). Marine Sancturaries Conservation Series ONMS 08-08. US Department of Commerce, National Oceanic and Atmospheric Administration, Office of National Marine Sancturaies, Silver Springs, Maryland, 181 pp.

CHANG, R. R., W. M. JARMAN, AND J. A. HENNINGS. 1993. Sample cleanup by solid phase extraction for ultratrace determination of polychlorinated dibenzo-p-dioxins and dibenzofurans in biological samples. Analytical Chemistry 65: 2420–2427.

COSTA, D. P., AND G. L. KOOYMAN. 1982. Oxygen consumption, thermoregulation, and effects of fur oiling and washing on sea otter (*Enhydra lutris*). Canadian Journal of Zoology 60: 2761–2767.

DEBIER, C., B. J. LE BOUF, M. G. IKONOMOU, T. DE TILLESSE, Y. LARONDELLE, AND P. S. ROSS. 2005a. Polychlorinated biphenyls, dioxins, and furans in weaned, free-ranging northern elephant seal pups from central California, USA. Environmental Toxicology and Chemistry 24: 629–633.

———, G. M. YLITALO, M. WEISE, F. GULLAND, D. P. COSTA, B. J. LE BOEUF, T. DE TILLESSE, AND Y. LARONDELLE. 2005b. PCBs and DDT in the serum of juvenile California sea lions: Associations with vitamins A and E and thyroid hormones. Environmental Pollution 134: 323–332.

DELONG, R. L., W. G. GILMARTIN, AND J. G. SIMPSON. 1973. Premature births in California sea lions: Association with high organochlorine pollutant residue levels. Science 181: 1168–1170.

DEROCHER, A. E., J. U. SKAARE, A. BERNHOFT, O. WIIG, K. R. NORUM, E. HAUG, AND D. M. EIDE. 2001. Relationships between plasma levels of organochlorines, retinol and thyroid hormones from polar bears (*Ursus maritimus*) at Svalbard. Journal of Toxicology and Environmental Health 62: 227–241.

DOROFF, A. M., J. A. ESTES, M. T. TINKER, D. M. BURN, AND T. J. EVANS. 2003. Sea otter population declines in the Aleutian Archipelago. Journal of Mammalogy 84: 55–64.

DUFFY, L. K., R. T. BOWYER, J. W. TESTA, AND J. B. FARO. 1994. Chronic effects of the Exxon Valdez oil spill on blood and enzyme chemistry of river otters. Environmental Toxicology and Chemistry 13: 643–647.

———, A. BLAJESKI, AND J. BIFELT. 1995. Porphyrins

as comparative biomarkers to evaluate ecological effects of environmental contaminants. *In* Landscapes: Human Ecology, Landscape Ecology, Earth System Science: Proceedings of the 46th AAAS Arctic Division Science Conference. American Association for the Advancement of Science, Arctic Division, Fairbanks, Alaska, 208 pp.

Estes, J. A., C. E. Bacon, W. M. Jarman, R. J. Norstrom, R. G. Anthony, and A. K. Miles. 1997. Organochlorines in sea otters and bald eagles from the Aleutian Archipelago. Marine Pollution Bulletin 34: 486–490.

———, M. T. Tinker, T. M. Williams, and D. F. Doak. 1998. Killer whale predation on sea otters: Linking oceanic and nearshore ecosystems. Science 282: 473–476.

———, B. B. Hatfield, K. Ralls, and J. Ames. 2003. Causes of mortality in California sea otter during periods of population growth and decline. Marine Mammal Science 19: 198–216.

———, M. T. Tinker, A. M. Doroff, and D. M. Burn. 2005. Continuing sea otter population declines in the Aleutian Archipelago. Marine Mammal Science 21: 169–172.

Frings, C. S., T. W. Fendley, R. T. Dunn, and C. A. Queen. 1972. Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction. Clinical Chemistry 18: 673–674.

Hall, A. J., F. M. D. Gulland, G. M. Ylitalo, D. M. Greig, and L. Lowenstine. 2008. Changes in blubber contaminant concentrations in California sea lions (*Zalophus californianus*) associated with weight loss and gain during rehabilitation. Environmental Science and Technology 42: 4181–4187.

Hanni, K. D., J. A. K. Mazet, F. M. D. Gulland, J. Estes, M. Staedler, M. J. Murray, and D. A. Jessup. 2003. Clinical pathological values and assessment of pathogen exposure in southern and Alaskan sea otters. Journal of Wildlife Diseases 39: 837–850.

Hartwell, S. I. 2004. Distribution of DDT in sediments off the central California coast. Marine Pollution Bulletin 49: 299–305.

———. 2008. Distribution of DDT and other persistent organic contaminants in canyons and on the continental shelf off the central California coast. Marine Environmental Research 65: 199–217.

Hothem, R. L., D. L. Roster, K. A. King, T. J. Keldsen, K. C. Marois, and S. E. Wainwright. 1995. Spatial and temporal trends of contaminants in eggs of wading birds from San Francisco Bay, California. Environmental Toxicology and Chemistry 14: 1319–1331.

Jarman, W. M., C. E. Bacon, J. A. Estes, M. Simon, and R. J. Norstrom. 1996a. Organochlorine contaminants in sea otters: The sea otter as a bio-indicator. Endangered Species Update 13: 20–22.

———, R. J. Norstrom, D. C. Muir, B. Rosenberg, M. Simon, and R. W. Baird. 1996b. Levels of organochlorine compounds, including PCDDs and PCDFs, in the blubber of cetaceans from the West Coast of North America. Marine Pollution Bulletin 32: 426–436.

Jepson, P. D., P. M. Bennett, R. Deaville, C. R. Allchin, J. R. Baker, and R. J. Law. 2005. Relationships between polychlorinated biphenyls and health status in harbor porpoises (*Phocoena phocoena*) stranded in the United Kingdom. Environmental Toxicology and Chemistry 24: 238–248.

Jessup, D. A., M. Miller, J. Ames, M. Harris, P. Conrad, C. Kreuder, and J. A. K. Mazet. 2004. The southern sea otter (*Enhydra lutris nereis*) as a sentinel of marine ecosystem health. EcoHealth 1: 239–245.

———, ———, C. Kreuder-Johnson, P. Conrad, T. Tinker, J. Estes, and J. Mazet. 2007. Sea otters in a dirty ocean. Journal of the American Veterinary Medical Association 231: 1648–1652.

Johnson, R. K. 1989. Canine hyperlipidemia. *In* Textbook of veterinary internal medicine, S. J. Ettinger (ed.). W. B. Saunders, Philadelphia, Pensylvannia, 203 pp.

Kajiwara, N., K. Kannan, M. Muraoka, M. Watanabe, S. Takahashi, F. Gulland, H. Olsen, A. L. Blankenship, P. D. Jones, S. Tanabe, and J. P. Giesy. 2001. 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. Archives of Environmental Contamination and Toxicology 41: 90–99.

Kannan, K., and E. Perrotta. 2008. Polycyclic aromatic hydrocarbons (PAHs) in livers of California sea otters. Chemosphere 71: 649–655.

———, S. Tanabe, A. Borrell, A. Aguilar, S. Focardi, and R. Tatsukawa. 1993. Isomer-specific analysis and toxic evaluation of polychlorinated biphenyls in striped dolphins affected by an epizootic in the western Mediterranean Sea. Archives of Environmental Contaminant Toxicology 25: 227–233.

———, K. S. Guruge, N. J. Thomas, S. Tanabe, and J. P. Giesy. 1998. Butyltin residues in southern sea otters (*Enhydra lutris nereis*) found dead along California coastal waters. Environmental Science and Technology 32: 1169–1175.

———, N. Kajiwara, B. J. Le Boeuf, and S. Tanabe. 2004a. Organochlorine pesticides and polychlorinated biphenyls in California sea lions. Environmental Pollution 131: 425–434.

———, ———, M. Watanabe, H. Nakata, N. J. Thomas, M. Stephenson, D. A. Jessup, and S. Tanabe. 2004b. Profiles of polychlorinated biphenyl congeners, organochlorine pesticides,

and butyltins in southern sea otters and their prey. Environmental Toxicology and Chemistry 23: 49–56.

———, T. Agusa, E. Perrotta, N. J. Thomas, and S. Tanabe. 2006a. Comparison of trace element concentrations in livers of diseased, emaciated and non-diseased southern sea otters from the California coast. Chemosphere 65: 2160–2167.

———, E. Perrotta, and N. Thomas. 2006b. Association between perfluorinated compounds and pathological conditions in southern sea otters. Environmental Science and Technology 40: 4943–4948.

———, ———, N. Thomas, and K. Aldous. 2007. A comparative analysis of polybrominated diphenyl ethers and polychlorinated biphenyls in southern sea otters that died of infectious diseases and noninfectious causes. Archives of Environmental Contamination and Toxicology 53: 293–302.

———, H. B. Moon, S. H. Yun, T. Agusa, N. J. Thomas, and S. Tanabe. 2008. Chlorinated, brominated, and perfluorinated compounds, polycyclic aromatic hydrocarbons and trace elements in livers of sea otters from California, Washington, and Alaska (USA), and Kamchatka (Russia). Journal of Environmental Monitoring 10: 552–558.

Krahn, M. M., M. B. Hanson, G. S. Schorr, C. K. Emmons, D. G. Burrows, J. L. Bolton, R. W. Baird, and G. M. Ylitalo. 2009. Effects of age, sex and reproduction status on persistent organic pollutant concentrations in "Southern Resident" killer whales from three fish-eating pods. Marine Pollution Bulletin 58: 1522–1529.

Kreuder, C., M. Miller, D. Jessup, L. Lowenstine, M. Harris, J. Ames, T. Carpenter, P. Conrad, and J. Mazet. 2003. Patterns of mortality in southern sea otters (*Enhydra lutris nereis*) from 1998–2001. Journal of Wildlife Diseases 39: 495–509.

Lahvis, G. P., R. S. Wells, D. W. Kuehl, J. L. Stewart, H. L. Rhinehart, and C. S. Via. 1995. Decreased lymphocyte responses in free-ranging bottlenose dolphins (*Tursiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. Environmental Health Perspectives 103: 67–72.

Levin, M., H. Leibrecht, C. Mori, D. Jessup, and S. De Guise. 2007. Immunomodulatory effects of organochlorine mixtures upon in vitro exposure of peripheral blood leulocytes differ between free-ranging and captive Southern sea otters (*Enhydra lutris*). Veterinary Immunology and Immunopathology 119: 269–277.

Miles, A. K., M. A. Ricca, R. G. Anthony, and J. A. Estes. 2009. Organochlorine contaminants in fishes from coastal waters west of Amukta Pass, Aleutian Islands, Alaska, USA. Environmental Toxicology and Chemistry 28: 1643–1654.

Moller, R. B. 2003. Pathology of marine mammals with special reference to infectious dieases. *In* Toxicology of marine mammals, J. G. Vos, G. D. Bossart, M. Fournier and T. J. O'Shea (eds.). Taylor and Francis Group, London, UK, pp. 3–37.

Monson, D. H., C. McCormick, and B. E. Ballachey. 2001. Chemical anesthesia of northern sea otters (*Enhydra lutris*): Results of past field studies. Journal of Zoo and Wildlife Medicine 32: 181–189.

Mulcahy, D. M., and B. E. Ballachey. 1994. Hydrocarbon residues in sea otter tissues. *In* Marine mammals and the Exxon Valdez, Loughlin T. R. (ed.). Academic Press, Inc., San Diego, California, pp. 313–330.

Murata, S., S. Takahashi, T. Agusa, N. J. Thomas, K. Kannan, and S. Tanabe. 2008. Contamination status and accumulation profiles of organotins in sea otters (*Enhydra lutris*) found dead along the coasts of California, Washington, Alaska (USA), and Kamchatka (Russia). Marine Pollution Bulletin 56: 641–649.

Murk, A. J., P. E. Leonards, B. Van Hattum, R. Luit, M. E. Van Der Weiden, and M. Smit. 1998. Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). Environmental Toxicology and Pharmacology 6: 91–102.

Nakata, H., K. Kannan, L. Jing, N. Thomas, S. Tanabe, and J. P. Giesy. 1998. Accumulation pattern of organochlorine pesticides and polychlorinated biphenyls in southern sea otters (*Enhydra lutris nereis*) found stranded along coastal California, USA. Environmental Pollution 103: 45–53.

Neale, J. C. C., F. M. D. Gulland, K. R. Schmelzer, J. T. Harvey, E. A. Berg, S. G. Allen, D. J. Greig, E. K. Grigg, and R. S. Tjeerdema. 2005. Contaminant loads and hematological correlates in the harbor seal (*Phoca vitulina*) of San Francisco Bay, California. Journal of Toxicology and Environmental Health 68: 617–633.

Newman, J. W., R. R. Chang, W. M. Jarman, and J. M. Vedder. 1994. A method for the determination of environmental contaminants in living marine mammals using microscale samples of blubber and blood. Chemosphere 29: 671–681.

Norstrom, R. J., W. M. Jarman, J. A. Estes, R. G. Anthony, C. E. Bacon, and A. K. Miles. 1997. Organochlorines in sea otters and bald eagles from the Aleutian archipelago. Marine Pollution Bulletin 34: 486–490.

Ricca, M. A., A. K. Miles, and R. G. Anthony. 2008. Sources of organochlorine contaminants and mercury in seabirds from the Aleutian Archipelago of Alaska: Inferences from spatial and trophic variation. Science of the Total Environment 406: 308–323.

Ross, P. S., S. J. Jeffries, M. B. Yunker, R. F.

Addison, M. G. Ikonomou, and J. C. Calambokidis. 2004. Harbor seals (*Phoca vitulina*) in British Columbia, Canada, and Washington State, USA, reveal a combination of local and global polychlorinated biphenyl, dioxin, and furan signals. Environmental Toxicology and Chemistry 23: 157–165.

Safe, S. 1990. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). CRC Critical Reviews in Toxicology 21: 51–88.

She, J., A. Holden, T. L. Adelsbach, M. Tanner, S. E. Schwarzbach, J. L. Yee, and K. Hooper. 2008. Concentrations and time trends of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in aquatic bird eggs from San Francisco Bay, CA, 2000–2003. Chemosphere 73: S201–S209.

Tanabe, S., N. Miyazaki, T. Mori, and R. Tatsukawa. 1983. Global pollution of marine mammals by PCBs, DDTs and HCHs (BCHs). Chemosphere 12: 1269–1275.

Tillitt, D. E., R. W. Gale, J. C. Meadows, J. L. Zajicek, P. H. Peterman, S. N. Heaton, P. D. Jones, S. J. Bursian, T. J. Kubiak, J. P. Giesy, and R. J. Aulerich. 1995. Dietary exposure of mink to carp from Saginaw Bay. 3. Characterization of dietary exposure to planar halogenated hydrocarbons, dioxin equivalents, and biomagnification. Environmental Science and Technology 30: 283–291.

Thomas, N. J., and R. A. Cole. 1996. The risk of disease and threats to the wild population. Endangered Species Update 13: 23–27.

Troisi, G. M., K. Haraguchi, D. S. Kaydoo, M. Nyman, A. Aguilar, A. Borrell, U. Siebert, and C. F. Mason. 2001. Bioaccumulation of polychlorinated biphenyls (PCBs) and dichlorodiphenylethane (DDE) methyl sulfones in tissues of seal and dolphin morbillivirus epizootic victims. Journal of Toxicology and Environmental Health 62: 1–8.

US Geological Survey. 2009. *Spring 2009 mainland California sea otter survey results*, http://www.werc.usgs.gov/otters/ca-surveyspr2008.htm. Accessed 24 June 2009.

Van Den Berg, M., L. S. Birnbaum, M. Denison, M. De Vito, W. Farland, M. Feeley, H. Fiedler, H. Hakansson, A. Hanberg, L. Haws, M. Rose, S. Safe, D. Schrenk, C. Tohyama, A. Tritscher, J. Tuomisto, M. Tysklind, N. Walker, and R. E. Peterson. 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Science 93: 223–241.

Young, D., M. Becerra, D. Kopec, and S. Echols. 1998. GC/MS analysis of PCB congeners in blood of the harbor seal *Phoca vitulina* from San Francisco Bay. Chemosphere 37: 711–733.

Ziccardi, M. H., J. A. K. Mazet, I. A. Gardner, W. M. Boyce, and M. S. Denison. 2002. Validation of a cell culture bioassay for detection of petroleum exposure in mink (*Mustela vison*) as a model for detection in sea otters (*Enhydra lutris*). American Journal of Veterinary Research 63: 963–968.

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