

## **Polycyclic aromatic hydrocarbon (PAH)-induced immune alterations in the harbor seal, *Phoca vitulina***

This project investigates PAH-induced alterations of the cell-mediated immune response, via effects on T cell activation, in the harbor seal. More specifically, we hypothesize that exposure of harbor seal peripheral blood mononuclear cells (PBMC) to a model PAH affects T cell proliferation and differentiation via modulation of cytokine expression, gene expression for protein tyrosine kinases (PTK), and activity of PTK enzymes. Our objectives are to expose harbor seal PBMC to a range of concentrations of a model PAH *in vitro* and to identify and characterize effects relevant to the cell-mediated immune response and host resistance to viral pathogens.

Specific aims and methodology include:

- (1) Characterize production of cytokines involved in the proliferation and differentiation of CD4 T cells, including IL-2, IL-12, and INF-gamma, following exposure to a model PAH, benzo[a]pyrene (B[a]P). Cell supernatant will be assayed for cytokine production using sandwich ELISA.
- (2) Evaluate changes in the molecular profile of PTK genes expressed by PBMC following B[a]P exposure. Protein tyrosine kinases represent a class of enzymes responsible for signal transduction via the antigen receptor of lymphocytes and are thus critical to the regulation of lymphocyte proliferation and differentiation. PTK gene expression will be assayed using a novel technique based on RT-PCR and restriction enzyme analysis.
- (3) Quantify and characterize the phosphorylation activity of PTK enzymes following exposure of PBMC to B[a]P. Phosphorylated proteins will be quantified and identified using gel electrophoresis and Western blotting.

This project addresses the research mission of the OWCN to investigate the effects of oil on wildlife by exploring mechanisms of immunotoxicity of an important class of petroleum compounds in a model coastal marine mammal. Anticipated results of this study will shed light on the highly relevant issue of contaminant-induced immunosuppression, with a focus on immunotoxic pathways leading to increased susceptibility to viral disease.