PERSISTENT ORGANIC POLLUTANTS (POPs) AND IMMUNE FUNCTION IN US ATLANTIC COAST HARBOR SEALS (*Phoca vitulina concolor*)

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Introduction

Polyhalogenated aromatic hydrocarbons (PHAHs), particularly the dioxin-like polychlorinated biphenyls (PCBs), dioxins and furans (PCDD/Fs) are potent immunotoxins that biomagnify in aquatic food chains and are associated with disease susceptibility and immune-disrupting effects in marine mammals and humans[1,2]. In 1979-1980, a large-scale outbreak of type A influenza virus resulted in the deaths of over 500 harbor seals (*Phoca vitulina concolor)* inhabiting the US Atlantic coast[3]. An immunomodulatory role of environmental chemicals was not investigated; however, studies conducted in the 1970s[4] reported that levels of PHAHs in blubber of these seals were comparable to those of seals in polluted areas of Europe. Recent data[5] indicate that PHAH levels have decreased since that time, but remain in concentration ranges known to be associated with impaired immune function in the species[6]. The sensitivity of harbor seals to the effects of PHAH exposure was previously demonstrated by a semi-field study in which numerous immune functions were suppressed in seals fed PHAH-contaminated fish from the Baltic Sea[7]. Similarly, a field study found associations between reduced immune responses of harbor seal pups and increasing blubber levels of dioxin-like PCBs following low-level perinatal exposure[8]. These observations support the hypothesis that dioxin-like PHAHs may play a role in the viral epizootics occurring among harbor seals, by impairing normal immune resilience to pathogens. The mechanisms of these effects are not yet clear, but are likely to involve interactions with the aryl hydrocarbon receptor (AhR) signaling pathway[9]. Here we report analysis of the relationship between body burdens of dioxin-like PHAHs and immune functions in free-ranging harbor seals from the US Atlantic coast.

Methods and Materials

Peripheral blood samples were collected from 29 free-ranging harbor seals captured during March 2001 in Chatham Bay, Massachusetts and April in Penobscot Bay, Maine. Seal blood samples were drawn from the extradural vein through a 20-gauge needle into four 10 ml heparinized vacuum tubes, placed on ice packs, and shipped overnight for next day analysis. **Lymphocyte Proliferation**. Peripheral blood mononuclear cells (PBMC) were isolated from the seal blood samples and lymphocytes (2×10^5) were cultured in flat-bottom 96 well tissue culture plates in the absence or presence of sub-optimal and optimal concentrations of Concanavalin A (Con A),

phytohemagglutinin (PHA), and lipopolysaccharide (LPS). Cells were cultured for 66 hours with 5-bromo-2'-deoxyuridine (BrdU), a thymidine analog, added during the last 16 hours. The Cell Proliferation ELISA, BrdU (colorimetric) assay was used to quantify proliferation as the incorporation of BrdU. Results were expressed as a stimulation index (SI), the proliferation ratio of stimulated to unstimulated cells. **Natural killer (NK) cell activity.** Lymphocytes were incubated for 150 minutes in round-bottom 96-well plates at 37°C with 5% CO_2 with labeled K-562 target cells at effector:target cells ratio of 100:1, 50:1, and 25:1. Target cell mortality (% cells lysed) was evaluated using propidium iodide and two color flow cytometry. The fluorescence of approximately 1000 target cells was read with a FACScan flow cytometer using the Cell Quest software. **CALUX Analysis**. PHAHs were extracted from seal plasma samples using acid-silica and XCARB as described[10]. The ability of each extract to induce Ah receptor-dependent gene expression was determined for each of the using the H1L6.1c3 CALUX cell bioassay (Xenobiotics Detection Systems, Inc., Durham, NC) and luciferase activity measured[10, 11]. The relative potency of each sample was determined by analysis of a dose (dilution) response for luciferase induction using a four-parameter Hill plot analysis of the dose curves, the EC50 values directly compared to a TCDD standard curve and the relative induction equivalents (CALUX-IEQs) for each sample calculated. CALUX-TEQ values are reported as pg/g lw.

Results and Discussion

Regression analysis showed a significant positive linear relationship between CALUX-TEQ values in the adult harbor seals (n=11) and lymphoproliferative responses to the T cell mitogen PHA (estimate = 0.039, SE = 0.011, F = 11.76, p = 0.008, r=0.753, r^2 = 0.566, r^2_{adj} = 0.518, Figure 1) and the B cell mitogen LPS (estimate = 0.008, SE = 0.003, F = 8.68, p=0.016, r=0.701, r^2=0.491, r^2_{adj} = 0.434, Figure 2). The PHA and LPS-induced responses in the adult seals were related only to the independent factor CALUX-TEQ and not to the factors sex or condition index. CALUX-TEQ values in adult seals were not significantly correlated with lymphoproliferative responses to the mitogen Con A or to NK cell activity. There were no significant relationships between CALUX-TEQ and mitogen responses or NK activity in juvenile seals.

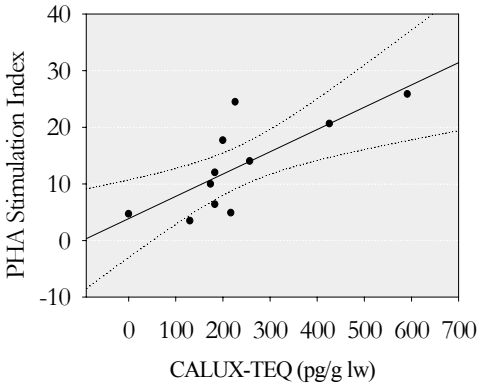

Fig. 1. Relationships between PHA response and CALUX-TEQs (± 95% confidence interval) in adult harbor seals

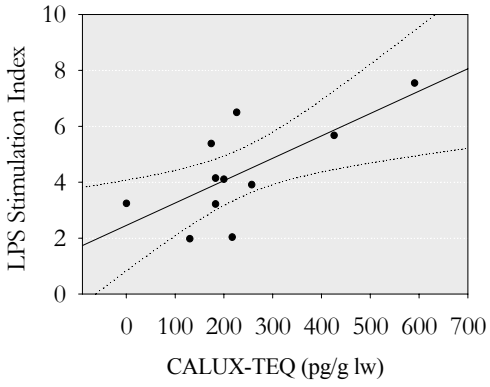

Fig. 2. Relationship between LPS response and CALUX-TEQs (± 95% confidence interval) in adult harbor seals

Thus, the CALUX-TEQ explained 57% and 49% of the variation in proliferative responses of harbor seal lymphocytes to the mitogens PHA and LPS, respectively. To our knowledge, a positive relationship between CALUX-TEQ values and mitogen-induced responses of peripheral lymphocytes of free-ranging phocid seals has not been previously documented. Recently, a positive correlation between PCBs in blubber and PHA and LPS-induced lymphocyte proliferation was reported in an experiment using captive harbor seal weanling pups (Levin, pers. comm.). Segre and coworkers[12] recently reported a significant positive correlation between PCB body burdens and mitogen responses of thymocytes in white-footed mice (*Peromyscus leucopus*).

Alterations in immune function can have devastating effects on the survival of an organism, particularly during critical phases of development. Whereas chemical-induced immune suppression may result in increased susceptibility, incidence, and severity of infectious diseases, conversely, immune enhancement may result in the loss of regulation within the immune system and can lead to adverse outcomes including as autoimmune disease, anergy (inability to mount antigen-specific immune responses), and cancer[9]. Because PHAHs can interfere with cell signaling pathways via interactions with the AhR, they may elicit a broad spectrum of immunologic and toxic responses[1,9]. For example, the significantly enhanced mitogenic responses observed in the harbor seals in this study might be explained by the presence of PCB-targeted regulatory cells such as CD4+CD25+ cells which act via contact or soluble factors to inhibit or down-regulate proliferating cells[13]. Alternatively, the production of cytokines, in particular IL-2 and the display of the IL-2 receptor on the surface of the activated T cells may be increased by exposure to PCBs[14], resulting in enhanced proliferation.

The CALUX-TEQ values detected in free-ranging US Atlantic coast harbor seals reflect relatively high burdens of dioxin-like compounds (Figure 3), although the relative contribution of individual PHAHs to the overall CALUX response remains to be determined. There were no correlations between the CALUX-TEQs and age, gender or condition of the animals. The levels of dioxin-like compounds in these seals (mean TEQ 235 ± 155 and 137 ± 94 pg/g lw for adults and juveniles, respectively) are comparable to or higher than those recently reported in Baltic ringed seals (*Phoca hispida baltica*) and gray seals (*Halichoerus grypus*) using a CALUX bioassay on liver extracts [15].

As a tool for measuring the toxic potency of dioxin-like compounds, the CALUX bioassay has several advantages, including its applicability to diverse tissues and its fast, relatively inexpensive, straightforward procedures. In the present study, logistical constraints prevented invasive sampling and thus correlations between the CALUX response and blubber burdens of dioxin-like compounds could not be examined. However, validation studies[10,16] have revealed a good correlation between the activity in these assays and TCDD/HAH concentrations in environmental and biologic samples as determined by GC/MS.

Although possible links between contaminant burdens and health status are not clear, the CALUX-TEQ values in adult harbor seals from the US Atlantic coast exceed the estimated threshold value of 160 pg TEQ/g lw in blubber for adverse effects on immune function in the species [6]. Dioxin-like PHAHs causing either inhibition of immunoregulatory cells or increased synthesis of cytokines may explain the observed positive correlation between lymphocyte mitogenesis and CALUX-TEQ burdens. While this relationship is not evidence *per se* of a direct cause-effect association, the results indicate that exposure to environmentally relevant levels of dioxin-like compounds may modulate immune responses in free-ranging harbor seals.

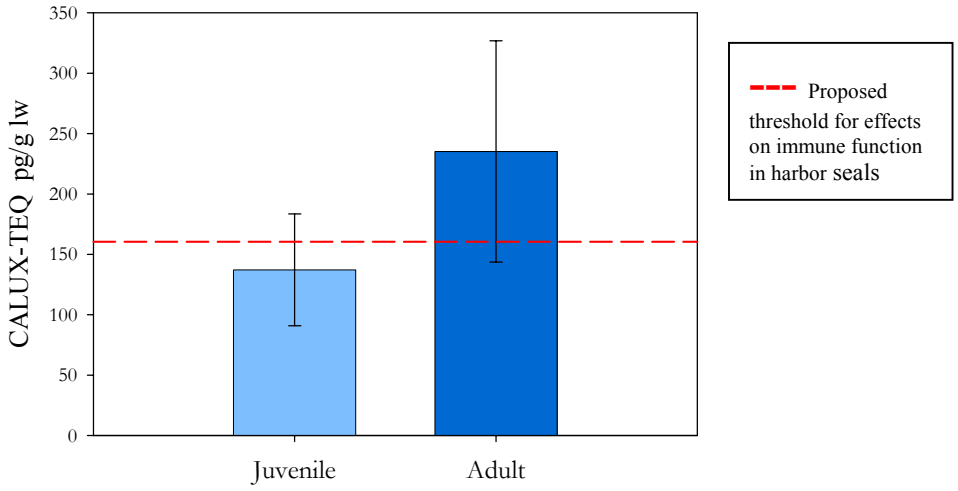

Fig. 3. CALUX-TEQ values (± 95% confidence interval) in juvenile and adult harbor seals

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