EFFECTS OF IN VITRO EXPOSURE TO PCBs ON POLAR BEAR (*Ursus Maritimus*) LYMPHOCYTE PROLIFERATION

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Introduction

The arctic marine ecosystem is contaminated with organochlorines (OCs) such as polychlorinated biphenyls (PCBs) and various pesticides [1]. Their high lipophilicity and resistance to biodegradation allow OCs to bioconcentrate in fatty tissues of organisms and to biomagnify as they move up the food chain, resulting in relatively high levels in predator species at the food chain summit, such as the polar bear [1,2,3]. Of all OC groups found in polar bear tissues, PCBs are found at the highest concentrations [1,2,3].

The Arctic ecosystem may be experiencing signs of immunotoxicity. Preliminary studies with northern fur seals (*Callorhinus ursinus*) of Alaska indicate OC-linked immunosuppression [4]. In some communities where the Inuit eat more "traditional" foods, these Inuit have similar tissue concentrations of OCs as polar bears [5], and there is evidence of immunotoxicity in their children, such as increased incidence of disease [6] and altered immune parameters [7].

To date there is no hard evidence that polar bears are experiencing any toxic effects due to their OC body burdens. In a recent study with polar bears, Letcher *et al*. [8] found strong positive correlations between PCBs and hepatic cytochrome P450 levels, indicating that PCBs can cause specific enzyme induction. In Svalbard, reported cases of pseudohermaphroditism in polar bears are speculated to be a result of endocrine disruption by OCs such as PCBs [9]. Data derived from studies with laboratory animals and non-human primates exposed to PCBs indicate that the immune system is perhaps the most sensitive target for PCB-induced toxicity [10,11]. Thus if concentrations of PCBs are high enough in polar bears to induce any toxic effects at all, the immune system would likely be affected.

The purpose of the present study is to determine the levels of PCBs that cause *in vitro* immunosuppression and to compare those with levels of PCBs found in polar bear tissues. As an *in vitro* indicator of immune response, the lymphoproliferation assay was chosen for its ease in execution considering the technical limitations of the field laboratory. This assay detects the competence of lymphocytes to be activated and to proliferate.

Materials and Methods

Polar bears were immobilized from a helicopter with Telazol® (a 1:1 mixture of tiletamine HCl and zolezapam HCl) by the methods established by Stirling *et al.* [12]. Peripheral blood was drawn from the femoral or jugular vein using variations on the methods of Ross *et al.* [13,14]. Blood was drawn into 60cc syringes pre-treated with 800 µL of heparin solution at 10,000 U/mL to reduce leukocyte agglutination. All syringes and sample collection tubes were kept warm (20 to 37°C) just before blood sample collection. Blood samples were stored at 4 to 20°C until arrival at the field laboratory. Within 8 hours of collection, blood samples were centrifuged at 300*g* for 6 to 7 minutes and leukocyte buffy coats were collected and pooled for each individual. Plasma was then pooled for each individual, aliquotted into cryogenic vials, and stored at between -20 and -70°C for chemical analyses.

Lymphocyte proliferation assays were conducted using modifications of existing methods [15,16]. Briefly, 9-10 mL of leukocyte suspension in autologous plasma was layered over 5mL Ficoll® density gradient in 15mL centrifuge tubes. The tubes were centrifuged at 300*g* for 30 minutes and cells were harvested from the interface. For cell washes, the cells were resuspended twice in 15mL RPMI 1640 culture medium and centrifuged at 250-300*g* for 6-7 minutes. The mononuclear cell suspension was adjusted to 2.5 x 10^6 cells/mL of complete CO_2-independent medium (containing 10% fetal calf serum and 100U penicillin/streptomycin), which was the culture medium used. A suspension of 2.5 x 10^5 cells (100µL) per well was cultivated in duplicate or triplicate in 96-well round-bottom culture plates with 50µL of concanavaline A (Con-A, a mitogen) at 3.0 µg/mL. Also added to each well was 50µL of one of 5 PCBs at concentrations of 5 to 75 ppm, or 50µL of the vehicle control, 1.5% DMSO (dimethyl sulfoxide).

For each PCB, the various treatment concentrations were prepared as follows. 1 mL of DMSO was added to each vial of 5mg PCB, for a concentration of 5000 ppm. If necessary, vials were then sonicated to aid dissolving. A small quantity of the solution was withdrawn and diluted to 500 ppm with culture medium, and this solution was then used to make subsequent dilutions. 50µL of these dilutions were added to each plate well. Total plate well volume was then 200 µL. The plate well concentrations of the PCBs were between 5 and 75 ppm. DMSO concentrations were greatest in the 75 ppm PCB treatment groups, with final DMSO concentrations of 1.5% (by volume). For DMSO control treatments, DMSO was added to plate wells for final concentrations of 1.5%. The PCBs chosen were PCBs 138, 153, and 180 which are highly abundant in polar bear tissues, and PCBs 156 and 157, which are the main PCBs contributing to total toxic equivalencies (TEQs) in polar bears [8,17].

The plates were then incubated at 37°C at atmospheric humidity. At 24 hours of incubation, 20 µL of alamar Blue®, a redox indicator, were added to each well. After total incubation of 72 hours, the plates were placed in a microplate reader where optical density was read at 540 and 620 nm wavelengths. Results as delta optical density (ΔOD) values were expressed as percent proliferation values with respect to mitogen control groups (0 ppm PCB), where the ΔOD value of -300 was equivalent to zero proliferation, and mitogen control groups are set to 100% proliferation.

Plasma OC concentrations were used as a general assessment of relative body burdens. Plasma samples from 11 individuals were analysed for PCBs, chlordanes, DDTs, chlorobenzenes, and

hexachlorocyclohexanes using modifications of established protocols [18]. Samples were dried with dried with andydrous sodium sulphate and isolated from the extracted lipid by gel permeation chromatography and chromatography on 33% potassium hydroxide/silica gels. OC groups were quantified using a gas chromatography/mass spectrum detector (GC/MSD). The percent lipid in the plasma was determined by colorimetric analysis using the method of Frings *et al*. [19]. Lipids were hydrolysed with sulphuric acid and reacted with a phospho-vanillin reagent, producing a chromophore with maximum absorbency at 540 nm, read with a diode array spectrometer.

Results and Discussion

Lymphocytes were isolated from the blood of only 17 polar bears due to excessive leukocyte agglutination occurring during blood processing. Control levels of lymphocyte proliferation without Con-A did not differ significantly from levels with Con-A. Consequently, the lymphoproliferation observed here is better described as spontaneous rather than mitogen-induced. No significant trends were found between plasma OC levels and immune function, nor between plasma OC levels and PCB effects on immune function.

PCBs 138, 153, 156, and 180 had similar effects on lymphoproliferation, which began to drop at approximately 20-30 ppm (Table 1). These reductions are more likely caused by inhibition of the capacity of the cells to proliferate then by cytotoxicity to the cells, as PCB cytotoxicity tests with seal lymphocytes revealed low cytotoxicity of PCBs [20]. The proliferative response was significantly ($p<0.05$) reduced by exposure to PCBs 153 and 156 at concentrations of 50, 60, and 75 ppm, and by exposure to PCBs 138 and 180 at 50 and 75 ppm, when compared to both the unexposed and vehicle (DMSO) controls. Conversely, treatment concentrations up to 75 ppm of PCB 157 did not differ significantly from controls.

For each PCB, immunotoxic concentrations that cause a 50% reduction in proliferation (IC_{50} values) were determined using regression equations of proliferation versus log-concentration relationships. PCB 138 had the lowest calculated IC_{50} value of 44 ppm. Those of PCBs 153, 180, and 156 were 67ppm, 73ppm, and 89ppm respectively. That of PCB 157 could not be calculated. IC_{50} values were also calculated for individual polar bears for use in comparison with plasma OC concentrations.

Plasma concentrations of total PCBs (15.7 ng/g wet weight and 1.75 μg/g lipid weight) found in these polar bears are lower than levels shown to cause in vitro immunosuppression. Lymphoproliferation began dropping at approximately 20 ug/g. Thus based on these tests, each PCB alone seems to not represent an immunotoxicological risk to polar bears. However, the combined effects of PCBs, other OCs, and other xenobiotics likely pose a greater risk than each individual PCB. More sensitive tests are necessary to reveal whether polar bears are experiencing any contaminant-induced immunotoxicity .

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Table 1: Percent Lymphocyte Proliferation Values for PCBs Tested

Percent proliferation with respect to individual controls (± standard deviation)											
PCB	Control	DMSO control	5ppm	10ppm	20ppm	25ppm	30ppm	40ppm	50ppm	60ppm	75ppm
138	94± 8	96± 8	102± 6	102± 6	99± 5	87± 12	73± 25	67± 24	39± 21	N/av	15± 8
153	97± 8	98± 7	101± 4	99± 4	99± 5	101± 5	94± 14	90± 9	68± 31	62± 26	34± 25
156	102± 4	103± 1	n/av	101± 1	98± 4	n/av	99± 3	89± 5	80±13	73±18	53± 21
157	102± 4	103±2	n/av	101± 5	101± 3	n/av	101± 4	103± 6	101± 6	103± 7	99± 6
180	94± 7	97±7	103± 3	100± 7	101± 5	100± 7	101± 5	91± 9	68± 30	74± 30	40± 30

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