LOW-LEVEL EXPOSURE TO PCBS IS ASSOCIATED WITH IMMUNE AND ENDOCRINE DISRUPTION IN NEONATAL HARBOR SEALS (*Phoca vitulina*) FROM THE CALIFORNIA COAST

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Introduction
Harbor seals (*Phoca vitulina*) appear to be sensitive to a wide range of toxic effects following exposure to PCBs and other dioxin-like compounds (*i.e.*, PCDDS, PCDFs) through the food chain. Results of feeding studies using captive harbor seals indicate that reproductive impairment (1), immunotoxicity (2, 3), and alterations of thyroid hormone and retinol (vitamin A) homeostasis (4) occur in adult harbor seals with low to moderate PCB burdens (mean ∑PCB ~17 to 25 ppm/lipid basis). The threshold levels at which PCB-induced effects may occur in young seals are not well understood. Since neonatal harbor seals appear to be less capable of metabolizing and excreting PCB congeners than adults (5,6), perinatally-exposed pups may be at special risk, even at low dose levels, to immunotoxicity, thyroid toxicity, and other adverse effects of these compounds.

Materials and Methods:
Sampling Approach. Peripheral blood and blubber samples were obtained from 11 harbor seal (HS) pups that had stranded as newborns along the central California coast and were housed at a rehabilitation facility. All pups were presumably exposed primarily *in utero*. Whole blood and serum samples were collected for immune function and endocrine studies after approx. 3 weeks of rehabilitation when pups were ~4 weeks old. Health status was evaluated at the same time point to control for disease. Blubber biopsies (1g) were taken from the dorsal region using a 6 mm dermal punch. Larger samples (10g) were collected from the mid-ventral region at necropsy, and frozen at -40°C until analysis. **Contaminant Analyses.** Concentrations of PCB congeners including 12 non- and mono-*ortho* substituted PCBs were analyzed in blubber by GC/ECD using a porous graphitic carbon (PGC) column as described elsewhere (7). Toxic equivalency factors (TEFs) recently proposed by WHO/IPCS for 12 PCBs in mammals (8) were used to calculate the contribution of TCDD toxic equivalents (TEQ) of the PCB congeners. **Lymphocyte Proliferative Responses to Mitogens.** Immune function was evaluated using a whole blood assay to measure *in vitro* lymphocyte proliferative responses to Conconavalin A (Con A), phytohemagglutinin (PHA) and poke weed mitogen (PWM) (9). Heparinized blood was shipped to the lab overnight at room temperature. Viabilities of lymphocytes were >95%. Diluted whole blood was cultured in triplicate wells with 3 concentrations each of Con A (0.4-25.6 μg/ml), PHA (2-18 μg/ml), and PWM (1-100 μg/ml). Plates were incubated 4-6 days and pulsed 18h before harvest with tritium-labeled thymidine. Proliferation was quantified by measuring the incorporation of ^{3}H-Trd at the

optimal mitogen concentration on the average peak day of response (day 4 for Con A and PHA and day 5 for PWM). Results are expressed as the net mean cpm ± SD of triplicate wells.

Thyroid Hormone Levels. Standard radioimmunossays (RIA) using commercially available kits (Amerlex-MAB, Amersham, England) were used to measure total and free thyroxine (T^4 ,FT^4) and free triiodothyronine (FT^3) concentrations in seal serum. Total triiodothyronine (T^3) was measured in seal serum using a standard T^3 assay previously applied and validated in domestic animals (10).

Statistical Analysis. Mean concentrations ± SD and ranges were calculated for contaminant levels and outcome markers. Data were examined for normalcy and where appropriate, log transformed. The Student's t test, Mann-Whitney Wilcoxon test, and chi square analysis were used to test for gender and regional differences in the distribution of markers. Bivariate Pearson correlation coefficients were calculated to determine the number and strength of correlations between groups of markers. Relationships between exposure and outcome measures were evaluated using chi square statistics, bivariate correlation statistics, and ANOVA and MANOVA with Bonferroni criteria applied. Multiple regression analyses were used to identify mathematical models for the relationships between contaminant levels and outcome measures.

Results and Discussion

Although mean ∑PCB concentrations in the pups were relatively low (3.3 μg/g, lipid basis), levels of the potent non-*ortho* PCBs 77, 81, and 126 contributed 46% of the ∑TEQ (88 pg/g, lipid basis) and were higher than levels reported in adult harbor seals from heavily polluted areas (13,14) and immunosuppressed captive harbor seals following dietary PCB exposure (2,3) (Figure 1). These higher levels may reflect the lower MC-type monooxygenase activities observed in neonatal seals (5,6). For most mammals, lymphocyte proliferative responses to polyclonal mitogens are critical during the neonatal period when humoral (antibody-mediated) and primary antigen responses may be poorly developed. At ~4 weeks of age, the pups exhibited relatively strong lymphocyte proliferative reponses to Con A and moderate responses to PWM and PHA, which is consistent with the pattern of responses in surviving rehabilitated HS pups (11) and healthy juvenile HS (2). Serum thyroid hormone levels in the pups were typical of levels reported in neonatal seals (12). Health status did not significantly affect contaminant levels or the markers of effect, although healthy pups had lower burdens of ∑PCB and ∑TEQ and showed stronger immune responses to Con A and PWM.

Regression analysis revealed significant associations between reduced immune responses in the pups and increasing levels of ∑TEQ (Con A: R^2= 0.42, p=0.04; PHA: R^2= 0.45, p=0.03), suggesting an effect of planar PCBs on T cell function. Reduced thyroid hormone levels in the pups were associated with increasing levels of ∑TEQ (FT^4: R^2= 0.53, p=0.02) and ∑PCB (T^3: R^2= 0.74, p=0.01), suggesting the involvement of planar and nonplanar congeners. Taken together, these data suggest that harbor seal pups may be at increased risk for *Ah* receptor-mediated as well as *Ah*-independent effects of PCBs following perinatal exposure. While the data presented here are preliminary, they are consistent with the results of laboratory studies demonstrating similar effects of PCBs in animals (15,16), and with captive studies showing that dietary organochlorine exposure can cause immune impairment and alterations of thyroid hormone homeostasis in adult harbor seals (2-4). We conclude that the threshold for immune and endocrine-disrupting effects of bioactive PCB congeners may be quite low (∑PCB ~3 ppm, lipid basis) for neonatal harbor seals.

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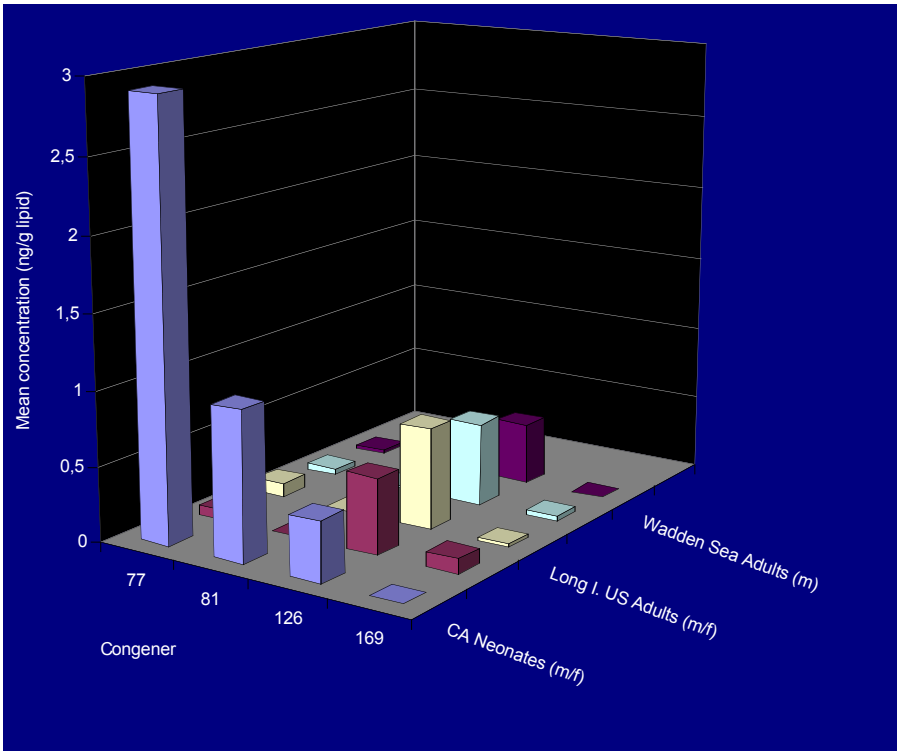

Figure 1. Non-Ortho Planar PCB Concentrations in Blubber of Neonatal and Adult Harbor Seals