POLYCHLORINATED BIPHENYLS, DIOXINS, AND FURANS IN WEANED, FREE-RANGING NORTHERN ELEPHANT SEAL PUPS FROM CENTRAL CALIFORNIA, USA

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Abstract—The aim of this study was to measure persistent organic pollutants in northern elephant seals ([NES], Mirounga angustirostris). We obtained blubber biopsy samples from six healthy, newly weaned NES pups from Año Nuevo, California (USA). Contaminant levels were lower than those of other pinnipeds studied on the west coast of North America. Blubber concentrations of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins, and polychlorinated dibenzofurans averaged 700 ± 130 μg/kg, 32 ± 23 ng/kg, and 17 ± 5 ng/kg (lipid wt), respectively. These contaminants originate from transplacental transfer and from maternal milk, which, in turn, reflect contaminants acquired by the mother from prey during long-distance foraging trips in the northeastern Pacific Ocean. The PCB profile in the blubber of NES pups mainly consisted of pentachloro-, hexachloro- and heptachlorobiphenyls, possibly reflecting the deep-sea nature of the mother's diet. Our results suggest that NES pups, in contrast to pups of other pinnipeds in the eastern Pacific Ocean, are exposed to low levels of persistent organic pollutants, reflecting an open ocean signal.

Keywords—Northern elephant seal Mirounga angustirostris Blubber Polychlorinated biphenyls Pup

INTRODUCTION

Northern elephant seals ([NES], Mirounga angustirostris) are large (400–2,000 kg), sexually dimorphic pinnipeds that frequent the waters off the west coast of North America. They gather on islands and remote continental shores of California and Mexico for reproduction between December and March, and molting during the spring (females) or summer (males) [1]. During the rest of the year, NES spend eight to 10 months dispersed at sea foraging over several thousands of kilometers in the northeastern Pacific Ocean [2].

Little is known about the degree to which NES are contaminated by persistent organic pollutants (POPs) of toxicological concern in high trophic level wildlife. High levels of these contaminants have been observed in several marine mammal species inhabiting or frequenting the coastal waters of the western United States and Canada [3–9]. The POPs are known to cause adverse effects such as endocrine disruption, oxidative stress, immunotoxicity, developmental abnormalities, and vitamin A impairment [10–13]. Given the deep pelagic feeding pattern of adult female NES in the open Pacific Ocean, one might expect that the contamination pattern of the NES may differ from those of other species, such as the coastal resident California sea lions (Zalophus californianus) or harbor seals (Phoca vitulina), that may be exposed to contaminants closer to point sources.

During the breeding period, NES exhibit the longest fasting durations of all phocid seals [1]. Lactating females remain with their pups throughout the entire 28-d nursing period, relying on the energy stored while feeding at sea during pregnancy to support milk secretion [14]. The POPs acquired during feeding trips in the open ocean are transferred to the pup through transplacental transfer and the fat-rich (up to 55% lipids) milk [15]. After weaning their pups, females return to sea for several months to feed while the weaned pups remain in the vicinity of the colony for a fasting period of up to 2.5 months, during which they develop their swimming and diving abilities [16,17]. The contaminant burden of weaned pups, therefore, is obtained entirely from transplacental and milk transfer. During the postweaning fast, blubber lipid reserves are used for maintenance, as well as muscle and skeletal growth. The utilization of lipid reserves during this fasting period results in the mobilization of the lipophilic POPs into circulation, which may increase risk of adverse health effects to the developing endocrine or immune system [18].

We measured for the first time congener-specific polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in blubber biopsies obtained from healthy, free-ranging, newly weaned, fasting NES pups from the colony of Año Nuevo, central California.

MATERIAL AND METHODS

Field techniques

Six northern elephant seal pups of normal body masses (~130 kg) were live-captured for sampling at Año Nuevo (37°06′30″N, 122°20′10″W) within 1- to 8-d postweaning in February and March 2002. Weaning dates were recorded by daily observations of the colony. Pups were captured on the beach with a bag fitted with straps and weighted using a 300 ± 0.2 kg digital scale suspended from a tripod. Pups were
sedated by an intravenous injection of Ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA, USA), 100 mg per 100 kg, and Diazepam (Elkins-Sinn, Cherry Hill, NJ, USA), 5 mg per 100 kg, in the extradural vein. A blubber biopsy extending the full depth of the blubber layer was taken in the lateral pelvic area with a 6 mm biopsy punch (Uni-Punch, Premier Medical, Plymouth, PA, USA) before being wrapped in aluminum foil and stored at −20°C until analysis. Each individual was released within 20 min of initial capture time.

**Chemical analyses**

Blubber samples ranging from 100 to 300 mg were analyzed for over 200 PCB, PCDD, and PCDF congeners at the Institute of Ocean Sciences (Fisheries and Oceans, Sidney, BC, Canada) by high-resolution gas-chromatography/high-resolution mass spectrometry as described elsewhere [6]. Many congeners were not detectable in the samples of NES pups. Total concentrations were calculated as the sum of the concentrations of the congeners that were detectable in at least 50% of the samples. Where congeners were detected in less than 100% but more than 50% of blubber samples, detection limit substitutions were made. Congeners that were detected in less than 50% of the samples were not included in further calculations.

Certain PCB congeners coeluted, such that we present the results for 141 congeners detected in 111 peaks.

**Data analyses**

Total toxic equivalents (ΣTEQs) were calculated for dioxin-like PCBs (nonortho and mono-ortho) as well as PCDDs/PCDFs data by using the most recent international toxic equivalency factors (TEFs) for mammals [19]. Body lipid content was estimated by using the equation established by Noren [20] from body condition measurements in NES pups at weaning

Lipid content (%): 24.0 ± 0.1 · body mass

Polychlorinated biphenyl body burden was then calculated by multiplying PCB concentrations expressed per unit of lipid weight by body lipid mass. This calculation is based on the assumption that PCBs are distributed equally within the body lipids. This hypothesis is supported by the fact that PCB blubber levels do not appear to vary between body areas in NES pups (C. Debier, unpublished data).

**RESULTS AND DISCUSSION**

Contaminant studies in marine mammals often are confounded by the influence of age, condition, body weight, and, in the case of reproductive-age adults, sex [6,21–23]. By selecting healthy animals of known and comparable age (29–36-d-old), body weight (125–145 kg) and condition (36.5–38.5% body fat), we were able to reduce or eliminate these factors that might confound the interpretation of POP results in our study of NES. By sampling recently weaned NES pups, our results reflect the accumulation of POPs in pup blubber that are exclusively of maternal origin (transplacental plus milk-derived), which, in turn, reflects the dietary intake of the mother over time. Given the pelagic feeding habits of adult female NES that spend up to 10 months in the open Pacific Ocean and only a limited time in coastal waters, we conclude that the contaminant levels reported derive largely from the open ocean, representing a global source signal.

<table>
<thead>
<tr>
<th></th>
<th>Conc. per wet wt</th>
<th>Conc. per lipid wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΣPCBs</td>
<td>550 ± 90</td>
<td>700 ± 130</td>
</tr>
<tr>
<td>ΣPCDDs</td>
<td>25 ± 17</td>
<td>32 ± 23</td>
</tr>
<tr>
<td>ΣPCDFs</td>
<td>14 ± 4</td>
<td>17 ± 5</td>
</tr>
<tr>
<td>ΣTEQs</td>
<td>15 ± 3</td>
<td>18 ± 4</td>
</tr>
</tbody>
</table>

**Table 1. Concentrations of total polychlorinated biphenyls (ΣPCBs) μg/kg, polychlorinated dibenz-p-dioxins (ΣPCDDs) ng/kg, polychlorinated dibenzofurans (ΣPCDFs) ng/kg, and toxic equivalents (ΣTEQs) ng/kg in blubber biopsies collected from newly weaned northern elephant seals from Ano Nuevo, central California (USA). Values are expressed on a wet weight and lipid weight basis as mean ± standard deviation**

**Total concentrations of PCBs, PCDDs, and PCDFs in NES pups**

Total PCB (ΣPCBs), PCDD (ΣPCDDs), and PCDF (ΣPCDFs) concentrations were calculated as the sum of the concentrations of the 111, 3, and 2 peaks, respectively, that were detectable in at least 50% of the seal samples. Total concentrations and ΣTEQs, expressed per unit of wet weight and lipid weight, are presented in Table 1. The lipid content of the blubber averaged 79.0 ± 2.5% (per wet wt). ΣPCDDs and ΣPCDFs accounted for 41.1 ± 1.6% and 19.6 ± 7.9% of ΣTEQ, respectively, and nonortho- and mono-ortho–substituted PCB congeners accounted for 25.7 ± 3.5% and 50.5 ± 6.5% of ΣTEQ, respectively. Details on TEQ calculated values for individual congeners are shown in Table 2.

Polychlorinated biphenyl levels in NES were considerably lower than those reported for other pinniped species that are resident in the Pacific coastal waters of North America throughout the year. For example, blubber PCB levels in NES pups ranged from 10 to 2,000 times less than those reported in California sea lion blubber [7,8]. However, one must underline that these sea lion samples were obtained from diseased, stranded individuals, and that some of these animals had a very low blubber fat content, which undoubtedly resulted in increased PCB concentrations in blubber. Blubber PCB levels in NES pups were 1.5 times lower than those reported in three- to six-week-old, healthy, biopsied harbor seal pups from the relatively remote Queen Charlotte Strait (50°44′N, 126°49′E), British Colombia, Canada [9]. By contrast, they were more than 25 times lower than those reported in harbor seal pups (same age class) from the industrialized Puget Sound (47°13′N, 122°40′E), Washington (USA) [9]. These results collectively suggest that NES seals are exposed to relatively low levels of POPs, which we reason reflects an open ocean contaminant signal.

Kajiwara et al. [7] reported PCB levels in the blubber of 4 NES yearlings that were stranded alive along the coast of central and northern California and did not survive. Polychlorinated biphenyl levels were 7 to more than 80 times higher than in our pups, likely reflecting an influence of starvation (blubber lipid concentrations ranged from 18 to 90% per wet wt). The POP levels increase by a factor of 2 in the blubber of gray seals sampled at Sable Island (Canada) between weaning and the end of their first year of life, this being attributed to the declining blubber weight as the weaned pups underwent a fasting period as they learned to feed on their own [24]. Another potential explanation for the higher blubber PCB concentrations in one-year-old NES may be related to their feeding ecology. The NES generally remain in coastal waters during their first year of life, moving northward where they are
Table 2. Mean concentrations, toxic equivalency factors (TEF), and calculated toxic equivalents (TEQ) of individual polychlorinated bienzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and nonortho and mono-ortho polychlorinated biphenyls (PCBs) in blubber biopsies collected from newly weaned northern elephant seals from Año Nuevo, central California (USA). Values are expressed on a lipid weight basis.

<table>
<thead>
<tr>
<th>Congener</th>
<th>Conc. (ng/kg)</th>
<th>TEF*</th>
<th>TEQ (ng/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCDDs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,3,6,7,8-hexaCDD</td>
<td>7</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-heptaCDD</td>
<td>8</td>
<td>0.01</td>
<td>0.08</td>
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<tr>
<td>Octa-CDD</td>
<td>17</td>
<td>0.0001</td>
<td>0.0017</td>
</tr>
<tr>
<td>PCDFs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4,7,8-pentaCDF</td>
<td>7</td>
<td>0.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Octa-CDF</td>
<td>10</td>
<td>0.0001</td>
<td>0.001</td>
</tr>
<tr>
<td>Nonortho PCBs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4,4',5-tetraCB (PCB-81)</td>
<td>12</td>
<td>0.0001</td>
<td>0.0012</td>
</tr>
<tr>
<td>3,3',4,4'-tetraCB (PCB-77)</td>
<td>60</td>
<td>0.0001</td>
<td>0.006</td>
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<tr>
<td>3,3',4,4',5-pentaCB (PCB-126)</td>
<td>41</td>
<td>0.1</td>
<td>4.1</td>
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<tr>
<td>3,3',4,4',5,5'-hexaCB (PCB-169)</td>
<td>60</td>
<td>0.01</td>
<td>0.6</td>
</tr>
<tr>
<td>Mono-ortho PCBs</td>
<td></td>
<td></td>
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<tr>
<td>2,3,3',4',4'-pentaCB (PCB-105)</td>
<td>12,500</td>
<td>0.0001</td>
<td>1.25</td>
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<tr>
<td>2,3,3',4',5-pentaCB (PCB-114)</td>
<td>1,440</td>
<td>0.0005</td>
<td>0.72</td>
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<tr>
<td>2,3,3',4,4',5-pentaCB (PCB-118)</td>
<td>54,000</td>
<td>0.0001</td>
<td>5.4</td>
</tr>
<tr>
<td>2,3,3',4,4',5-hexaCB (PCB-123)</td>
<td>3,900</td>
<td>0.0001</td>
<td>0.039</td>
</tr>
<tr>
<td>2,3,3',4',5,5'-hexaCB (PCB-156)</td>
<td>1,960</td>
<td>0.0005</td>
<td>0.98</td>
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<tr>
<td>2,3,3',4',5,5'-hexaCB (PCB-157)</td>
<td>1,750</td>
<td>0.0005</td>
<td>0.875</td>
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<tr>
<td>2,3,3',4',5,5'-hexaCB (PCB-167)</td>
<td>2,140</td>
<td>0.0001</td>
<td>0.021</td>
</tr>
<tr>
<td>2,3,3',4',5,5'-heptaCB (PCB-189)</td>
<td>150</td>
<td>0.0001</td>
<td>0.015</td>
</tr>
</tbody>
</table>

\* From Van den Berg et al. [19].

thought to feed in the neritic zone [25]. Their contamination status, therefore, may be more representative of coastal contamination sources as opposed to the situation with pups, which are exposed to transplacental and milk-derived contaminants from their ocean-feeding mothers. Further research into the effects of fasting and body condition on contaminant concentrations in pinnipeds, and the influence of the life history-based feeding habits of the NES, will help to provide a better characterization of POP sources to this phocid species.

The PCB concentrations in the blubber lipids of our NES were similar to those reported in the milk lipids of NES in another study [15]. This is in accordance with the literature on Canadian gray seals, where similar contaminant levels in milk lipids and pup blubber lipids were reported [26,27].

We estimated the total PCB body burden of our NES pups to average 35 ± 8 mg, which corresponds to the quantity of PCBs that are transferred via transplacental and milk transfer by the mother to her offspring following one cycle of gestation and lactation. This burden lies in the range of the total estimated PCB burdens reported for much smaller gray seal pups (8.8–58.9 mg) from the United Kingdom [28]. When considering the entire colony of Año Nuevo (2,250 births per year in 1991 [29]), we estimate that 80 g of PCBs are transferred each year from lactating females to the next generation.

The XPCDD levels in NES pups were 3 to 9 times lower than those reported in harbor seals from Washington state and British Columbia [9]. XPCDF levels in NES pups were 1.5 times lower than those found in harbor seal pups from British Columbia. Perhaps surprisingly, our NES were more than 1.5 times more PCDF-contaminated than harbor seals from the industrialized Puget Sound [9]. This pattern may result from differences in the contaminant sources, food chain biomagnification, or, more importantly, contaminant metabolism and excretion. The relatively low PCDF levels encountered in harbor seals from Puget Sound partly may be due to their heavy PCB contamination, which, in turn, may have induced a higher cytochrome P450 1A-associated metabolism and excretion for the planar dioxin–like compounds [9]. Lower relative levels of PCDDs and PCDFs in captive harbor seals compared to their dietary intake was thought to reflect a relatively rapid metabolic removal of these contaminants [18], providing support for the notion of a role for metabolism in shaping the pattern of contaminants in pinnipeds.

Profiles of PCBs, PCDDs, and PCDFs in NES pups

Given the relatively low contamination of NES pups, which we attribute in large part to open ocean sources, one might anticipate a pattern that is dominated by lesser chlorinated congeners that disperse more readily through atmospheric and other processes [9]. However, contrary to our expectations, the PCB profile (by homologue group) in NES pups mainly was composed of penta-, hexa-, and hepta-chlorobiphenyls, which represented more than 90% of total PCB concentration (Fig. 1). This profile is very similar to the one observed in non-migratory harbor seals from coastal British Columbia and Washington state [9], although a lighter pattern was observed in seals from the more remote site in this latter study.

Adult female NES forage on vertically migrating prey in the offshore mesopelagic zone, where NES conduct dives at depths of 300 to 800 m. Several major prey items that have been identified by stomach lavage include bioluminescent cephalopods inhabiting the darkness of the bathyepelagic zone [30]. The deep-sea nature of the adult female NES diet, therefore, partly may explain the relative dominance of higher-chlorinated congeners and the low occurrence of lesser-chlorinated congeners. Indeed, a relatively higher contribution of heavier PCB components has been observed in deep-sea organisms as compared to surface species, as a consequence of the sinking of PCB-bound particles [31–33].
CONCLUSION

This pilot study suggests that, healthy, newly weaned NES have a low-level body burden of PCBs, PCDDs, and PCDFs, which we attribute to open ocean sources. The dominance of the higher-chlorinated PCB congeners probably ensues from the deep-sea diet of NES adult females. Given the importance of atmospheric transport in delivering POPs to remote regions [34,35], the prevailing westerlies from Asia, and the extent to which adult female NES forage in the deep waters of the North Pacific Ocean, we propose that the NES provides a unique measure of global environmental contamination.

Despite the fact that our NES pups are less contaminated than other pinnipeds from the west coast of North America, they still may be at risk for low-level, chronic exposure to PCBs, PCDDs, PCDFs, and other endocrine-disrupting chemicals. The period during the postweaning fast may represent an especially sensitive time, when blubber POPs are released into circulation and the animals lose approximately 25% of their initial fat stores [20].

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