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## Coastal Environmental Quality Initiative

### Title

Contaminant-induced immune alterations in the Pacific harbor seal, *Phoca vitulina richardsi*, of the central coast and San Francisco Estuary

### Permalink

<https://escholarship.org/uc/item/1t41h8zi>

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### Publication Date

2003-12-01

## FINAL REPORT

### Contaminant-induced immune alterations in the Pacific harbor seal, *Phoca vitulina richardsi*, of the central coast and San Francisco Estuary

Proposal # 02 T CEQI 03 0104

Award period: Jan 1 2002—Dec 31 2002 extended to Aug 31 2003

#### Summary of research findings:

#### 1. PCBs and DDE In Blood Of Free-ranging Harbor Seals (*Phoca Vitulina*) From Central California and Bristol Bay, Alaska

We sampled 100 individual harbor seals from four sites in California and 79 seals from three sites in a reference population of Bristol Bay, Alaska and analyzed contaminant levels and blood lipid in whole blood. As expected, levels of PCB and DDE were far greater in California samples (mean  $\Sigma$ PCB = 11.11 +/- 1.69 (SE) ppb wet weight (ww), DDE = 34.22 +/- 5.43 ppb ww) than in Alaska samples (mean  $\Sigma$ PCB = 0.74 +/- 0.07 ppb ww, DDE = 0.67 +/- 0.09 ppb ww). However, the relative importance of PCBs relative to DDE was quite different for the two regions. In California seals, average DDE levels were approximately three times higher than  $\Sigma$ PCB; whereas in Alaska seals, DDE levels were somewhat lower than  $\Sigma$ PCB. Profiles of the 10 PCB congeners were similar for the two populations, and CB 153 and CB 180 contributed most to  $\Sigma$ PCB both populations. In general, lipid normalization of organochlorine levels did not affect results qualitatively but rather changed the scale of measurement (to low parts-per-million range), and a tight, linear relationship was observed between wet weight and lipid weight concentrations for  $\Sigma$ PCB and DDE.

Within each population, we investigated factors of contaminant levels in multiple regression analyses which included age, sex, the interaction of age and sex, condition, and capture site. In Alaska harbor seals, contaminant concentrations tended to increase with age in males but decrease with age in females. Others have reported similar findings and have attributed such differences to female reproduction, in particular the off-loading of stored fat and associated organics from adult females to their offspring during pregnancy and especially lactation. Although independent data on contaminant levels in pups with varying exposure to milk-borne organics suggest that residue dumping during lactation by females does occur in coastal California seals (see below), the expected sex-age trends were not detected in the California sample. For harbor seals in California, age class alone was a significant factor in DDE levels, with levels highest in pups. Levels for PCBs showed a similar trend as for DDE, i.e., pups having the highest loads, although this effect was not significant. Capture site, however, was a significant factor for  $\Sigma$ PCB, with highest loads in seals captured in the San Francisco Bay. The relatively high levels of organochlorine contaminants found in seal blood provides a strong basis for continued monitoring of contaminant loads in harbor seals of the California coast and investigation of their potential health effects.

## **2. Contaminant Exposures and Health Correlates in Harbor Seals (*Phoca Vitulina*) of the San Francisco Bay, California**

An expanding body of research supports the hypothesis that exposure to contaminants impacts marine mammal health and contributes 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, we quantified in 39 free-ranging harbor seals blood levels of PCBs, DDE, and polybrominated diphenyl ethers (PBDEs)—a novel class of global environmental contaminants used as flame retardants and which are currently used in large quantities and have an environmental dispersion similar to that of PCBs and DDT. We examined relationships between contaminant exposures and six key hematological parameters (white and red blood cell counts, neutrophil and lymphocyte proportions, hematocrit, and hemoglobin). In addition, we compared PCB levels in the present study with levels determined in SFB seals a decade ago.

Males tended to have higher loads than females; age class and condition index (an estimate of relative fat stores) were not significantly correlated with contaminant levels in seals. PCB residues in harbor seal blood apparently declined slightly during the past decade. Nevertheless, levels remained high enough that reproductive and immunological effects might be expected. We found a positive association between white blood cell counts and PBDEs, PCBs, and DDE residues in seals, and inverse relationships between contaminant concentrations and red blood cell counts; hematocrit and hemoglobin also were negatively correlated with PCBs in males. Taken together, the data provide correlational support for an hypothesis of contaminant-induced alterations of harbor seal health in the SFB; continued monitoring of contaminant levels and especially hematological and other biomarkers of health in this at-risk population is indicated.

## **3. Proliferative Responses Of Harbor Seal (*Phoca Vitulina*) T Lymphocytes To Model Marine Pollutants**

In recent years, population declines related to viral outbreaks in marine mammals have been associated with polluted coastal waters and high tissue concentrations of certain persistent, lipophilic contaminants. Such observations suggest a contributing role of contaminant-induced suppression of cell-mediated immunity leading to decreased host resistance. Here, we assessed the effects of the prototypic polycyclic aromatic hydrocarbon (PAH), benzo[a]pyrene (B[a]P), and two polychlorinated biphenyls, CB-156 and CB-80, on a standard measure of immunocompetence—the T-cell proliferative response to mitogen—in harbor seal peripheral lymphocytes. Despite the variability associated with our free-ranging sample, we observed a clear suppressive effect of B[a]P (10 uM) exposure on T cell mitogenesis. Exposures to 10 uM CB-156 and CB-80, as well as 1.0 uM and 0.1 uM B[a]P, did not produce significant depression in lymphoproliferation. Exposure to the model PAH at 10 uM resulted in a 61% (range 34-

97%) average reduction in lymphoproliferation. We were able to rule out a direct cytotoxic effect of B[a]P, indicating that observed effects were due to altered T cell function. Based on our *in vitro* results, we hypothesize that extensive accumulation of PAH by top-trophic-level marine mammals could lead to altered T cell activation *in vivo* and impaired cell-mediated immunity against viral pathogens.

#### **4. Molecular Cloning And Sequencing Of Tyrosine Kinases From The Harbor Seal (*Phoca Vitulina*)—in prep.**

Contaminant-induced immunosuppression has been speculated to play an important contributing role in the declines of several marine mammal species, most notably the massive die-offs of harbor seals in the 1988 distemper epizootic of Europe's north coast. Decreased host resistance to viral pathogens implies depressed cell-mediated immunity and altered T cell function. The enzyme family of protein tyrosine kinases is largely responsible for T-cell receptor signaling leading to the differentiation and proliferation of antigen-specific clones required for proper immune response to viruses.

Using polymerase chain reaction, protein tyrosine kinase cDNA fragments from harbor seal were cloned and sequenced. Nine distinct proteins were identified using NCBI's BLAST program based on cDNA fragments of 166-194 bp. Predicted amino acid residues are currently being analyzed for comparison with other mammalian species. Kinases identified included Syk, Lyn and a longer variant of Lyn (Lyng), Itk, Fyn, Mapkk3, Ret, FeVSR, and DDR2. Several of these are key enzymes regulating signal transduction via the T-cell receptor; Fyn of the Src family of kinases, plays a critical role in early signaling events, and Itk and Mapkk3 are important in downstream signaling. Syk, of the Syk/ZAP-70 family and Lyn of the Src family are responsible for similar early signaling events in B cells. The sequence information obtained in this study can be applied in field studies or *in vitro* experimentation, in conjunction with restriction enzyme digestion maps, to identify effects on particular tyrosine kinases related to contaminant exposure of the individual or *in vitro* exposures of harbor seal lymphocytes.

#### **5. Maternal Transfer Of Persistent Organic Pollutants Via Lactation In Harbor Seals (*Phoca Vitulina*)—in prep.**

Sex and age differences in contaminant burdens of marine mammals have been attributed to female reproduction, in particular the off-loading of stored fat and associated organics from adult females to their offspring during pregnancy and especially lactation, although this has rarely been directly investigated in free-ranging populations. We hypothesized that blood concentration of organohalogenes in harbor seal pups would increase with duration of nursing. We sampled whole blood of 17 pups admitted to the Marine Mammal Center (TMMC; Sausalito, CA) Mar-Jun of 2001 and 2002 and analyzed levels of  $\Sigma$ PCB,  $\Sigma$ PBDE, and DDE to see if pups admitted later in the season (and that had presumably nursed longer) and had higher pollutant burdens than pups admitted early.

Along the west coast of North America, time of parturition in harbor seals varies with latitude, with pupping occurring earlier in more southerly locations and later in northern

areas. Although the pups included in this study all came from a relatively restricted geographical area, we wanted to rule out the possible confounding factor of latitude (i.e., of stranding location) on date of admission (our surrogate for time spent nursing). To this end, we regressed admit date of pups on latitude of stranding site. Blood concentrations of  $\Sigma$ PCB,  $\Sigma$ PBDE, and DDE all increased significantly with date of admission to TMMC. After correcting for physical condition of the animal, all regressions remained significant. Date of admission was not correlated with latitude of stranding location.

## Appendix

**Note: reprints of published manuscripts will be sent as they become available.**

### I. Research activities during award period (2002-2003)

#### Honors/awards:

2002 First Place-Student Oral Presentation, Wildlife Society-Western Section Annual Meeting, Visalia, CA

2001-present NIEHS Predoctoral Fellowship-Advanced Training in Environmental Toxicology, University of California, Davis

#### Additional Grants:

2002-2003 Oiled Wildlife Care Network, *Polycyclic aromatic hydrocarbon-induced immune alterations in the harbor seal, Phoca vitulina*

2002 San Francisco Estuary Institute-Regional Monitoring Program *Blood Levels of Selected Persistent Organic Marine Pollutants in Harbor Seals of the South San Francisco Bay, CA, USA*

#### Professional activities:

**Reviewer:** Journal of Wildlife Management, Journal of Mammalogy, Canadian Journal of Zoology

#### **Conference Presentations/Posters and Proceedings (all first-authored):**

2002 *Pollutant Burdens And Contaminant-Induced Immune Alterations In The Harbor Seal*. Oral presentation, Wildlife Society-Western Section Annual Meeting, Visalia, CA

2002 *Contaminant-Induced Immune Alterations In The Harbor Seal*. Oral presentation, Defenders of Wildlife-Carnivores 2002, Monterey, CA

#### **Invited lectures:**

2002 *Harbor Seals In The San Francisco Estuary: Tissue Burdens Of Persistent Organic Pollutants And The Potential For Contaminant-Induced Immune Suppression*, Regional Monitoring Program-Exposure and Effects Working Group, San Francisco Estuary Institute, Oakland, CA.

#### Publications:

**Neale, J.C.C.**, Van de Water, J.A., Harvey, J.T., Tjeerdema, R.S., and M.E. Gershwin. 2002. Proliferative responses of harbor seal (*Phoca vitulina*) T lymphocytes to model marine pollutants. Developmental Immunology 9: 215-221.

Sacks, B.N., and **J.C.C. Neale**. 2002. Foraging strategy of a generalist predator toward a special prey: coyote predation on sheep. Ecological Applications 12: 299-306.

### II. Participants

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