POLAR BEAR CASE STUDY: RATIONALE FOR FUTURE RESEARCH

Lie E[1], Skaare JU[1,2]

[1]Norwegian School of Veterinary Science, P.O. Box 8146 Dep, 0033 Oslo, Norway; [2]National Veterinary Institute, P.O. Box 8156 Dep, 0033 Oslo, Norway

Introduction

The polar bear has received particular attention relative to contaminants in the Arctic. As an apex predator in Arctic ecosystem, biomagnification of persistent organic pollutants (POPs), particularly polychlorinated biphenyls (PCBs), has resulted in higher concentrations in polar bear than in most other mammals[1]. At Svalbard, alarmingly high PCB levels have been found in polar bears[2]. By comparing the PCB levels found in polar bears with thresholds for biological effects, it is reasonable to assume that polar bears at Svalbard may be at risk[1]. The Norwegian School of Veterinary Science, the National Veterinary Institute, the Norwegian Polar Institute and the University of Oslo have collaborated since 1987 with the aims to provide comprehensive data for risk assessment of the high PCB exposure in polar bear in the Norwegian Arctic. Collaboration with Canadian Wildlife Service was initiated in the early 1990s. In summary, this polar bear study have revealed knowledge of biomagnification of POPs in the polar bear food chain, geographic and time trends of POPs in the Arctic, and exposure-effects relationship of POPs in polar bear. Thus, the highest levels of PCBs and the organochlorine pesticides (OCPs) chlordanes and DDE are found in polar bears from Franz Josef Land and the Kara Sea with decreasing trends eastwards and westwards from this region[3,4]. Furthermore, associations between POPs and physiological parameters such as retinol, thyroid hormones, sex hormones and IgG have been found[5,6,7,8,9,10]. An experimental study to investigate associations between POPs and several immunological parameters was performed. High levels of PCBs and pesticides were found to influence both humoral and part of the cell-mediated immunity in polar bears[11,12]. Thus, the results indicate alteration of the endocrine system and decreased resistance to infections. Furthermore, ecological studies found relatively low cub survival at Svalbard and there are indications that the reproduction cycle was less than 3 years[13,14]. Hence, ecological studies suggest that reproduction and cub survival in polar bears at Svalbard may be impaired. Based on the results of the ecological, physiological and immunological studies it is reasonable to conclude that the population and health of polar bears with high levels of POPs are at risk.

Obviously, this polar bear study has resulted in a comprehensive data base, comprising levels of individual POPs, biological parameters of individual bears (age, gender, nutritional condition) and effect parameters (endocrine hormones and immune parameters). At present, we aim to estimate threshold concentrations for POPs for the demonstrated effects by using multiple regression analyses followed by classical mathematic function analyses. Also, we aim to establish various models as valuable tools for monitoring and risk assessment of POPs in the Arctic by using the existing data-set, to validate, improve and integrate various already developed approaches for this purpose (Quantitative structure-activity relationships (QSAR), food web models, fingerprinting, a.o.). These studies will contribute to our understanding of mammalian bioaccumulation and effects of POPs and to improve monitoring and risk assessment of POPs in the Arctic.

Materials and methods

We will use the existing data-set, which is unique both with regard to geography (the Arctic), species (the marine top predator), the number of bears, the number of analysed POPs, toxicological (endocrine and immunological) and biological parameters. Sampling procedures, analyses of POPs and physiological parameters have been reported in detail elsewhere[2-10]. Details on the design of the experimental study, analyses of immunological parameters and the statistical analyses are presented elsewhere[11,12,15].

By using multiple regression analyses followed by classical mathematic function analyses thresholds for immunotoxic effects were estimated. Classical mathematic function analyses are in this case partiell derivation of the estimated equations between the dependent variables and the set of independent variables. Furthermore, based on the results of these multiple regressions[11,12], a simplification of the result interpretation and presentation of individual immunological parameters was possible by keeping the biological factors such as gender, age, condition, body mass, and percentage extractable fat of the polar bears constant. This step moderated the equations and, thus, the presentation of the results[15].

Results and discussion

We have previously demonstrated that high levels of PCB impair the ability to produce protective antibodies following immunization and reduce serum IgG levels[11] and that high POP exposure affects lymphocyte functions and part of the cell-mediated immunity following *in vitro* stimulation with different mitogens and antigens[12]. These findings indicate that POP exposure may suppress vital part of the immune system.

We have estimated threshold concentrations for the immunotoxic effects found in polar bears. The estimated threshold concentrations were found to occur at concentrations ranging from 25-89 ng/g wet weight (ww) and 14-27 ng/g ww for sum PCBs (ΣPCBs) and sum pesticides (ΣOCPs) respectively, for selected immunological responses (Table 1). The demonstrated effects and estimated thresholds are based on results of multiple regressions, a statistical approach that studies the contributions of independent variables as ΣPCBs, ΣOCPs and biological factors and combinations of these, to the variation in dependent immunological variables[11,12,15].

Table 1. Estimated threshold concentrations of ΣPCBs and ΣOCPs in plasma (ng/g wet weight) for effects on serum IgG concentration, increased antibody titres against influenza virus and reovirus following immunization, lymphocyte responses to phytohemagglutinin (PHA) and concanavalin A (Con A).

	ΣPCBs	**ΣOCPs**
serum IgG levels	54	18
antibody titres to influensa virus	71	15
antibody titres to reovirus	25	27
lymphocyte responses to PHA	40	14
lymphocyte responses to Con A	89	14

The estimated thresholds indicate the plasma levels of POPs that may result in an immunotoxic effect. The range in the estimated thresholds may indicate that an effect on different parameters occur at different levels of PCBs and OCPs. This suggests that immunological parameters vary in their sensitivities to the effects of POPs. The individual estimated threshold concentrations are either at, below or above the mean levels of ΣPCBs and ΣOCPs in our study[11]. Hence, this may indicate that some parameters are more at risk than others to POP exposure.

Figure 1 illustrates how the sums of PCBs and pesticides influence serum IgG levels[15]. It can be seen that serum IgG levels (z-axis) decrease when ΣPCBs (x-axis) or ΣOCPs (y-axis) levels increase (Fig. 1a). However, the interaction of PCBs and pesticides seem to reduce the decreases in the IgG levels seen by either ΣPCBs or ΣOCPs. Thus, a decrease in serum IgG levels is observed when either the ΣPCBs or ΣOCPs is high. The figure is constructed from equation given in Lie et al. 2004 and by maintaining the biological factors and variables such as gender, age, condition, body mass and percentage extractable fat constant[15]. In addition, the breakpoints at the ΣPCBs-axis (ln ΣPCBs=4.0 ng/g ww) and ΣOCPs-axis (ln ΣOCPs=2.9 ng/g ww) in Figure 1 show the estimated thresholds for effects on serum IgG levels (Table 1).

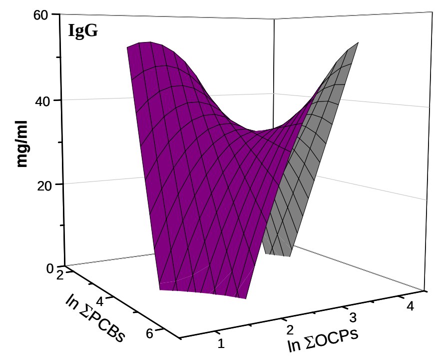

Figure 1. Predicted serum IgG levels (mg/ml) in relation to ln sum PCBs (ΣPCBs) and ln sum pesticides (ΣOCPs) from an average animal with regards to gender, age, condition, body mass and percentage extractable fat. ΣPCBs and ΣOCPs are presented in ng/g wet weight.

In our experimental study plasma concentration of PCBs, as the predominant contaminants, ranged from 14.8-200 ng/g ww (1.3 % lipid) in polar bears from Svalbard[11]. This level is of the same order of magnitude as reported earlier[10], which found decreased plasma IgG with increasing PCB levels. Again we found that PCBs was the predominant contributor to the variation in IgG levels. In addition, PCBs made a negative contribution to IgG levels[11]. In general, the plasma PCB levels in our study are at or above PCB levels associated with immunosuppressive effects in various marine mammal species (Table 2). Furthermore, the present PCB levels exceed the lowest-adverse-effect-levels (LOAEL) of immunosuppression reported in rhesus monkeys[1,16]. However, due to numerous limitations in the thresholds data, in quantification of PCBs and problems with extrapolating such data across tissues and species, this comparison should be used with caution. Similar approach could have been taken regarding the pesticides. However, at the present time, this is difficult due to lack of information of pesticide levels in several studies, and the thresholds for effects of the pesticides are unfamiliar. Nevertheless, it has been demonstrated that pesticides also contribute to adverse immunotoxic effects. Even if a causal relationship between POP exposure and impaired immune functions has not been proven, adverse immunotoxic effects of high levels of PCBs and chlorinated pesticides have been demonstrated[11,12].

Table 2. Mean ΣPCBs[a] levels (ng/g) in blood (wet weight) and adipose tissue or blubber (lipid_weight) in various marine mammals extracted from present and previous studies. Lowest-adverse-effect-levels (LOAEL) for immunotoxic effect reported in rhesus monkey are included for comparison.

Species	**Gender**	**Tissue**	**ΣPCBs[a]**
Rhesus monkey (*Macaca mulata*)[16]	♀	blood	10 ng/g ww
Polar bears (*Ursus maritimus*) (Franz Josef Land)[3]	♀	blood	58 ng/g ww
Polar bears (*Ursus maritimus*) (Svalbard and Canada)[11, 12]	♀♂	plasma	45 ng/g ww
Polar bears (*Ursus maritimus*) (Svalbard)[10]	♀♂	plasma	67 ng/g ww
Polar bears (*Ursus maritimus*) (Svalbard)[17]	cubs	plasma	160 ng/g ww
Harbor seal seals (*Phoca vitulina*) fed highly contaminated fish[18]	♀♂	blood	33 ng/g ww [b]
Polar bears (*Ursus maritimus*) (Svalbard)[2]	♂	adipose tissue	21 700 ng/g lw
Beluga whales (*Delphinapterus leucas*) (St. Lawrence Estuary, 1987-1990)[19]	♂	blubber	78 900 ng/g lw

[a] ΣPCBs consist of various # of PCB congeners; thus comparison must be done with great caution.
[b] Concentration in pooled blood sample of 11 seals[18].

The present investigation has estimated threshold concentrations for the demonstrated immunotoxic effects. These threshold concentrations indicate at which PCB and pesticide levels in plasma immunotoxic effects may occur in free-ranging polar bears. To our knowledge information on threshold concentrations in blood for immunotoxic effects is limited for marine mammals. In wildlife, such thresholds may be valuable in identifying species that may be at risk for the effects of POPs. Hence, our study introduces a potential approach for estimating thresholds concentrations in wildlife.

So far we have demonstrated effects of ΣPCBs and ΣOCPs, which were considered to represent the exposure, as an alternative to examining the effects of individual compounds. However, these two groups of contaminants consist of components with different molecular structure and toxic effects. The mixture of persistent pollutants in polar bears consists of contaminants that can exert various reproductive and immunotoxic effects via several possible mechanisms of actions. It has, however, so far been difficult to isolate certain individual compounds in the polar bear samples as the cause of the effect. The effects could, as well, be due to an exposure of a mixture of the compounds that in combination exert the effect. By using multivariate techniques including PCA and PLS, many parameters can be examined simultaneously. Such techniques can be used to identify which factors contribute most to bioaccumulation and effects. Thus, they will provide the key to which variables of the bioaccumulation models are most important for this specific type of food web. Moreover, it can identify parameters that can be improved or can be added to existing models such as exposure variation, metabolic degradation and life-history traits. Furthermore, using multivariate techniques the polar bears can be classified/ordinated in different groups, according to the patterns of compounds. This grouping can then be related to the physical/chemical properties of the main occurring compounds. These compound specific data (for instance vapour pressure, $\log K_{ow}$, metabolic resilience and others) may direct to reveal the origin of the compounds, and the route of entrance. At present, we use this unique existing data-set resulting from previous polar bear studies, to validate, improve and integrate various already developed methodologies and approaches for this purpose (QSAR, food web models, fingerprinting, a.o.). We aim to identify significant explanatory variables for exposure, bioaccumulation, biotransformation, and effects of POPs to be included in national and international monitoring programs and risk assessment. Additionally, the modelling will integrate the data from the present project to verify whether current model approaches also apply to other types of compounds, such as persistent flame retardants (BFRs). Thus, our present study may establish various models as valuable tools for monitoring and risk assessment of POPs in the Arctic. These models and the approach to estimate thresholds for effects could be useful for other studies and should be evaluated in data-set from other mammal species as well as other POPs.

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