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CONTAMINANT LOADS AND HEMATOLOGICAL CORRELATES IN THE HARBOR SEAL (*PHOCA VITULINA*) OF SAN FRANCISCO BAY, CALIFORNIA

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An expanding body of research indicates that exposure to contaminants may impact marine mammal health, thus possibly contributing to population declines. The harbor seal population of the San Francisco Bay (SFB), California, has suffered habitat loss and degradation, including decades of environmental contamination. To explore the possibility of contaminant-induced health alterations in this population, blood levels of polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (DDE), and polybrominated diphenyl ethers (PBDEs) were quantified in free-ranging seals; relationships between contaminant exposure and several key hematological parameters were examined; and PCB levels in the present study were compared with levels determined in SFB seals a decade earlier. PCB residues in harbor seal blood decreased during the past decade, but remained at levels great enough that adverse reproductive and immunological effects might be expected. Main results included a positive association between leukocyte counts and PBDEs, PCBs, and DDE in seals, and an inverse relationship between red blood cell count and PBDEs. Although not necessarily pathologic, these responses may serve as sentinel indications of contaminant-induced alterations in harbor seals of SFB, which, in individuals with relatively high contaminant burdens, might include increased rates of infection and anemia.

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Fish-eating seals are long-lived, maintain large adipose deposits, and occupy high trophic levels in the marine food web thus potentially accumulating high levels of lipophilic contaminants (Boon et al., 1987; Tanabe, 1988; Young et al., 1998). Among marine mammals, harbor seals (*Phoca vitulina*) typically experience relatively high exposures to such chemicals, as they frequent coastal habitats often altered by industrial centers, heavy marine traffic, and ubran and agricultural runoff. Harbor seals are nonmigratory and use bays and estuaries for resting, foraging, and reproduction, and thus are excellent indicators of the health of estuarine systems (Brown & Mate, 1983; Kopec & Harvey, 1995; Grigg et al., 2002).

Available reports indicate that harbor seals were numerous in San Francisco Bay (SFB), California, before the late 1800s; intensive hunting most likely led to the dramatic declines observed by the 1920s (Fancher, 1979; Grigg, 2003). Systematic surveys in SFB were not conducted until the 1970s, when they indicated that harbor seals had suffered extensive habitat loss and degradation, including food web contamination. Numbers stabilized (at around 400 to 500 seals) throughout the 1970s and 1980s (Risebrough et al., 1980; Allen, 1991). During recent decades, the harbor seal population in SFB has been increasing at a slower rate than most others along the Pacific Coast (Harvey et al., 1990; Stewart & Yochem, 1994; Sydeman & Allen, 1999). Along with disturbance, habitat loss, and changes in prey availability, pollution is a likely cause for this apparent delayed recovery (Reijnders, 1986; Harvey et al., 1990; Grigg, 2003).

Halogenated organic contaminants, in particular, could be especially important. They have been suggested as factors altering health in several marine mammal species (Addison, 1989; Tanabe et al., 1994), and contaminant-induced immunosuppression may have contributed to the high mortality observed in several marine mammal populations during recent morbillivirus epizootics (Hall et al., 1992; Aguilar & Borrell, 1994). Organochlorines such as polychlorinated biphenyls (PCBs) and DDT and its metabolites are ubiquitous environmental contaminants and are associated with various physiological disorders, such as impaired immunological function and reproductive success in harbor seals (Reijnders, 1980, 1986; Ross et al., 1996), decreased circulating vitamin A and thyroid hormone levels in juvenile habor and gray (*Halichoerus grypus*) seals (Brouwer et al., 1989; Jenssen et al., 2003), depressed humoral immune responses in northern fur seal (*Callorhinus ursinus*) pups (Beckmen et al., 2003), and decreased lymphocyte responses in bottlenose dolphin (*Tursiops truncatus*; Lahvis et al., 1995). In addition, Neale et al. (2002) found that model PCBs depressed proliferation of harbor seal T lymphocytes in vitro. Brominated flame retardants are a novel class of environmental contaminants; within this group, the polybrominated diphenyl ethers (PBDEs) are currently used in large quantities, disperse similarly to PCBs and DDT, and bioaccumulate and biomagnify (Sellstrom et al., 1993; Meerts et al., 2001). Adverse effects of PBDEs include hepatotoxicity, embryotoxicity, and thyroid hormone alterations; some congeners also are estrogen receptor agonists (Meerts et al., 2001; Hall et al., 2003). Similar to PCBs, PBDEs exhibit dioxinlike

Ah-receptor-mediated induction of cytochrome P-450 1A1 and 1A2 enzymes (Meerts et al., 2001).

San Francisco Bay has been heavily contaminated with organochlorines for decades (Jarman et al., 1997; SFEI, 1998, 1999). Although progress has been made in reducing new inputs to the bay, recent analyses of sediments and water for PCBs indicated no significant decreases during recent decades and only modest declines for DDTs (Jarman et al., 1997; SFEI, 1998). PBDE levels are rising in many parts of the world (Meironyte et al., 1999; Noren & Meironyte, 2000). PBDEs have been recently identified in SFB water, sediments, and biota, and are likely increasing there also (Oros & David, 2002; She et al., 2002).

Here, free-ranging seals were sampled in SFB and analyzed for blood levels of organohalogens, including 10 congeners of PCBs, 3 congeners of PBDEs, and dichlorodiphenyldichloroethylene(DDE). Temporal changes in blood contaminant levels in seals were assessed via a comparison of our data to levels determined in SFB seal blood a decade earlier (1989–1992). Contaminant-induced health alterations were investigated in the SFB harbor seal population through analysis of several key hematological parameters: white blood cell count and differential, red blood cell count, hemoglobin, hematocrit, albumin, globulins, and serum iron. It was hypothesized that contaminant exposure in harbor seals of SFB alters health, and it was predicted, accordingly, that values for blood parameters would be systematically related to contaminant concentrations.

MATERIALS AND METHODS

Sample Collection and Storage

Harbor seals were live-captured near primary haul-out sites of SFB during summer months (July and August) of 2001 and 2002, using beach seine and tangle net techniques (Yochem et al., 1987; Jeffries et al., 1993; NMFS Scientific Research Permit Nos. 555–1565, 373–1575). Seals were physically restrained, and gender, mass, and standard length (SL) were recorded. Blood samples were collected from 22 females (2 pup, 15 yearling/subadult, 5 adult) and 13 males (3 pup, 5 yearling/subadult, 5 adult). Blood was drawn from the extradural venous sinus into sterile evacuated blood collection tubes containing ethylenediamine tetraacetic acid (EDTA; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Samples for hematology were kept cool and analyzed within 24 h of collection. Samples for analytical chemistry were transferred to glass vials with tetrafluoroethylene (TFE)-lined screw caps and stored frozen.

Target Chemicals and Standardization

Specific PCB and PBDE congeners were selected for analysis based on toxicity information and reported abundances in the environment and in biota (Van den Berg et al., 1998; Valoppi et al., 2000). For PCBs, these included

CBs (IUPAC) 128, 153, 156, 167, 169, 170, 180, 189, 195, 206, and 209. Selected PBDE congeners represented the major groups, including a tetrabromodiphenyl ether (BDE 47), a pentabromodiphenyl ether (BDE 99), and a hexabromodiphenyl ether (BDE 153). For all sample runs, CBs 14, 65, and 166 were used as surrogates and CBs 30 and 204 were used as internal standards. PCBs and DDE were purchased from AccuStandard, Inc. (New Haven, CT), and PBDEs were purchased from Cambridge Isotope Laboratories (Andover, MA). All solvents (highest analytical grade) were purchased from Fisher Scientific (Pittsburgh, PA).

Sample Extraction and Cleanup

Sample extraction generally followed procedures of Young et al. (1998). Briefly, whole blood samples were thawed and briefly vortexed. Three surrogates (in methanol) were mixed into a 5-ml aliquot of blood from each seal and allowed to equilibrate for 1 h. Acetonitrile (10 ml) was then added and the sample was briefly vortexed, then sonicated for 4 min to ensure membrane disruption and homogenization of the sample. Next, 15 ml of Nanopure-DI water was added along with 5 ml of a solution of 10% isooctane in hexane (isooctane/hexane). The sample was mixed on a wrist-action shaker for 1 h and then centrifuged at 35 x g for 5 min. The supernatant was collected into a 15-ml centrifuge tube. The aqueous layer was then reextracted twice more, as already described. The supernatant was collected and concentrated to approximately 1 ml under N_2 gas.

The sample was transferred to a 1-g Maxi-clean Florisil SPE cartridge (Alltech, Deerfield, IL) that had been preconditioned with 20 ml isooctane/hexane. Three successive rinses, each using 1 ml isooctane/hexane and brief vortexing, were preformed to ensure quantitative transfer of the sample, which was then eluted with 8 ml isooctane/hexane. The final volume was concentrated to approximately 100 μl under N_2 gas, spiked with 30 ng each of the internal standards (in isooctane), and analyzed by electron capture detection gas chromatography (ECD-GC).

Gas Chromatography

Quantitative analysis of samples was performed using a Hewlett Packard (HP) 5890 series II GC equipped with a ^{63}Ni electron-capture detector. Injections were made in splitless mode using an HP model 7673 autosampler. Instrument set points and data acquisition were under the control of HP 3365 Chemstation software. The injection port and detector temperatures were 275 and 310°C, respectively. The injection volume was 2 μl, and the purge time was 1.2 min. Analyses were conducted on a fused-silica Rtx-5MS capillary column (60 m × 0.2 mm ID × 0.25 mm film thickness; Restek, State College, PA). Helium (1.4 ml/min) was the carrier gas, and N_2 was the ECD makeup gas. After sample injection at 100°C, the temperature was held constant for 1.5 min, then increased at 15°C/min to 165°C, followed by 20°C/min to 285°C. The final temperature was maintained until a total run time of 114 min

had been completed. Multilevel internal standard calibrations using a minimum of five standard concentrations were used as the basis for quantification.

Method Performance and Quality Control

A standard solution including all analytes, surrogates, and internal standards was reanalyzed every sixth or eighth injection to ensure optimal instrument performance. Reagent blanks (Nanopure water) were processed in a manner identical to whole blood samples and were included in each batch of nine duplicated samples. Surrogates were monitored in samples, blanks, and standard solutions to ensure that recoveries were at acceptable levels. The method limit of detection and quantification (LOD and LOQ, respectively) for each target analyte was determined as follows. For each analyte, the six lowest (nonzero) mean values (i.e., of duplicate samples) were identified. The LOD was calculated as 3x the standard deviation of these 6 values, and the LOQ was established at 3x the LOD. The LOD values ranged 0.003 to 0.064 ppb wet weight, and all LOQ values (0.010–0.192 ppb) were below the target of 1 ppb.

Peak identities were confirmed by separate analysis of several samples via gas chromatography–mass spectroscopy (GC-MS), using an HP 6890 GC coupled to an HP 5972 mass-selective detector (MSD; Agilent Technologies, Palo Alto, CA). The GC was equipped with a ZB-50 capillary column (30 m × 0.25 mm ID × 25 μm film thickness; Phenomenex, Torrance, CA). Helium was the carrier gas (0.6 ml/min). Injections were made in splitless mode using an HP model 7673 autosampler. Column and GC conditions were identical to those already described. The MSD was run in both SIM and SCAN modes for identity confirmation and to identify unknown peaks occurring near peaks of interest. The HP MS library confirmed one of the unknowns as the pesticide Mirex, which coeluted with CB-169; therefore, this congener was excluded from quantitative analysis.

Lipid Determination

Blood lipids were measured using the colormetric technique of Frings et al. (1972). Briefly, using 25 μl whole blood from each seal sample, lipids were hydrolyzed with sulfuric acid and then reacted with phospho-vanillin, producing a chromophore (absorption maximum 540 nm). Sample absorption was determined using ultraviolet/visible (UV/Vis) spectroscopy (Lambda 25; Perkin Elmer, Boston), and lipid concentration was quantified using a linear, five-point calibration curve of olive oil standards (0.25–10 mg/ml) that bracketed all analyzed samples.

Hematology

White and red blood cell counts, hemoglobin concentration, and hematocrit were determined using a Vet ABC hematology analyzer (Heska Corp., Fort Collins CO), and differential white cell counts were performed manually on blood smears as described in Jain (1986). A Vet Test 8008 (Idexx Laboratories Inc., Westbrook, ME) was used to analyze serum chemistry panels.

Data Analysis

Age of seals was estimated based on standard length as follows: class 1 ("pup"; M and F < 100 cm); class 2 ("yearling/subadult"; M 101–134 cm, F 101–129 cm); and class 3 ("adult"; M > 135 cm, F > 130 cm). This classification agrees closely with published relationships of standard length and age for known-age harbor seals from British Columbia and Canada (Bigg, 1969; Boulva & McLaren, 1979) as well as age estimates for harbor seals from Prince William Sound, AK, and the Washington/Oregon coast (Krahn et al., 1997).

An index of total fat reserves or general condition was desired. Unfortunately, the index used in previous studies—the simple ratio of mass to SL (Ricker, 1975; Kopec & Harvey, 1995)—results in an age-related bias that systematically overestimates condition of older (longer) animals and underestimates condition of younger (shorter) animals (Figure 1). To correct this problem, a condition index was established based on the assumption that body mass (a three-dimensional parameter) increases proportionally to the cube of body length (a one-dimensional parameter). Specifically, the $\sqrt[3]{\text{mass}}$

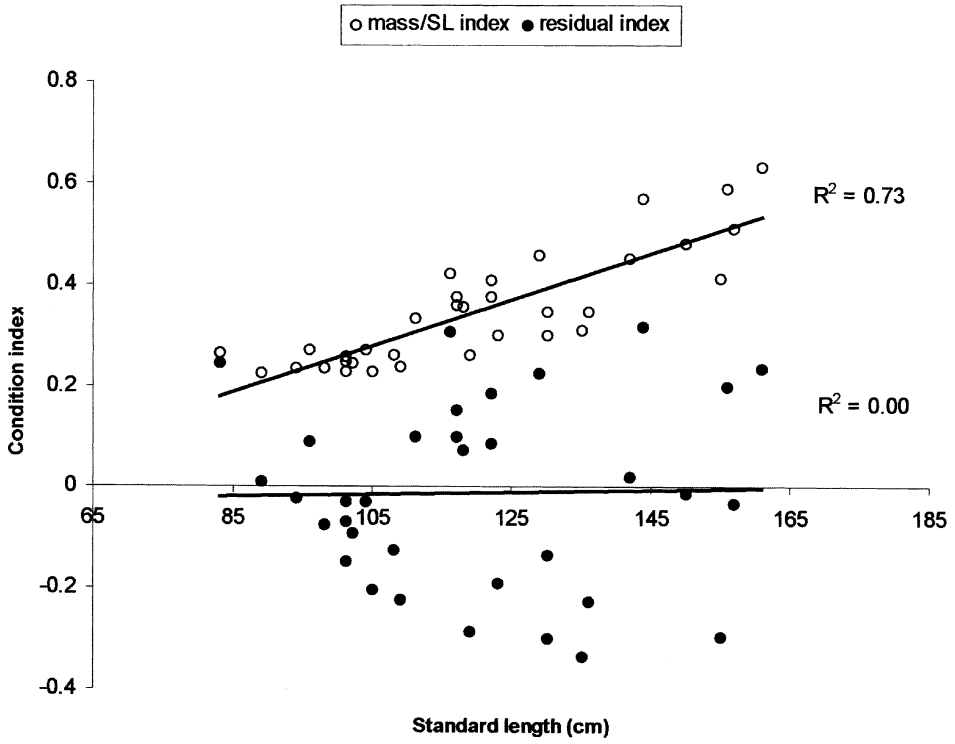

FIGURE 1. Relationships between standard length (SL) of harbor seals (n = 35) and two condition indexes, illustrating that the mass/SL index is highly correlated with SL and therefore age, while the residual condition index $\left[\sqrt[3]{\text{mass}} - (0.0241)\text{SL} + 0.5578\right]$ is not.

was regressed on SL for all seals and the regression line [$\sqrt[3]{\text{mass}} = (0.0241)\text{SL} + 0.5578$] used as the expected value (i.e., of a seal in average body condition). The condition index was defined as the observed $\sqrt[3]{\text{mass}}$ minus expected $\sqrt[3]{\text{mass}}$. All seals were used because no difference in this relationship was found between genders (unpublished data). While it is uncertain that body mass correlates exactly to the cube of SL (Hayes & Shonkwiler, 2001), this assumption resulted in an index that was not biased by (i.e., was not correlated with) SL (Figure 1).

Analyte concentrations determined in whole blood were expressed on wet-weight (ng/g whole blood) and lipid-adjusted (ng/g blood lipid) bases. For each sample, the measured concentrations of individual congeners were totaled to obtain ΣPCB and ΣPBDE values. Before statistical procedures, certain variables were transformed to better approximate normality. Proportions (neutrophils, lymphocytes, and hematocrit) were transformed as arcsin[sqrt(x)] (Zar, 1984). Contaminant concentrations, hemoglobin, albumin, the albumin:globulin ratio, and red blood cell counts were transformed as $\log(x + 1)$.

Hematological parameters may vary in relation to factors such as gender, age, season, and body condition. Only summer samples were included in this study, thus eliminating the possible confounding effect of season. The other factors were incorporated in statistical testing of relationships via analyses of covariance using the General Linear Model (GLM) procedure in SYSTAT (version 9, SPSS, Chicago). Thus, all analyses of hematological parameters included contaminant concentration (ΣPBDE, ΣPCB, or DDE) as well as gender, age class, the interaction of gender and age class (gender x age), and condition index as factors/covariates. These variables were included to control for any potentially confounding effects; however, because sampling was not designed to test for their importance (which would minimally require more even distribution among gender and age groups), lack of statistical significance should not be taken to mean that they do not have an important effect in nature. All statistics reported are from the full, conservative models (i.e., no variables were dropped; direction and strength of relationships determined while controlling for all other variables). The criterion for significance was set at $p < .05$. Sample size was 35 seals for all analyses of DDE and PCBs on hematological variables and 33 for PBDEs, with the exception of serum iron and proteins analyses ($n = 33$ for DDE and PCB models, 32 for PBDE).

RESULTS

Organohalogens in Harbor Seal Blood

Significant linear relationships were observed between wet weight and lipid weight concentrations for ΣPCB, DDE, and ΣPBDE ($R^2 = .96$, .94, and .89, respectively). Although lipid normalization of organohalogen levels changed the scale of measurement (to low ppm range for ΣPCB, DDE and ΣPBDE; Table 1), it did not appreciably affect relationships among variables. Therefore, for economy and comparison with earlier studies (Kopec & Harvey,

TABLE 1. Concentrations (ng/g) of ΣPCB, ΣPBDE, and *p,p′*-DDE in Harbor Seal Whole Blood

Contaminant	Mean	SD	SE	*n*
ΣPCB—wet weight	18.6	18.1	3.1	35
ΣPCB—lipid weight	5334.9	5548.7	951.6	35
ΣPBDE—wet weight	2.8	1.8	0.3	33
ΣPBDE—lipid weight	760.0	530.1	93.7	33
DDE—wet weight	24.3	21.5	3.7	35
DDE—lipid weight	6805.8	5996.2	1028.3	35

1995; Young et al., 1998), the following analyses were based on wet-weight measurements only.

Congener profiles of PCBs and PBDEs generally agreed with relative concentrations of congeners in marine mammal tissues reported in the literature (Boon et al., 1987; Lake et al., 1995; Hong et al., 1996; Ikonomou et al., 2000; Troisi et al., 2001; She et al., 2002). The rank order of PCB congeners was consistent among seals for the PCBs contributing most to ΣPCB: CB 153> 180 > 170 > 128, with each of these four congeners averaging a little more than twice the value of the next largest. CBs 153 and 180 constituted an average of 79% of ΣPCB. The remaining congeners (CBs 156, 167, 189, 195, 206, 209) had average wet weight concentrations <1 ppb, and were more variable in their rankings (which is expected, by chance, for small values). Among the PBDEs, BDE 47 dominated the load in all samples and comprised an average of 84% of ΣPBDE. BDE 99 and BDE 153 levels were relatively low in all cases, with BDE 153 typically less or equal to BDE 99.

The current contaminant data were compared with previous data for SFB harbor seals (Kopec & Harvey, 1995; Young et al., 1998), which were obtained in a very similar fashion; peripheral blood samples were taken from free-ranging seals and analytical techniques were nearly identical. The earlier PCB data were limited to wet-weight, whole-blood concentrations of PCB congeners in a relatively small sample of male and female seals of age classes 2 and 3 collected during 1991–1992 (Young et al., 1998). For these two age classes, (log-transformed) sums of the six PCB congeners measured in both studies were compared statistically using the GLM procedure as already described. The model included study, gender, age class, gender x age, and condition (condition indexes as described earlier were calculated for the 1991–1992 sample). PCB levels in the 1991–1992 samples (mean sum of 6 PCBs = 27.33 ppb wet weight) were significantly greater than those in 2001–2002 samples (mean = 17.73 ppb wet weight). Gender also was a significant factor (M > F). The PCB congener pattern was the same between the two studies (CB153 > CB 180 > CB 170 > CB 128; Figure 2). DDE residues were determined in blood plasma of harbor seals sampled during 1989–1990: 36 (88%) of 41 samples had detectable levels (detection limit = 5 ppb wet weight) and the mean of these was 12.6 ± 1.5 (SE) ppb (Kopec & Harvey, 1995). In the present study, all samples had DDE residue levels >5 ppb and the average

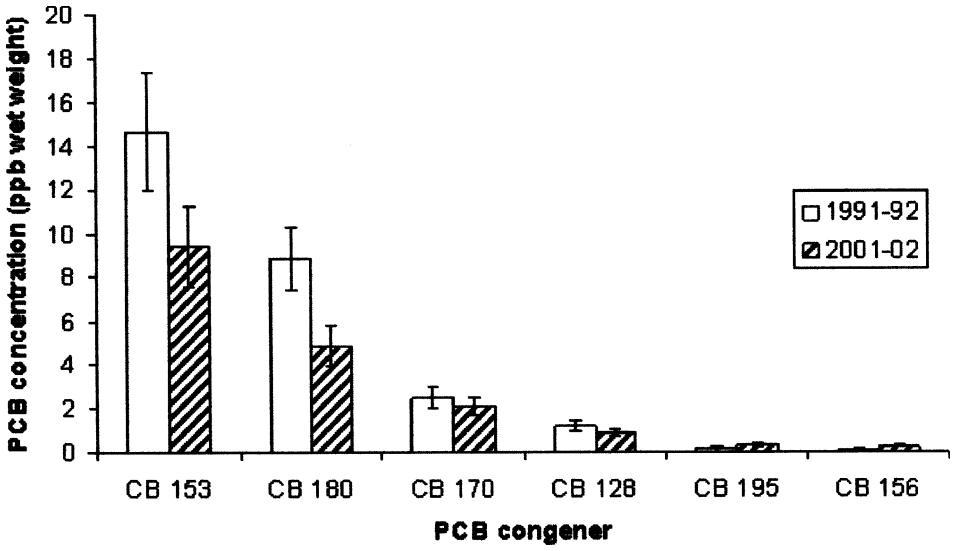

FIGURE 2. Concentrations (mean ± SE) of six PCBs determined in whole blood of subadult and adult harbor seals from San Francisco Bay, California sampled during 1991–1992 (Young et al., 1998; $n = 14$) versus 2001–2002 (Present study; $n = 30$).

concentration (24.27 ± 3.68) was significantly greater. However, because of the different blood compartments analyzed, it was not possible to determine if this difference reflected an actual increase in DDE in seal tissues during the past decade.

Contaminants and Hematology

Leukocyte count was positively significantly related to ΣPBDE, ΣPCB, and DDE. The use of a linear test (i.e, assuming linearity) was conservative given that these relationships appeared nonlinear (i.e., threshold or exponential; e.g., PCBs vs. leukocytes, Figure 3). None of the factors examined produced significant results in analyses of neutrophils and lymphocytes. However, the proportion of band (immature) neutrophils was nearly three times greater in seals with above-average leukocyte counts and contaminant levels (mean = 0.22 ± 0.16 SE, $n = 9$) compared with seals of average (or below-average) leukocyte and contaminant levels (mean = 0.08 ± 0.05 SE, $n = 26$). Total leukocytes also increased significantly with condition index. This relationship was greatly influenced by two individuals (one male pup and one female yearling/subadult), and removal of these two samples from statistical analysis resulted in loss of significance of condition as a factor of leukocyte count in the PCB model, whereas PCB concentration remained a significant factor.

Erythrocyte count was significantly inversely related to ΣPBDE; a similar (nonsignificant) inverse relationship was found for ΣPCB and DDE. Age class was a significant factor of erythrocyte counts ($1 > 2 > 3$), with roughly even

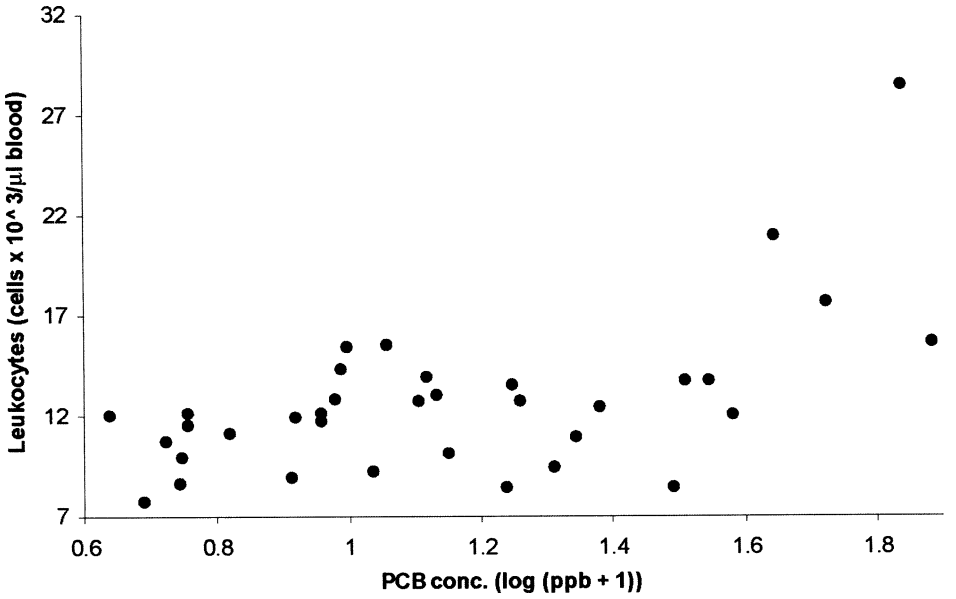

FIGURE 3. Total leukocyte count as a function of (wet weight) ΣPCB ($n = 35$).

decreases among classes. Significant interaction (gender x age) was observed in the analyses of hemoglobin and hematocrit; therefore, these models were decomposed and run for males and females separately, with age class, contaminant concentration, and condition index as factors. For males, age class ($1 \geq 2 > 3$) was a significant factor of both hemoglobin and hematocrit in all three contaminant models. For females, no significant factors were determined for either parameter, although class 1 females tended to have greater values.

In serum chemistry analyses, albumin and serum iron were highly variable with no significant factors. Total globulin was significantly positively correlated with age class; this was expected (Thomas, 2000). The albumin:globulin ratio was significantly inversely correlated with age; this was due to the significant increase with age in globulins (albumin showed no relationship with age class).

DISCUSSION

Current Contaminant Levels in SFB Seals

The relatively high contaminant levels in SFB seals remain cause for concern. Although the comparison of PCB levels in the present study with those reported by Young et al. (1998) indicated a decline in PCB loads in harbor seals, this analysis was limited by the reduced number of congeners compared and small sample sizes of limited age classes. In fact, DDE and PCB levels in the present study were roughly in the same range as most values obtained for other tissues (liver, brain, muscle, blubber) of dead harbor seals recovered

from SFB in the mid-1970s (Risebrough et al., 1980). PBDE residues determined in blubber from a small sample ($n = 10$) of stranded dead harbor seals collected along the SFB shoreline during 1989–1998 (She et al., 2002) were similar to levels (lipid basis) reported here.

ΣPCB values in the present study (mean = 18.6 ppb wet weight) equaled the estimated 18 ppb ΣPCB average in whole blood of captive seals fed PCB-contaminated Wadden Sea fish (Young et al., 1998; Boon et al., 1987). Indeed, the comparison is conservative, given the greater number of congeners analyzed by Boon et al. (1987). Seals in that study and a similar follow-up experimental feeding study had reduction in serum retinol and thyroid hormone levels, and impaired immune function associated with PCB loads (Brouwer et al., 1989; De Swart et al., 1994; Ross et al., 1996). Kopec and Harvey (1995) also found depressed serum retinol levels in SFB harbor seals, indicating possible PCB toxicity. Our data also were comparable with other reported whole-blood contaminant concentrations for free-ranging marine mammals for which organochlorine loads were associated with impaired immunological responses and unexplained population declines (Lahvis et al., 1995; Beckmen et al., 2003).

Hematology and contaminant levels

Overall, hematological parameters in this study were generally within normal ranges for this species (Reidarson et al., 2000), and all seals appeared clinically healthy at time of capture. Nevertheless, our examination of blood health parameters as they related to contaminant levels provided some indication that current environmental exposures of harbor seals to persistent organohalogens in SFB may be sufficiently high to produce adverse health effects, at least in those individuals with the greatest burdens.

The relationships between total leukocyte count and loads appeared either exponential or reflecting threshold effects, such that below a certain concentration of contaminant (e.g., around 1.6 ppb ΣPCB), leukocyte counts remained relatively unchanged (around 12,000 cells/μl). At contaminant concentrations above the apparent thresholds, leukocyte counts increased markedly (Figure 3). Although the overall average leukocyte count (12,650 ± 3890 SD, $n = 35$) was similar to reported values for rehabilitated wild pup and yearling harbor seals (12,700 ± 3550 and 12,000 ± 3770, respectively; Reidarson et al., 2000), the average value among seals with the greatest blood levels of pollutants was considerably greater (16,600 ± 5275, $n = 9$).

In this high-contaminant/high-leukocyte group, a relative prominence of immature neutrophils was also observed, indicative of a mild "left shift" associated with inflammation (Smith, 2000). Taken together with the increase in leukocytes with increasing organohalogen levels, this finding indicates the possibility of increased infection rates in seals with greater contaminant loads in SFB (Zinkl & Kabbur, 1997; Schultze, 2000; Smith, 2000). Inflammation occurring in response to mediators related to infections agents or to products of tissue injury or necrosis may be experienced as acute or chronic, and is generally associated with declines in serum iron and albumin, an increase in

globulins, and a corresponding decrease in the albumin:globulin ratio, relative to normal levels (Andrews & Smith, 2000; Thomas, 2000). However, plasma protein concentrations are also influenced by factors such as diet, stress, hydration status, etc. Here, serum iron and plasma proteins were not found to correlate with contaminant concentrations. It is possible that the reduced sample size for these analyses prevented detection of more subtle effects and/or other relationships (e.g., the expected increase in globulins with age) confounded our analyses.

Depressed red-cell parameters in seals with greater loads also could reflect inflammatory disease (Gossett, 2000; Waner & Harrus, 2000), and such anemia might negatively affect the ability to respond to oxygen demands, such as during foraging dives (Harvey, 1997). Our results indicated that such effects might be most prominent in adult males and could be exacerbated if male harbor seals tend to make deeper dives than females in SFB. Previous researchers speculated that depressed erythrocyte values and thus reduced oxygen capacity of SFB seals might be expected in the relatively shallow SFB, because feeding dives might not sufficiently stimulate increased oxygen capacity typical of pinnipeds in deeper environments (Kopec & Harvey, 1995). However, SFB harbor seals regularly forage outside the bay in deeper waters (Green et al., 2003). Furthermore, that hypothesis cannot explain the negative relationship between erythrocyte counts and ∑PBDE determined in this study. Although not significant, ∑PCB and DDE had similar inverse relationships to erythrocyte count, and hemoglobin and hematocrit also decreased with increasing ∑PCB, ∑PBDE, and DDE. Reduced plasma volume of dehydration (experienced to some degree by seals during capture and sampling) and the associated increase in red-cell density can mask anemia, and this may have reduced our ability to detect these relationships (Watson, 2000). The observed decreases in erythrocyte parameters with age observed here were consistent with previously reported trends in other seals (Geraci et al., 1979; Kuiken, 1985).

Although the few previous studies on health correlates of chronic contaminant exposure in free-ranging marine mammals typically have been based on biased (stranded/dead animals) or very small samples, collectively, they may indicate a causal link. For example, a suite of pathological conditions was documented in necropsied beluga whales (*Delphinaterus leucas*) found dead in the highly polluted St. Lawrence Estuary (Martineau et al., 1994). Decreased lymphocyte responses to mitogen in vitro were correlated with increasing concentrations of PCBs and DDT in five bottlenose dolphins from the west coast of Florida (Lahvis et al., 1995). Few studies investigated relationships between contaminant levels and hematological parameters. Hematology was conducted on SFB seal samples (1989–1992) previously by Kopec and Harvey (1995), although they did not attempt to relate blood health parameters to contaminant levels. Average values for white and red cell parameters for those seals were very similar to values obtained here—erythrograms were somewhat lower, and leukocyte counts higher, than values reported for other wild seals (McConnell & Vaughan, 1983); Kopec and Harvey (1995) speculated that

either disease or environmental contamination was responsible. In a captive feeding study of harbor seals, De Swart et al. (1995a, 1995b) reported greater leukocyte counts in the group exposed to greater organochlorine levels. Similarly, Beckmen et al. (2003), working with northern fur seal pups, found a positive correlation between contaminant loads and total leukocytes and immature neutrophils.

Physiological hemeostasis, is however, a powerful phenomenon, and the immune system in particular is highly resilient to insult. The relationships observed here between hematological parameters and contaminants may be best seen as indicative of sentinel responses to contaminant exposure, responses that were not necessarily pathologic. Hematological parameters of marine mammals also can be affected by parasite load, diving behavior, stress, dietary shifts, and other individual differences (Bryden & Lim, 1969; Roletto, 1989; Geraci & Medway, 1993; Thompson et al., 1997). If such factors covaried with contaminant levels, it would not be possible to distinguish their relative contributions or causal proximity to alterations in hematological parameters. Other environmental contaminants also were present in SFB, such as polycyclic aromatic hydrocarbons and heavy metals, which could affect immune function and blood values in these seals (Turk & Casteel, 1997; Neale et al., 2002).

Finally, although evidence in support of contaminant-induced health alterations in marine mammals appears to be mounting, it is not clear *how* organohalogens might affect blood parameters such as those measured. For example, although research on the adverse effects of halogenated aromatic hydrocarbons such as PCBs has elucidated the role of the *Ah* receptor in mediating many such events, the cellular basis for PCB immunotoxicity is unclear (Kerkvliet & Burleson, 1994). It is likely that many effects of chronic exposure to PCBs and related compounds are indirect, producing subclinical infection and other pathological processes via multiple pathways, such as depression of plasma retinol (Kerkvliet & Burleson, 1994; De Swart et al., 1995a, 1995b; Jenssen et al., 2003).

CONCLUSION

Persistent environmental contaminants have been previously implicated as possible contributing agents in several declines of marine mammals, including the severe reduction in the harbor seal population in the Wadden Sea during 1988 (Dietz et al., 1989; Ross et al., 1996). However, this hypothesis has been difficult to test and remains an open question (Olsson et al., 1994; O'Shea, 2000; Ross et al., 2000). To our knowledge, this study is the first to investigate hematological correlates of chronic contaminant exposure in free-ranging harbor seals. Although PCB levels may be on the decline in harbor seal tissues, contaminant declines as a whole in SFB appear modest, and in some cases (e.g., PBDEs) may be on the increase. Although results presented here are not inconsistent with contaminant-induced alterations of seal health, predictive power of such field studies for population-level effects is severely limited by

the log-normal distribution of "high-load" individuals in the population (i.e., very few individuals have high contaminant burdens). In light of our findings, it is recommended to continue periodic monitoring of contaminant levels and hematological and other biomarkers of health in this at-risk population, along with targeted in vitro experimentation to explore causal links between chronic exposure to marine contaminants and seal health. Such efforts should be coupled with monitoring of the population to detect changes in overall numbers, productivity, and distribution.

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