An Individual Based Modelling Approach to Investigate the Impact of Pollutants on Cetacean Population Dynamics – Effects on Calf Survival and Immunity.

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I. ABSTRACT

This paper reports on progress and findings of the Phase II Pollution 2000+ project. The main objective was to investigate how contaminant induced effects on other target systems, such as immune function, could also be incorporated into the existing model framework constructed to assess the impact of polychlorinated biphenyls (PCBs) on cetacean populations. The model already links maternal blubber PCB concentrations to an increased risk of calf mortality as its main toxicological effect. However, since other significant PCB related health effects have been reported in cetaceans which would similarly affect population vital rates, such as survival, and therefore population dynamics, it was important to be able to include these in the model. Of the major physiological systems affected, such as the endocrine and skeletal systems, effects on immune function have been widely reported since the 1990s. Perturbations in immunity are highly likely to have influence at the population level following exposure of immune suppressed animals to environmental pathogens. Thus we first thoroughly reviewed the literature to determine which of a broad spectrum of immune functions, both innate and acquired, might be impacted by PCBs in cetaceans. In addition, we needed to determine the nature and functional form of the link between these immune function measures and *quantifiable* survival risks that could be incorporated into the model framework.

A large body of literature associating increased PCB exposure with reduced immune function exists and a number of papers have been published which demonstrate that this effect is also seen in marine mammals, both seals and cetaceans. However, only one study by the National Toxicology Program (NTP), carried out in the early 1990s (Luster *et al*. 1993) was available which allowed us to *quantitatively* link immune function assays to decreased host resistance and thus reduced survival probability. This was crucial to us being able to modify the estimated survival parameters in the model. Of the wide range of immune function assays reported only T-lymphocyte proliferation in response to stimulation with a mitogen known as Concavalin A (Con A) was used in both the NTP study and in a study published by Schwacke et al. (2011) on the impacts of PCBs on wild bottlenose dolphins. Nonetheless, these two studies allowed us to convert PCB induced immune suppression in bottlenose dolphins to impacts on survival following exposure to a pathogen.

Once the additional concentration-response relationships were established, the effect of additional immune impacts on potential population growth for bottlenose dolphins was investigated. Following model simulations when 10% of the population were exposed each year to a class 2 pathogen with similar pathogenicity and virulence to *Listeria monocytogenes* used in the mouse

model experiments, significant effects on the potential population growth were seen at an annual accumulation in the blubber of the dolphins of > 1 mg/kg. When a more virulent and pathogenic organism such as *Streptococcus pneumoniae* used in the mouse experiments was included, effects were seen at annual accumulations of < 0.5 mg/kg

Further simulations found that at lower annual accumulations of <1 mg/kg exposing 20% of the population each year to the lower virulent organism had a severe effect on potential population growth, causing a long term decline. However, when 10% or 20% of the animals were exposed each year impacts at the population level were seen only when annual accumulation concentrations were >2 mg/kg. And at the lowest pathogen encounter of 5% of the animals effects were not seen until annual accumulation rates of 3 mg/kg. This represents between a further 0.3% to 3 % decrease in potential population growth, depending on the proportion exposed each year and the annual accumulation rate (i.e. degree of contamination in the prey) compared to a model with only effects on calf survival.

By determining how the blubber PCB annual accumulation rates relate to levels in mature, breeding females (available as an output from the model simulations), comparisons with empirical data can be made and predictions about effects on various populations formulated. For example, data from Schwacke *et al*. (2011) and Wells *et al.* (2005) would suggest that at the current blubber PCB concentrations seen in the breeding females the populations in Sarasota Bay and St Joseph Bay, Florida would remain stable or increase slightly over the 50 – 100 year timescales projected, but that the population in Brunswick, Georgia where levels in breeding females were 10 times higher, would decline over the same period without external population inputs through immigration.

We have demonstrated that this model framework can be used to include other contaminant induced health effects in addition to the impact of calf survival previously investigated (Hall *et al*. 2011). The simulations reported here used the bottlenose dolphin as a demonstration species because sufficient population dynamic data available for the Sarasota Bay population to parameterise the underlying population. Thus generalised potential population dynamic predictions can be made for specific populations when estimates of PCB concentrations in mature, breeding females are known. These impacts can then be compared to the effects of other population pressures such as interactions with boats, shipping and fisheries so that the overall effect of pollutant insults can be placed into a relative management context.

Impacts on other populations and species, such as Humpback whales from the Gulf of Maine as were included in the previous simulations (Hall *et al.* 2011), could easily be investigated. And future developments of this model will include a sensitivity analysis; incorporation of a bioaccumulation model to estimate blubber concentrations for populations or species in which only levels in prey are known and making the model available online with a user-friendly interface.

II. Introduction

At the IWC Intersessional Phase II Pollution 2000+ workshop held at the Marine Mammal Center, Sausalito, CA, USA from 22nd-24th February, 2010, the objectives for the cetacean pollutant exposure, risk assessment modelling work were agreed by the Steering Committee. This involved the development and implementation using two demonstration species in the risk assessment framework outlined by Hall, Schwacke and colleagues (Hall *et al*. 2006). Objectives 1 and 3 have been completed and were reported to the 2011 IWC Scientific Committee (Hall *et al*. 2011; SC/63/E5). The results presented in this paper are focussed on Objective 2, with additional refinements to the model built under Objective 3. Progress towards the completion of Objective 4

has been made but suitable datasets that will allow us to address the impact of other contaminants of concern are still lacking.

Relevant Phase II Objectives

Objective 2. Derive additional CR functions to address other endpoints (i.e., survival) in relation to PCB exposure. This requires a multi-stage modelling approach, e.g., a series of functions that provide a connection from PCB exposure → functional immune endpoints → increased pathogen susceptibility → increased likelihood of mortality.

Objective 4. Implement a CR component for at least one additional contaminant of concern (COC). This was determined by the Steering Committee given knowledge for likelihood of exposure and toxicity. The group of brominated flame retardants, the polybrominated diphenyl ethers (PBDEs) was highlighted as the additional contaminant to be investigated.

III. Additional Concentration Response Functions – Review of the literature.

A wide range of physiological effects of persistent organic pollutants (POPs) on mammalian systems and therefore risk indicators for cetaceans have been reported in the literature. These include effects on thyroid hormone function and homeostasis (Murai et al. 1987, Brouwer et al. 1989, Morse et al. 1992, Gray et al. 1993, De Guise et al. 1995, Schwacke et al. 2011), skeletal integrity (Bergman et al. 1992, Gould et al. 1997, Routti et al. 2008, Cocchi et al. 2009), growth and development (Gould et al. 1997, Restum et al. 1998, Crofton et al. 2000), nervous system abnormalities (Boegner *et al.* 1994, Ribas-Fito *et al.* 2001, Boersma & Lanting 2000) and immune system dysfunction (Thomas & Hindsill 1980, Silkworth et al. 1984, DeSwart et al. 1994, De Guise et al. 1995, Lahvis et al. 1995, Ross et al. 1996, Tryphonas 2001, Wade et al. 2002, Hammond et al. 2005, Schwacke et al. 2011). Whilst many of these impacts may severely compromise an individuals' health and ultimately cause direct mortality or reduce its capacity to reproduce, the published studies often do not take the impacts to the level of severity required to determine their ultimate effect on population dynamics. Of primary interest here are those responses which can be *quantified* in terms of their effects on fecundity and survival and where model parameter ranges can be estimated for use in the overall risk assessment. This requirement means we are limited to including those responses for which suitable data are available. In addition, one of the most widely reported responses to POPs in general, and polychlorinated biphenyl (PCB) exposure in particular, has been effects on immunity ((De Swart et al. 1996, Ross et al. 1996, Luebke et al. 1997, Neale et al. 2002, Lie et al. 2004, Hammond et al. 2005, Schwacke et al. 2011). With increasing reports of infectious diseases affecting cetacean populations (Gulland & Hall 2007) clearly a physiological response to a pollutant stressor which lowers the ability of animals to react normally to foreign agents could be critical at the population level. We therefore chose to focus on incorporating effects of PCBs on the immune response, in addition to the effects of maternal exposure on calf survival already included in the individual based model.

1. Immune parameters and PCB exposure in laboratory animal models and marine mammals

Initial studies in this field used indirect approaches whereby the rate or risk of infectious disease morbidity or mortality associated with exposure to PCBs was investigated. Such studies were carried out using laboratory animal models where exposure to pollutants and subsequently to disease agents could be controlled. Other indirect studies specifically focussing on cetaceans used an epidemiological approach to determine the increased odds or risk associated with PCB exposure and infectious disease mortality. More recently however, research has focussed down at the cellular and molecular level to investigate the extent to which the immune system is compromised following

direct exposure. The extent to which each of these approaches can be used to generate concentration-response functions for the risk model are outlined.

1) Indirect measures

A. PCB-treated laboratory animal models with increased infection rates

It has been shown in a variety of experimental studies using a range of different species that exposure to PCBs can render animals more susceptible to viral and bacterial infections. PCB-induced immunosuppression has been shown to result in a higher sensitivity of experimental animals to various infectious agents including gram-negative bacteria (endotoxin), protozoa and viruses. For example, PCB-treated mice are more sensitive to endotoxin, malaria (Loose *et al*. 1978) and bacteria (Thomas and Hinsdill, 1978). Mice have also been shown to be more sensitive to challenge by herpes simplex and ectromelia (mousepox) (Imanishi *et al*. 1980). PCB-treated rabbits have been shown to synthesise fewer antibodies after being challenged by pseudorabies virus (Koller and Thigpen, 1973), and the resistance of PCB-treated ducks to duck hepatitis virus was also shown to be impaired (Friend and Trainer, 1970). Together, these results clearly demonstrate the association between increased levels of pathogen infection with exposure to PCBs, but the underlying immunosuppressive mechanisms were not investigated and the experimental designs did not generate useful concentration-response functions.

B. Determining the risk of mortality from infectious disease using a case-control approach

A study published by Hall *et al*. (2006b) used a case-control approach to determine whether the risk of mortality from infectious disease in harbour porpoises in UK waters increased with higher uptake of PCBs. Using long-term strandings data from the UK, porpoises were categorized into two groups; those that died as a result of physical trauma, the 'controls', and those that died of an infectious disease, the 'cases'. An exposure odds ratio was generated from a logistic regression which showed that for each 1 mg/kg increase in blubber PCBs, the average increase in risk of infectious disease mortality was 2%. It was seen that a doubling of risk occurred at approximately 45 mg/kg lipid weight. As other contaminants, such as DDTs and PBDEs for example, also accumulate in the blubber, it is possible that this increase in risk is due to a combination of contaminants, rather than a consequence of exposure to PCBs alone. However, it has been demonstrated in a wide variety of species that PCBs are among the most immunotoxic of the POPs. It is also assumed that the increased risk is mediated through effects on immunity. However, since the true incidence of infectious disease in the population that gave rise to the cases is not known it is difficult to translate the observed increase in risk from the odds ratio estimates into a realistic modification of the survival probability that is required for the model.

2) Direct measures

The next stage was to directly investigate relationship between immune function and PCB exposure, using both *in vivo* and *in vitro* laboratory animal models and studies on marine mammals.

A. Characterising acquired and innate immunity in marine mammals

At the cellular and molecular levels assays to measure and evaluate the functioning of both the humoral (acquired) and cell-mediated (innate) immune responses in marine mammals have been carried out. Parameters of primary interest have included natural killer cell activity, phagocytosis response, cytokine expression and lymphocyte proliferation among others (De Guise *et al.* 1995. Pillet *et al*. 2000. Lalancette *et al*. 2003. Hammond *et al.* 2005. Camara Pellisso *et al.* 2008. Fonfara *et al.* 2008. Frouin *et al.* 2008). Through a combination of *in vitro* and *in vivo* studies using blood

samples taken from laboratory animals, as well as in captive and wild marine mammals, studies have investigated changes in cellular immunity in response to POP, PCB and heavy metal exposure. Overall, these studies suggest that marine mammals exposed to high levels of environmental contaminants will be immunocompromised, and consequently suffer from reduced resistance to disease. Table 1 below summarises the immune parameters that have been measured in marine mammals and the assays used to do so. Reported effects become more severe as exposure increases and at the highest exposure levels effects on both cell-mediated and humoral functions are reported resulting in more severe immunosuppression and thus premature mortality upon exposure to infection. However, despite a growing body of research in this field, well characterized dose-response curves are generally lacking.

Table 1. Summary of immunological parameters measured in various *in vivo* and *in vitro* studies on marine mammals exposed to environmental contaminants.

Parameter	Measurement	Assay
Natural Killer Cells	Activity	^{51}Cr release assay Flow cytometric assay
Neutrophils	Phagocytic activity	Leukocyte incorporation of fluorescent latex beads Leukocyte incorporation of fluorescein labelled *Escherichia coli* (K-12 strain)
	Respiratory burst	BioParticles Flow cytometric assay Flow cytometric assay
Cytotoxicity	Cytotoxic Activity	YAC-1 mouse lymphoma cells labelled with fluoroscein and incubated with peripheral blood mononuclear cells (PBMCs).
Lymphocyte proliferation	T-lymphocyte proliferation	Mitogen-induced proliferation (ConA) Antigen-induced proliferation Mixed lymphocyte reaction Delayed-type hypersensitivity skin test
	B-lymphocyte proliferation	Mitogen-induced proliferation (PHA) Specific serum antibody responses Ex vivo/in vitro immunoglobulin production
Lymphocyte signalling		Responsiveness to thymosin α1.
Hematology	Leukocyte counts	Lymphocyte counts in peripheral blood Neutrophil counts in peripheral blood

B. Functional immune endpoints

Of the published studies investigating cellular immunity in marine mammals most research effort has focused on three parameters; neutrophil function, natural killer cell activity and lymphocyte proliferation. Unfortunately, even within these more common responses there is very little information on normal reference ranges for these parameters in marine mammals, and even less data on dose-response relationships associated with PCB exposure, or threshold values that suggest a level below which an animal would be incapable of generating an immune response to infection. For this reason, immunosuppression studies on other mammal species, including humans, have been included here.

a) Neutrophil Function and Quantification

Neutrophils are the most abundant of the white blood cells known as granulocytes, making up approximately 50-60% of the total white blood cells. They are the first line of defence against invading organisms, especially bacteria, and therefore play a prominent role in acute inflammatory reactions (Dierauf, 1990). Neutrophils continually circulate in the blood stream until triggered by a series of inflammatory stimuli, including phospholipids and peptides derived from bacteria for example, when they then move to sites of infection and inflammation. Ingestion of foreign materials through the process of phagocytosis, and destruction of phagocytized particles are the major functions of neutrophils, and thus they play a critical role in the defence of many bacterial pathogens (Dierauf, 1990).

The Absolute Neutrophil Count (ANC) is a measure of the number of neutrophils present in the blood. The ANC is calculated from measurements of the total number of white blood cells in a blood sample, and the combined percentages of the mature neutrophils, called 'segs', and the immature neutrophils called 'bands'. Neutropenia occurs when there is a decrease in the circulating number of neutrophils in the peripheral blood to below normal levels. Neutropenia has many causes that can be divided into conditions attributable to intrinsic disorders of the hematopoietic cells, and disorders associated with extrinsic factors. Intrinsic bone marrow diseases include leukaemias and lymphomas while extrinsic factors include infection with viral, bacterial, protozoan and fungal agents, or it can be drug induced. A reduction in the neutrophil counts will result in the failure to deliver sufficient numbers of neutrophils to sites of injury or infection, and thus make the animal more susceptible to disease (Boxer and Dale, 2002).

Another parameter that is monitored is the *activity* of the neutrophils, as well as their number. One important innate immune function of peripheral blood neutrophils and monocytes is respiratory burst which generates reactive oxygen species that are used to kill engulfed microorganisms (Levin *et al.* 2007). A reduction in the respiratory burst activity of these cells could result in the reduction in the killing of pathogens engulfed through phagocytosis (Kuby, 1997), and will thus affect the organism's ability to rid itself of invading pathogens. It must be recognised however that the presence of infection itself can cause secondary alterations in the phagocytic performance of neutrophils (Dierauf, 1990), so neutrophil activity should be used in conjunction with other tests to assess the immune function of an animal.

b) Neutrophil Counts and Respiratory Burst in Marine Mammals Exposed to OCs and PCBs

In a two and a half year feeding experiment, 22 young harbour seals (*Phoca vitulina*) were split into two groups, and fed herring from either the relatively unpolluted Atlantic Ocean or the heavily polluted Baltic Sea as part of an immunotoxicological study (de Swart *et al.* 1995). Blood samples

were taken at regular intervals and analysed for routine haematology and clinical chemistry to monitor potential indicators of immunotoxic stress as well as general health state. The results found a significant increase in the neutrophil counts in the seals fed the Baltic herring, which became more pronounced towards the end of the feeding experiment. It was speculated that this increase in neutrophil count may have been caused by the increased occurrence of subclinical bacterial infections in these animals due to the impairment in other aspects of their immune response.

The potential differences in disease susceptibility following exposure to various organochlorines (OCs) between captive and free-ranging sea otters (*Enhydra lutra*) from the same genetic population were assessed following an *in vitro* study (Levin *et al.* 2007). The effects of individual and different combinations of PCB congeners were tested using *in vitro* immune assays to evaluate both innate and acquired immune functions. Five of the 26 different mixtures of PCB congeners induced a significant decrease of between 23-39% in the respiratory burst of the neutrophils isolated from the captive otters. However, there was no decrease in neutrophil respiratory burst seen in the wild otters. It was speculated that the differences in susceptibility of the two groups could be due to the acute stress of capture, the chronic stress of captivity, nutritional differences or a combination of these.

The immunomodulatory potential of different OC mixtures, and individual OCs on the respiratory burst in several marine mammal species, humans and mice have also been characterised using flow cytometry (Levin *et al*. 2006). Both significant enhancement and suppression of respiratory burst occurred in all species tested, but the pattern was different between species. While the neutrophil and monocyte respiratory burst was significantly enhanced in many of the cetacean species, it was significantly reduced in the harbour seal. Using regression analysis, the authors were not able to elucidate which OCs were involved in modulating the responses, highlighting the difficulty of developing models to predict the immunotoxic effects attributed to different OC mixtures.

c) Relationship between Neutrophils, Immune Function and the Risk of Disease

As neutropenia is most often caused by cancer therapies, including chemotherapy and radiation therapy, there has been considerable research effort focused on the effects of neutropenia in human patients with cancer in terms of better diagnostic tests and improved treatment. There are five severity or grading levels for neutropenia, associated with a relative risk of infection as defined by the National Cancer Institute (2003), which are summarised in the Table 2 below. The percentage ANC compared to healthy individuals was calculated to make these data potentially applicable to other species and incorporation into modelling approaches.

Table 2 – Summary of the classification of different levels of neutropenia and associated risks of infection published by the National Cancer Institute (2003).

Classification of Neutropenia	Risk of Infection	ANC Range ANC / µL	Average ANC	% ANC compared to Healthy Individuals
None	None	≥2000	2000	100
Mild	Slight	1500 - 2000	1750	87.5
Moderate	Moderate	1000 - 1500	1250	62.5
Severe	High	500 - 1000	750	37.5
Profound	Extremely High	≤500	500	25

As the number of circulating neutrophils decreases below 1000/µL and the severity of neutropenia increases, the risk of infection progressively increases. In cancer patients, if the ANC falls below

500/µL, infections may be life-threatening. Normal ANC ranges vary between species but it may be possible to use these data to calculate the percentage ANC compared to healthy individuals for each category of infection risk, allowing us to apply these relationships to other species. While there is no quantitative risk assessment available to describe the likelihood of contracting an infection with varying degrees of neutropenia, classifying risk into 5 different categories, from 'none' to 'extremely high' in this way could assist in modelling the probability of infection that would then be useful in terms of prediction.

While the possibility of a reduced ANC increasing the risk of infection in marine mammals is a highly plausible one, to date there is no published evidence of neutropenia occurring in marine mammals exposed to PCBs. Neutrophils have been shown to be elevated by stress and bacterial infection, but decreased by *overwhelming* bacterial infections and toxaemia (Dierauf, 1990). For example, in a study on sick and healthy pinnipeds published by Roletto (1993), the neutrophil counts were increased in sick sea lions, elephant seals and harbour seals compared to healthy ones. As such, the ANC is perhaps not a good indicator of immunosuppression as it appears to fluctuate quite considerably and a high ANC obviously does not always indicate a healthy animal. Furthermore, if the ANC were to be used to assess the circulating neutrophils in wild marine mammals, other assays and tests would have to be run in parallel to ascertain that the animals were not suffering from an overwhelming bacterial infection for example that caused the neutropenia rather than as a result of PCB exposure. It would be difficult to determine if a low ANC was the result of PCB exposure, allowing a bacterial infection to develop, or due to the bacterial infections themselves. For example, in their study on harbour seal pups Mos *et al.* (2006) suggested that elevated neutrophil counts and respiratory burst activity was more indicative of expose to bacteria and pathogens, as a result of biological pollution for example, as counts were not correlated with high blubber PCB contamination.

Finally, the effects of PCBs on the respiratory burst of neutrophils remains confusing as it would appear that the burst is enhanced in some species, but suppressed in others when exposed to different OCs (Levin *et al*. 2007). This may be an artefact of *in vitro* studies, and it is entirely possible that *in vivo* studies may suggest otherwise, but this has yet to be investigated. While a recent study by Keogh *et al.* (2011) published reference values for normal respiratory burst activity in healthy bottlenose dolphins, there is no published data on the extent to which changes in respiratory burst activity would affect resistance. In conclusion, while there is a large body of literature detailing the importance of neutrophils as part of the mammalian immune system, and the increased risks of infection associated with neutropenia in humans, there is still a lack of evidence to suggest that this is the case in marine mammals contaminated with PCBs. As such, there is not enough data published to use the ANC or respiratory burst as immune endpoints on which to build dose-response functions to predict the risk of infection.

d) Natural Killer Cell Activity and Assays

Natural killer (NK) cells are heterogeneous populations of large, granular lymphocytes that contribute to protective responses against a variety of infections and tumour cells. They form an important part of the innate immune system. The NK cells migrate directly into peripheral lymphatic organs and the blood following development in the bone marrow. While some of their functions overlap with those of T-cells, they can be distinguished by their involvement in innate immunity and defence in the early phase of infection (Biron *et al*. 1999). As such, they are vital in the non-specific immunological defence against a variety of pathogens that the animal has not previously encountered, and therefore play a role in limiting the spread of infection while a more effective specific antibody and cellular response can be mounted. Although it is now known that NK cells can respond to infections caused by a number of different agents, the best evidence for their importance in defence is against viral infections (Biron *et al.* 1999).

Routine testing for NK cell activity in different animal species involves tumour cell-directed cytotoxicity assays. The target tumour cells are radiolabelled with ^{51}Cr. When incubated with an animals' peripheral blood mononuclear cells (which include the NK cells), the specific release of the ^{51}Cr by the target tumour cells can be quantified. This leak of the radioactive isotope from the cells occurs once they have died and their membranes have ruptured, and thus reflects the natural cytotoxic activity of the NK cells, and is recorded usually as 'counts per minute' (De Guise *et al.* 1997). Another method of assessing NK cell activity is through the use of flow cytometric assays. The basis of these assays is the measurement of the loss of membrane integrity of target cells which allow a dye to penetrate the cell (De Guise *et al*. 1997).

e) NK Cell Activity in Marine Mammals and Exposure to PCBs

In the long term feeding experiment involving 22 captive harbour seals (De Swart *et al.* 1994. Ross *et al.* 1996), blood and blubber biopsy samples were taken throughout the experiment and a series of immune function assays and tests were performed, including for the first time the evaluation of NK cell activity. The results showed significant seasonal patterns of NK cell activity which in winter was approximately half of that observed in the summer months. They also showed that the NK-cell cytotoxic activity of the seals fed Baltic herring was consistently and significantly reduced to a level approximately 25% lower than that observed in seals fed Atlantic herring. Their analysis suggested an inverse relationship between NK cell activity and environmental contaminants. It was impossible to identify any contaminant in particular which led to the suppression of NK cell activity as the Baltic Sea herring had elevated levels of all PCB, PCDD and PCDF congeners measured.

De Guise *et al*. (1997) were the first to demonstrate NK cell activity in cetaceans. The NK cell activity against two different tumour cell lines was investigated in beluga whales (*Delphinapterus leucas*) using two different methods: ^{51}Cr release assays and flow cytometry. It was determined that while NK cell activity was readily detected using both methods, the flow cytometric method was deemed the best alternative. It was also concluded that the NK cell activity observed was similar to that of other species. De Guise and colleagues had previously suggested that deficient NK cell activity could explain why the isolated population of St. Lawrence belugas have such a large number of tumours (De Guise *et al.* 1994).

f) Relationship between NK Cell Activity, Immune Function and the Risk of Disease

A number of additional *in vitro* exposure studies have been conducted on various species to assess the effects of PBCs on NK cell activity. For example, exposure of rat spleen cells to PCBs resulted in a significant depression of splenic NK cell activity. It was suspected that this exposure may in part explain the tumour-inducing effect of these chemicals, through compromising the immune surveillance system (Talcott *et al.* 1985). Various studies in both humans and other animals have also demonstrated that NK cell deficiencies can lead to increased susceptibility to a number of different viral infections. For example, a human patient suffering from recurring and life-threatening viral infections including varicella, hepatitis, cytomegalovirus, and herpes simplex, lacked functional NK cells, but all other immune parameters examined were normal (Biron *et al*. 1989). It has also been seen that young, NK-deficient mice (Boos *et al.* 1971), and beige mice (Shellam *et al*. 1981), are less resistant to mouse cytomegalovirus (MCMV). Similarly, mice with severe combined immunodeficiency (SCID) showed a depletion of NK cells and NK cell activity and eventually succumbed to infection by MCMV while immunocompetent mice did not (Welsh *et al.* 1991).

Despite the amount of evidence confirming the role of NK cells as an important component of the innate immune system, and the reduction in their activity associated with exposure to

environmental contaminants, there appears to be little information on reference values of normal ranges of NK cell activity in animals or indeed humans. There is even less information on the NK cell activity levels that categorise an animal as immunocompromised, or thresholds of activity that suggest a level below which an animal would be incapable of generating an immune response to infection.

In one study however, Ojo-Amaize and colleagues (Ojo-Amaize *et al.* 1994) investigated the decreased NK cell activity associated with Chronic Fatigue Immune Dysfunction Syndrome (CFIDS) in humans. The study monitored the NK cell activity of patients split into 3 clinical groups; a group with the least severe clinical condition, a clinical condition of intermediate severity, and a group with the most severe clinical condition as determined by the criteria established by the Centres for Disease Control and Prevention. Briefly, these clinical conditions were grouped according to the severity of symptoms including fatigue, short-term memory and concentration ability as well as physical flu-like symptoms. Their NK cell activity, as well the NK cell activity of a group of 50 healthy control patients, was assessed by the number of lytic units (LU). The results are summarised in Table 3 below. The average NK cell activity and the percentage NK cell activity compared to the healthy controls were calculated to potentially make these data applicable to other species.

Table 3 – NK Cell Activity of Groups of Patients with Varying Levels of Clinical CFIDS symptoms.

Health Class	NK Cell Activity Range (LU)	Average NK cell Activity (LU)	% NK Cell Activity of the Healthy Controls
	20 – 250		
Healthy Controls	50% 20-50 32% 51-100 6% 101-130 12% > 150	66.6	100
Least Severe Clinical Condition	61 ± 21.7	61.0	91.6
Intermediately Severe Clinical Condition	18.3 ± 7.3	18.3	27.5
Most Severe Clinical Condition	8.0 ± 5.3	8.0	12.0

The authors concluded that these data suggested a correlation between low levels of NK cell activity and the severity of CFIDS, which, if confirmed by additional studies would be useful for subgrouping patients and monitoring therapy and/or the progression of CFIDS. In terms of applying these data to other species, it may be possible to use the percentage NK cell activity compared to healthy controls to classify the extent to which individuals are immunocompromised. If we have information on the average NK cell activity levels of 'healthy' individuals, then these percentage reductions in activity could be used to classify individuals with varying levels of immunosuppression. There will undoubtedly be species specific and individual differences in response, nevertheless, this is useful starting point for further investigation.

g) Lymphocyte Function and Proliferation

Lymphocytes are small white blood cells that arise and develop in the thymus and the bone marrow, and once mature, continually circulate around the body between the blood, body tissues and

lymphoid organs. Lymphocytes can be split up into two classes : T-cells, which make up 75% of lymphocytes in mammals, and B-cells. T-cells are responsible for cell-mediated immunity in that they attack cells of the body that have been taken over by viruses or have become cancerous, while B-cells are responsible for humoral immunity as they produce antibodies that attack bacteria and toxins. Stimulation of these lymphocytes by specific antigens should result in the recognition of a pathogen and the mounting of an effective immune response, without which, the pathogen may not be eliminated.

In vitro evaluation of cellular immunity is done using purified populations of peripheral blood mononuclear cells (PMBCs) (Dierauf, 1990). One assay that is often used to evaluate cellular immunity is lymphocyte proliferation. The basis of the assay is that the incorporation of radiolabelled thymidine into PMBCs cultured with a mitogen is a relative measure of the number of cells dividing in response to the mitogen. Thus, the activation of T lymphocytes results in rapid proliferation, which can be quantified *in vitro* by the incorporation of a radioactively-labelled precursor into the newly synthesised DNA, RNA or protein (Dierauf, 1990). Mitogens include plant lectins and other substances that non-specifically stimulate lymphocytes to proliferate. The lectins, phytohemagglutinin (PHA), and concanavalin (Con A) are two of the main mitogens used for such assays. PHA preferentially stimulates the proliferation of helper T cells, while Con A primarily stimulates the proliferation of cytotoxic/suppressor T-cells (Dierauf, 1990).

h) Lymphocyte Proliferation in Marine Mammals and Exposure to PCBs

a. <u>In vitro Studies</u>

The effects of *in vitro* exposure to 4 different PCB congeners found in St Lawrence beluga blubber were evaluated on phagocytosis and cell proliferation (De Guise *et al.* 1998). Both PHA-stimulated and unstimulated beluga whale leukocytes were exposed to varying concentrations of the 4 PCB congeners, only one congener, PCB 138 (the most immunotoxic single congener in this study), significantly reduced the proliferative response of the lymphocytes. However, when cells were exposed to a mixture of 3/4 congeners at the lowest final concentration of 5ppm, the PHA-stimulated proliferation of beluga splenocytes was also significantly reduced. These data suggest a possible synergistic effect of some PCB compounds on beluga whale splenocytes at concentrations that are within the range of those measured in the wild in St. Lawrence beluga whale blubber.

In the study on captive and free-ranging sea otters (Levin *et al*. 2007), the acquired immune functions were evaluated through assessing mitogen-induced B and T lymphocyte proliferation. Four individual PCB congeners were tested, as well as the 26 possible combinations of these. It was seen that at 5ppm, six mixtures significantly increased ConA-induced lymphocyte proliferation from free-ranging sea otters, but that there was no effect on the lymphocytes from the captive otters. As for the neutrophil results it was thought that these differences in susceptibility may have been due to the acute stress of capture, the chronic stress of captivity, or nutritional differences between the two groups.

The immunomodulatory effects of four different PCB congeners were assessed through changes in mitogen-induced B lymphocyte proliferation in pilot whales (*Globicephala melaena*), beluga whales (*Delphinapterus leucas*), and bottlenose dolphins (*Tursiops truncatus*) in cetaceans, harbour seals (*Phoca vitulina*) and Northern fur seals (*Callorhinus ursinus*) in pinnipeds, and also sea otters (*Enhydra lutris*) (Mori *et al.* 2008). Any changes in lymphocyte proliferation for these species were also compared to mice mitogen-induced lymphocyte proliferation under the same conditions. In the mice, *in vitro* exposure to all the PCB mixtures and individual congeners at 5ppm induced significant

decreases in lymphocyte proliferation. But in the marine mammals, the in vitro exposures exerted no effect on the lymphocytes of the belugas or the sea otters. Some mixtures however induced a significant increase in the lymphocyte proliferation of pilot whales, bottlenose dolphins, harbour seals, and Northern fur seals. Interestingly, it was also seen that simple additive effects of the PCB congeners in mixtures were found only in mice, while both synergistic and antagonistic interactions between congeners were found in marine mammals. Importantly, the commonly used mouse model failed to predict the immunotoxicity associated with PCB contamination in marine mammals. It was concluded that these findings may be important in more accurately characterising the immunotoxic potential of organochlorines in different target species to develop a more relevant risk assessment.

Frouin et al. (2010) investigated the *in vitro* effects of PCBs and various heavy metals on the proliferation of T lymphocytes from the thymus, the lymph nodes and the blood of a juvenile female grey seal (*Halichoerus grypus*). Exposure to 7 different PCB Aroclor mixtures led to reduced proliferation of the thymocytes, and the T lymphocytes from the lymph nodes and the blood. Overall, it was seen that lymph node lymphocytes are more sensitive to PCBs than peripheral blood lymphocytes, but they are less sensitive than thymocytes. This suggests that T-lymphocyte proliferation is tissue/matrix dependent. However, it was noted that this individual response may not be representative of the species as a whole, and a greater number of animals should be sampled to confirm these results.

The effects of one of the most common PCB mixtures, Aroclor 1254, and heavy metal chlorides, were investigated on lymphocyte proliferation in two phocid species; harbour seals and grey seals (Dufresne *et al.* 2010). Blood samples were taken from harbour seals that were live captured in the St Lawrence estuary in Canada, and grey seals held at the Zoological Society of Granby, Canada. Only grey seal PBMCs were exposed to Aroclor 1254 (15ppm and 50ppm) which resulted in a dose-dependent inhibition of Con A-induced T-lymphocyte proliferation. However, for heavy metal compounds tested, it appeared that harbour seal cells were less sensitive to methyl mercury at concentrations that had detrimental effects on lymphocyte proliferation in the grey seals. These observations were in contrast to those seen in a previous *in vitro* study whereby the innate immune functions of harbour seals were more greatly reduced than those of grey seals when exposed to PCBs (Hammond *et al.* 2005).

b. *In vivo studies*

In the long term harbour seal feeding experiment (Ross et al. 1994), blood samples were taken throughout the experiment, and mitogen-induced T-lymphocyte responses and other immune functions were assessed. The T-lymphocyte proliferative responses to the mitogens Con A and PHA were seen to decline in the Baltic seals between 6-10 months after the start of the experiment, indicating a reduction in T-cell function. There was a more rapid appearance of an impairment of NK cell activity after only 4 months, and it was thought that this difference in timing may be due to the ontogeny of these two leukocyte subpopulations.

Peripheral blood samples were obtained from a number of free-ranging, male bottlenose dolphins along the west coast of Florida (Lahvis *et al*. 1995). The mitogen-induced lymphocyte responses of individual dolphins were determined *in vitro*, and compared by regression analysis to contaminant concentrations in the blood from a subset of these animals. Results indicated that a reduced immune response was correlated with increasing whole blood concentrations of several of the contaminants quantified. Of the PCB classes quantified, the tetra-chlorinated and octa-chlorinated PCBs showed the strongest inverse correlation with lymphocyte proliferative responses to Con A. Regression analyses between lymphocyte proliferative responses to Con A and the levels of DDT and

DDT metabolites showed a similar inverse relationship. Interestingly, weaker correlations, or no correlation at all was observed in PHA-induced lymphocyte proliferation.

Pacific harbour seals, aged between three to five weeks were captured at four haulout sites, two urban and two remote sites of varying sizes, human population density and agricultural activity in British Columbia and Washington State (Mos *et al*. 2006). Blood and blubber samples were collected to quantify hematology, innate immune function, adaptive immune function and PCB accumulation. Along with the other immune parameters Con A induced T-lymphocyte proliferation was negatively correlated with blubber PCB concentrations in the seal pups. In addition to this reduced functionality, the more contaminated seals had decreased circulating concentrations and decreased percentages of lymphocytes in the WBC counts. It was concluded that PCBs appear to be affecting both the quality and quantity of lymphocytes, and therefore, the adaptive immune system as a whole may be less able to respond to infectious agents.

Health evaluations were conducted on 29 free-ranging bottlenose dolphins sampled from Brunswick along the Georgia coastline in the United States which is known to be heavily contaminated by Aroclor 1268, a relatively uncommon PCB mixture which is primarily made up of octa- through deca-chlorobiphenyl congeners (Schwacke *et al.* 2011). These dolphins suffered from anaemia and reduced thyroid hormone levels, consistent with the strong evidence from other studies that PCBs cause thyroid hormone disruption. In addition, mitogen-induced T-lymphocyte proliferation, using Con A also showed a negative correlation with blubber PCB concentration. Of the dolphins sampled, the adult females had the highest PCB concentrations. These data demonstrate that bottlenose dolphins are vulnerable to several PCB-related health effects, and that the reduction in lymphocyte proliferation is highly likely to be indicative of a decrease in host resistance.

The significant negative relationship between Con A induced T-lymphocyte proliferation and PCBs in bottlenose dolphin blubber was then used in our individual based risk assessment model as the most robust dataset providing a quantifiable concentration-response relationship (see methods below).

Table 4. Summary of the *in vitro* and *in vivo* studies on lymphocyte proliferation

Increased Lymphocyte Proliferation	Decreased Lymphocyte Proliferation
Pilot whales *(in vitro)* (Mori *et al.* 2008)	Bottlenose dolphins *(in vivo)* (Lahvis *et al.* 1995)
Bottlenose dolphins *(in vitro)* (Mori *et al.* 2008)	Harbour seals *(in vivo)* (Ross *et al.* 1996)
Harbour seals *(in vitro)* (Mori *et al.* 2008)	Beluga whales *(in vitro)* (De Guise *et al.* 1998)
Northern fur seals *(in vitro)* (Mori *et al.* 2008)	Harbour seals (Mos *et al.* 2006)
Sea otters *(in vitro)* (Levin *et al.* 2007)	Grey seal *(in vitro)* (Frouin *et al.* 2010)
	Grey seals (*in vitro*) (Dufresne *et al*. 2010)
	Bottlenose dolphins *(in vivo)* (Shwake *et al.* 2011)

These studies indicate that cetaceans are sensitive to the effects of PCBs on their lymphocyte proliferation capacity, and this is an appropriate and effective immune parameter to for assessing the immune function of an animal. In these various studies, the lymphocyte proliferation was shown to either increase or decrease, but not always to the same magnitude, and not always in response to the same concentrations of the contaminants. These very different responses of the lymphocytes could be a consequence of the differences between the *in vitro* and the *in vivo* studies. Of the five studies conducted *in vitro* mentioned here, comprising of seven different species, five of them showed increased lymphocyte proliferation when exposed to varying concentrations of PCBs (see Table 4). Of the four *in vivo* studies discussed here on harbour seals and bottlenose dolphins, they all showed a decrease in lymphocyte proliferation correlated with increased concentrations of blubber PCBs.

The significance *in vivo* of the changes reported in these *in vitro* exposure studies are hard to assess. At the cellular level, the effects of PCBs are probably mediated by other aspects of the animals' physiology including their metabolism, other tissue components, matrix structure etc. In addition, mixtures of several organochlorines rather than individual PCB congeners alone are likely to affect the ability of either quiescent or stimulated lymphocytes to proliferate. There may also be possible synergistic effects of complex mixtures, long-term exposure, health and nutritional status that will affect the cells of the immune system within the whole animal and interactions with the endocrine system, particular the thyroid gland, are paramount. These other influences on the immune cells are possibly the reason why differing results have seen between *in vitro* and *in vivo* studies. It is therefore hard to use results of *in vitro* studies to assess the potential consequences on individuals in the wild, because a cellular response *in vitro* may not necessarily reflect the response taking place within the whole organism. For this reason, when modelling the effects of PCBs in wild populations, as here, it is probably more representative to use the data on lymphocyte proliferation from *in vivo* studies wherever possible.

i) Relationship between Lymphocyte Proliferation, Immune Function and the Risk of Disease

Lymphocyte proliferation plays an important role in the clonal expansion response following encounter with an antigen, so a reduction in the proliferative potential of lymphocytes may create a significant weakness in a functional immune system. A small defect in immune responsiveness may then provide an opportunity for a pathogen to invade an organ system and create a clinical disease (De Guise *et al.* 1998). As such, a lowered T-lymphocyte response could increase susceptibility to infectious disease, particularly of viral origin (Schwacke *et al.* 2011).

Quantitative, objective data on the magnitude of changes in host resistance following immunosuppression is lacking. However, one comprehensive study published by Luster *et al.* (1993) carried out by the National Toxicology Program, quantitatively determined the relationships between a variety of immune function parameters, including reduced responsiveness of T-lymphocytes to stimulation with Con A and host resistance in controlled experiments in laboratory mice. The aim was to develop statistical models that examined both the qualitative and quantitative relationships between immune function and host resistance pathogen challenge tests which could be translated into changes in survival probabilities. The authors reported the coefficients from the statistically significant relationships between changes in many of the immune tests and altered host resistance. Host resistance was defined as being the likelihood that an animal was unlikely to survive to the next time point following challenge with a pathogenic agent and was interpreted as a proportional reduction in survival probability. There were no instances where host resistance was altered without affecting an immune test.

Groups of mice were treated with a broad-spectrum immunosuppressant, cyclophosphamide, at a range of dose levels. The immune and host resistance assessments were initiated within 48 hours of injection. These tests included 12 different immune function assays and resistance tests against various bacterial pathogens as well as transplantable tumour cells. Of interest for our model was the specific relationship between the proportional decrease over the controls in the T lymphocyte response to Con A and the proportional decrease in survival after challenge with two bacterial pathogens.

Using either logistic or standard regression, linear and linear quadratic models were fitted to the immune assay and host resistance data to establish which model accurately described the relationship. Most of the immune-host relationships appeared to approximate a linear model. Parameter estimates derived from the most appropriate model for the relationship between each

assay combination were reported. Finally, for each pair of host resistance and immune function tests that could be fit by one of the two logistic models, the mathematical function representing the relative difference was developed (see Appendix 1 for the form of the equation).

The parameter estimates relating Con A-induced T-lymphocyte proliferation to host resistance against two bacterial pathogens *Listeria monocytogenes,* which causes listeriosis and often manifests itself as sepsis, meningitis and encephalitis and *Streptococcus pneumoniae,* which is recognised as a major cause of pneumonia are shown in Table 5. These two pathogens have contrasting virulences with *S. pneumoniae* being more pathogenic than *L. monocytogenes*. This gave us the opportunity to investigate what impact exposure to different pathogens could have at the population level.

Table 5 – Parameter estimates for the host resistance models

Host Resistance Assay	Immune Test	Intercept Parameter	Linear Parameter
L. monocytogenes	Con A	-2.5639	2.7903
S. pneumoniae	Con A	-1.9401	5.8436

IV. Population level effects of PCB exposure including impacts on calf survival and immune function

The approach taken to determine the effects of PCB exposure on cetacean populations builds on the individual based model primarily constructed to investigate the impact of maternal PCB exposure on calf survival and consequently on potential population growth rate (Hall et al. 2006, Hall et al 2011). The effect of additional immune suppression impacts affecting juvenile and adult mortality has now been added to the model. However, for this to have an effect on the population animals must be subsequently exposed to an infection. The model simulations presented here allow us to investigate the population consequences of varying the proportion that encounter an infectious pathogen each year. By integrating the relationship between reduced T lymphocyte Con A proliferation and exposure to PCBs in bottlenose dolphins with the results of the Luster *et al.* (1993) studies quantifying the link between this immune assay and host resistance in mice, we have been able to include both PCB-related impacts on immunity as well as on calf survival into the model framework.

1. Methods and Results

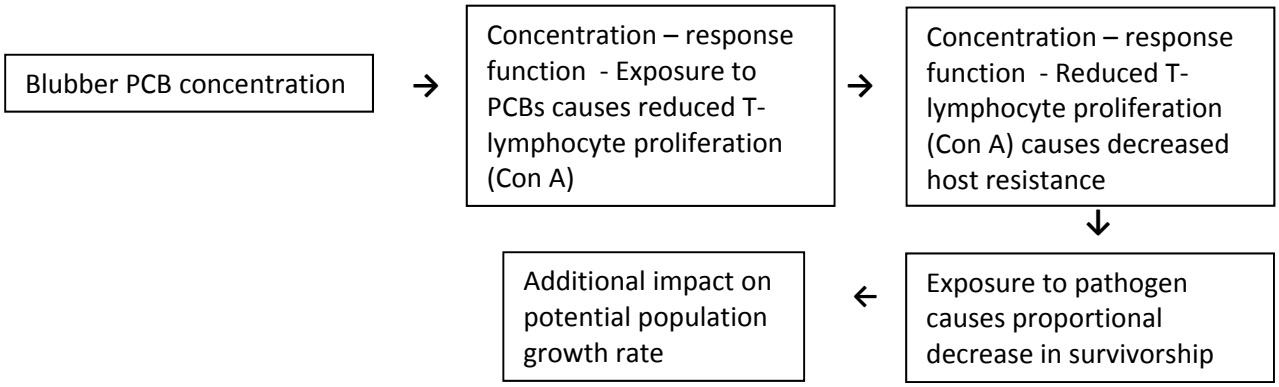
Full details of the modelling approach are given in Hall *et al.* (2006) and Hall *et al.* (2011). Briefly, the model is a female-only individual based population model. When females reach breeding age they become pregnant with a certain probability then during gestation and lactation offload a proportion of their blubber PCB to the calf. The probability of survival of the offspring is modified by a concentration response function relating maternal PCB to offspring survival estimates (see Hall *et al.* 2011 for details). Juvenile and adult survival is then also modified using the blubber PCB immune suppression concentration response function following exposure of a specified proportion of the population to a pathogen. Population trajectories are generated for 100 years and after 40 years of simulations the mean potential population growth is calculated.

1) <u>Relationship between T-lymphocyte proliferation (Con A response) and PCBs in bottlenose dolphins</u>

A two stage process was implemented whereby the functional response between the proportional decrease in T-lymphocyte response to Con A stimulations and proportional decrease in survival

(from Luster *et al*. 1993) was combined with the function relating T-lymphocyte proliferation response to Con A to blubber PCB concentrations from wild bottlenose dolphins from several sites along the east coast of the US using the data from Schwacke *et al*. (2011). The steps involved in this process are shown in Fig. 1.

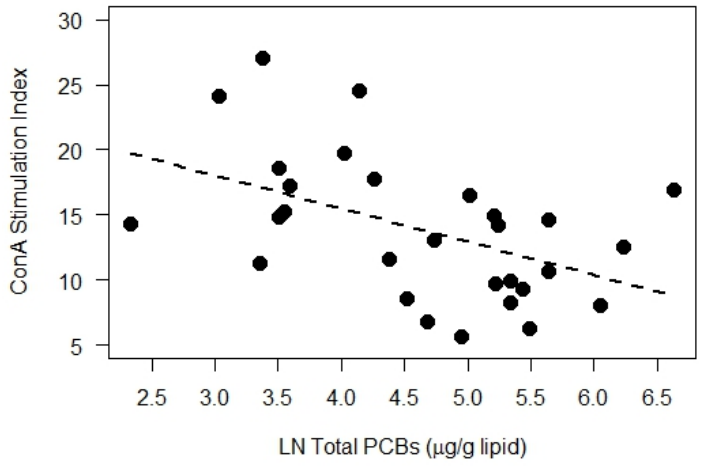
c. Fig 1. Steps involved in estimating change in survival probability in relation to exposure to PCBs through immune suppression.



In order to utilise the Luster *et al*. (1993) predictive relationships between the proportional change in T-lymphocyte proliferation in response to Con A and proportional change in survival probability, data from Schwacke et al. (2011) were converted to a *proportional* change in response to Con A in relation to an estimated maximal response. Thus the "control" was taken as the T-lymphocyte response to Con A at the intercept in the relationship shown in Figs. 2a and 2b. This was then used to convert the Con A stimulation index originally calculated to a change in Con A over the control, making it directly comparable to the data published by Luster and colleagues. This relationship was then used to estimate the additional proportional decrease in survival probability following pathogen exposure for given concentrations of blubber PCBs (see Fig 3.).

Fig. 2. Relationship between change in T-Lymphocyte response to ConA and log blubber PCBs in bottlenose dolphins (a) Stimulation index – maximal "control" response taken at the intercept (b) proportional change in index over control

(a)



(b)

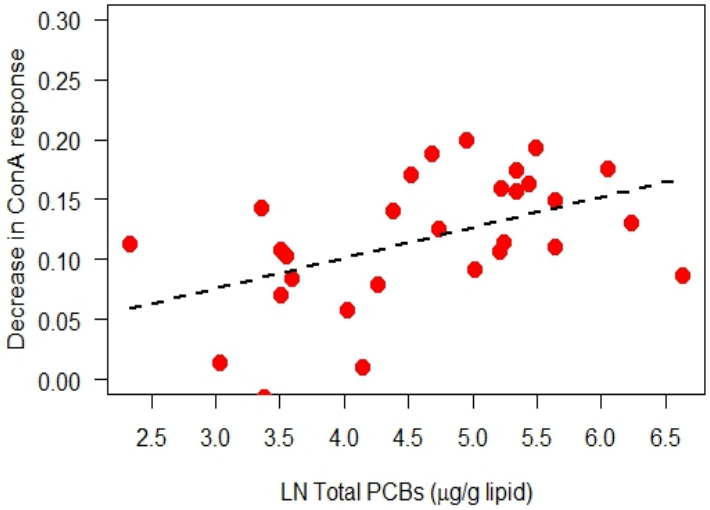
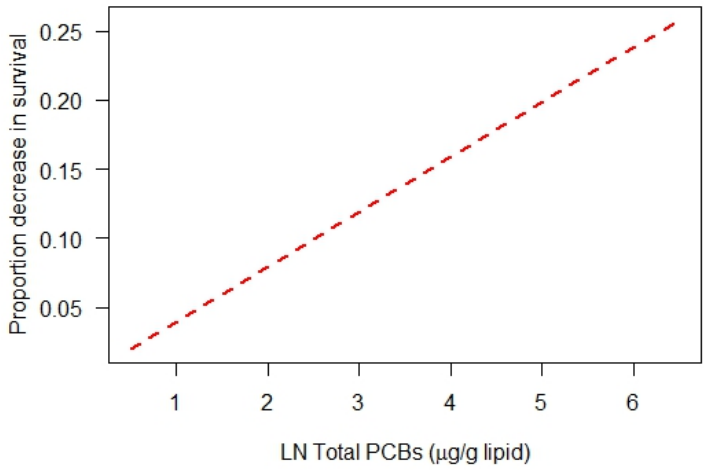


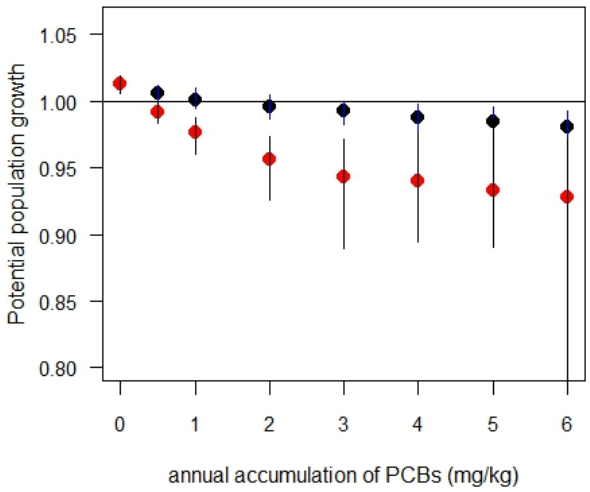
Fig. 3. Relationship between blubber PCBs in bottlenose dolphins and proportional decrease in survival probability following exposure to a class 2 pathogen, *L. monocytogenes*.



2) Bottlenose dolphin population model simulations including effects on calf survival and immune function

Model simulations with varying PCB annual accumulations were run with 100 iterations using the vital rates estimated for the Sarasota bay population as previously reported (Hall *et al.* 2011). The concentration response function for the impacts of maternal PCBs estimated from the mink laboratory model data were incorporated, with uncertainty (Hall *et al.* 2011). The additional effect of immune suppression with subsequent exposure of 10% the population each year to a pathogen was initially investigated. Fig. 4 shows the results of simulations for annual accumulations of between 0.5 to 6 mg/kg for exposure to a pathogen with similar virulence and pathogenicity to *L. monocytogenes* compared to *S. pneumoniae.*

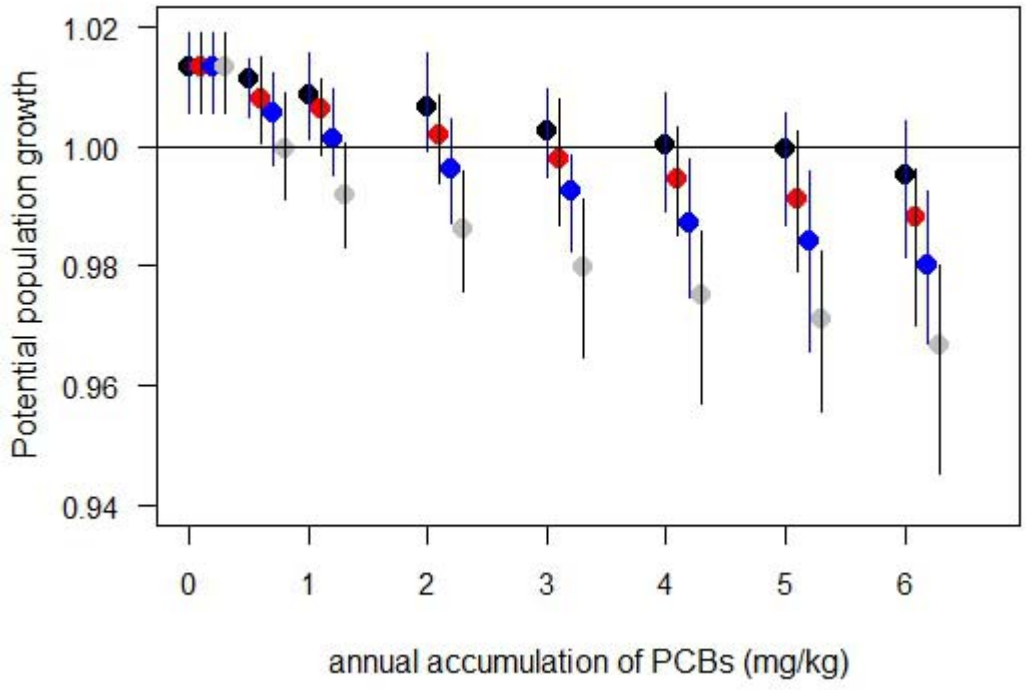
Fig. 4. Change in potential population growth for different annual accumulations of PCBs in the blubber of bottlenose dolphins following exposure to L. monocytogenes (black circles) and S. pneumoniae (red circles). The horizontal line shows a stable potential population growth.



At this level of exposure effects on the potential population growth are seen at an annual accumulation of > 1 mg/kg for the pathogen with lower virulence but for the more pathogenic organism effects are seen at annual accumulations of < 0.5 mg/kg.

Further simulations to determine the impact of exposing different proportions of the population each year to a lower virulence pathogen were carried out and the results are shown in Fig. 5. The model outputs (potential population growth rates) are compared to simulations in which only maternal PCB effects on calf survival were included so the magnitude of the additional immune suppression on potential population growth can be seen. At lower annual accumulations of <1 mg/kg major population impacts and population growth rates of less than one were seen when 20% of the population each year were exposed to a pathogen. However, for annual accumulation concentrations of >2 mg/kg immune impacts were seen when 10% or more of the animals were exposed each year. At 3 mg/kg then effects were seen at 5% or more of the population encountering a pathogen each year. Thus the additional effect of incorporating immune suppression effect in the model represents a further 0.3 to 3 % decrease in potential population growth, depending on the proportion exposed each year and the annual accumulation rate (i.e. degree of contamination in the prey).

Fig. 5. Change in potential population growth for different annual accumulations of blubber PCBs in bottlenose dolphins with different proportions of the population exposed to a class 2 pathogen. Calf survival effects only = black, immune effects with 5% exposed to a pathogen = red circles, 10% exposed to a pathogen = blue circles and 20% exposed to a pathogen = grey circles. Horizontal line = stable population growth.



3) Validation using empirical data from bottlenose dolphins

Additional data are now available on concentrations of PCBs in the blubber of bottlenose dolphins from the various populations around the US coast (Schwacke et al. 2011) which will allow us to investigate model predictions, now and into the future. One output from the model is the concentration of PCBs in each individual female at the end of the simulation run (see Fig. 6). By comparing these with concentrations found in the mature females (those that have already given birth to a calf and have depurated their contaminants during at least one lactation) within a population we can estimate what these represent in terms of their annual accumulation of PCBs and therefore what that level of exposure would represent for the population dynamics. Fig. 7 shows the relationship between blubber PCBs in the mature females and the annual accumulation concentration from a baseline model incorporating fecundity and immune effects with 10% of the population exposed to a pathogen. Also shown are the geometric mean concentrations of PCBs measured in bottlenose dolphins from Sarasota Bay, Florida, St Joseph Bay, Florida and Brunswick, Georgia (Schwacke *et al*. 2011 and Schwacke *et al*. unpublished data). Thus, for populations that have underlying vital rates similar to those published for the Sarasota Bay population and used in these simulations, the resulting estimated annual accumulation would be approximately 0.5 mg/kg for the lower exposed populations whereas it would be almost 6 mg/kg for the highly exposed populations. Populations such as those monitored in Florida are thus likely to be stable or increasing slightly whereas for the much higher exposed population such as Brunswick we would predict a decline in the abundance of dolphins over time, given no compensatory population inputs or changes in vital rates over time.

Fig. 6. Example of model output showing concentration of blubber PCBs in females by age. Individuals are shown as open circles. Red line indicates the mean concentration.

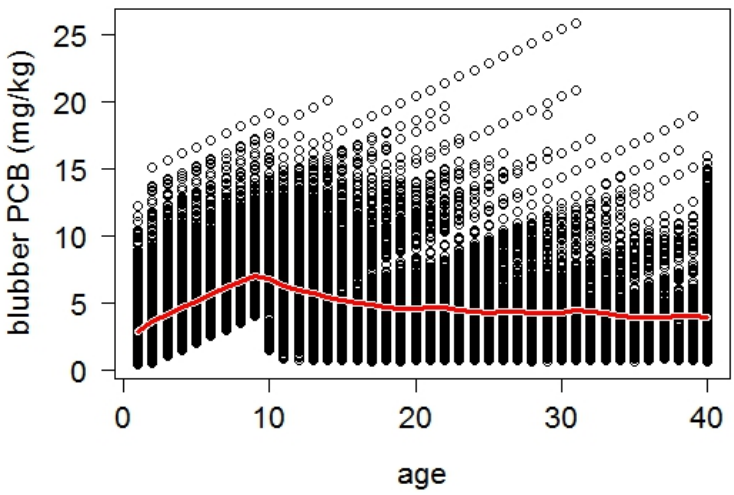
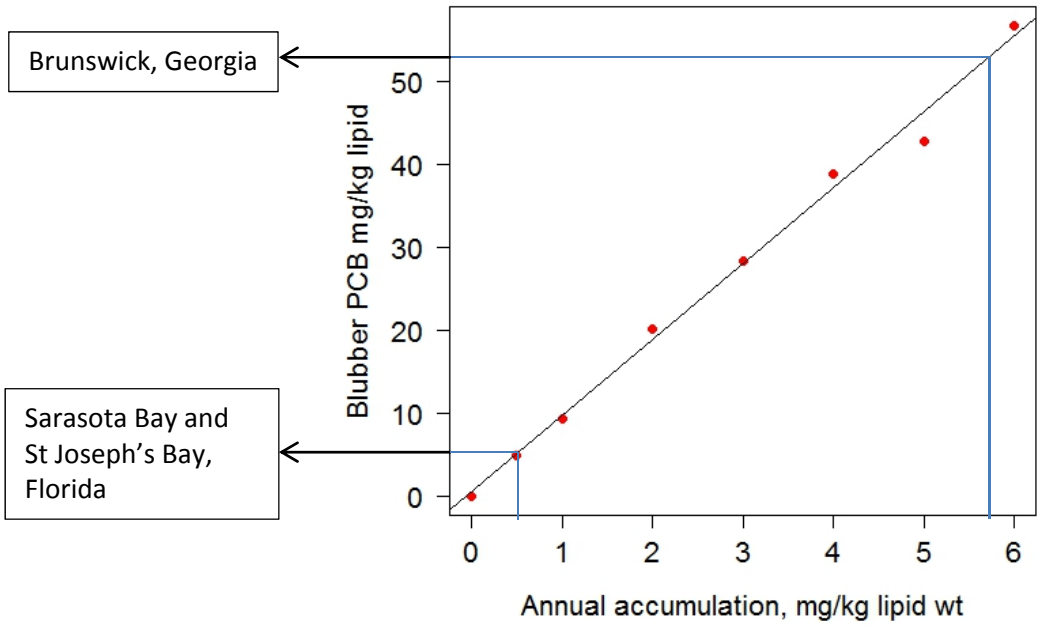


Fig. 7. Relationship between blubber PCB concentrations in mature, breeding females and annual accumulation of PCBs. The line shows the fitted linear regression model with slope = 9.16, SD = 0.31, R^2=0.9932, p<0.0001. Two populations of bottlenose dolphins in which blubber PCBs have been measured in known age animals (Schwacke et al. 2011 and unpublished data) are shown.



4) Conclusions

We have demonstrated that this model framework can include other contaminant induced health effects in addition to the impact of calf survival previously investigated (Hall *et al*. 2011) and we have modified it for future continued development and flexibility. The simulations reported here used the bottlenose dolphin as a demonstration species for which sufficient population dynamic data were available. Additional effects of PCB-induced immune suppression were incorporated by modelling their impact on survival following exposure to a pathogen. However, we still need to determine a realistic level at which to set the proportion of the population exposed. The laboratory model data are based on controlled exposure of caged mice in which pathogen uptake is highly likely due to the dosing regime. In a wild population of bottlenose dolphins even exposing 5% of the population each year to a class 2 pathogen may be an over estimation. However, this gives us a degree of precautionary approach and further data on disease occurrence in this species will allow us to investigate this in future.

In addition, the Luster et al. (1993) studies relating immune function assays to proportional changes in host resistance and survival probability suggested that given the different magnitude of responses between different immune function assays and between innate and acquired immunity that more than one assay should be included in a battery of tests. As such, we would recommend inclusion of a second assay. For example the investigation of NK cell activity in relation to blubber PCB concentrations in bottlenose dolphins would provide a further insight into the impact on an arm of the innate immune system important in defence against viral infection.

Broad and general potential population dynamic predictions can be made for specific populations when estimates of PCB concentrations in mature, breeding females are known. These impacts can then be compared to other population pressures (such as interactions with boats, shipping and fisheries so that the overall effect of pollutant insults can be placed into a relative management context.

Impacts on other populations and species, such as Humpback whales from the Gulf of Maine, could easily be investigated. Future developments of the model will include a sensitivity analysis to determine which parameters have most influence on model outputs; incorporation of a bioaccumulation model to determine blubber concentrations from prey so that species for which blubber PCB data are lacking could be investigated and availability of the model online with a user-friendly interface.

V. Additional Contaminant of Concern

The additional group of contaminants of concern identified by the Steering Committee were the polybromiated diphenyl ethers or PBDEs which have similar lipophilic properties, structural activities and potential adverse health effects to the PCBs. Concentrations measured in marine mammals and specifically in cetaceans are generally orders of magnitude lower than the PCBs. But some correlations with immune effects in particular have been reported in marine mammals and including cetaceans and selected references are summarised in Appendix 2.

However, we were unable to find mammalian studies linking immune effects of PBDEs to host resistance as was available for the PCBs. One study by Arkoosh *et al*. 2010 exposed Chinook salmon to PBDEs and then investigated their susceptibility to *Listonella*. The results indicated that salmon exposed at the lower dose level showed higher susceptibilities to *Listonella* infection than those exposed to the higher concentration of PBDEs. The authors suggest that this dichotomous effect has been reported for other species including mink (Martin *et al*. 2007, although this was a mixed exposure study so not directly comparable). However, given the differences between fish and mammalian immunity it is difficult to use these results in our model framework. Future work on this project will continue to search the literature, particularly papers as they become available that could provide relevant data in the correct form for inclusion in a future version of our individual based model.

VI. References

Arkoosh, M.R., D. Boylen, J. Dietrich, F. Anulacion, G. Ylitalo, C. F. Bravo, L.L. Johnson, F. J. Loge and Collier, T.K. 2010. Disease susceptibility of salmon exposed to polybrominated diphenyl ethers (PBDEs) . Aquat. Toxicol. 98:51-59.

Bergman, A., M. Olsson and S. Reiland. 1992. Skull-bone lesions in the Baltic grey seal (*Halichoerus grypus*). Ambio 21**:** 517-519.

Boegner, F., D. Franke, H. Altenkirch, G. Stoltenburg and M. Wagner. 1994. PCBs have a predominantly neurotoxic effect on dissociated cultures of the nervous system. Neurotoxicology 15**:** 593-596.

Biron, C. A. Byron, K. S. Sullivan, J. L. 1989 Severe herpes virus infections in an adult without natural killer cells. New Engl. J. Med. 320: 1731-1735.

Biron, C. A. Nguyen, K. B. Pien, G. C. Cousens, L. P. Salazar-Mather, T. P. 1999. Natural Killer Cells in Antiviral Defence: Function and Regulation by Innate Cytokines. Annu. Rev. Immunol. 17: 189-220.

Boersma, E. R. and C. I. Lanting. 2000. Environmental exposure to polychlorinated biphenyls (PCBs) and dioxins. Consequences for longterm neurological and cognitive development of the child lactation. Adv Exp Med Biol 478**:** 271-287.

Boos, J. Wheelock, E. F. 1971. Correlation of survival from MCMV infection with spleen cell responsiveness to concanavilin A. Proc. Sot. Exp. Biol. Med. 149: 443-446.

Brouwer, A., P. J. H. Reijnders and J. H. Koeman. 1989. Polychlorinated biphenyl (PCB)-contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (*Phoca vitulina*). Aquatic Toxicology 15**:** 99-106.

Boxer, L. Dale, D. C. 2002. Neutopenia : Causes and Consequences. Seminars in Hematology. 39: 75-81

Camara Pellisso, A. Munoz, M. J. Carballo, M. Sanchez-Vizcaino, J. M. 2008. Determination of the immunotoxic potential of heavy metals on the functional activity of bottlenose dolphin leukocytes *in vitro*. Veterinary Immunology and Immunopathology. 121 : 189-198.

Cocchi, D., G. Tulipano, A. Colciago, V. Sibilia, F. Pagani, D. Vigano, T. Rubino, D. Parolaro, P. Bonfanti, A. Colombo and F. Celotti. 2009. Chronic treatment with polychlorinated biphenyls (PCB) during pregnancy and lactation in the rat Part 1: Effects on somatic growth, growth hormone-axis activity and bone mass in the offspring. Toxicology and Applied Pharmacology 237**:** 127-136.

Crofton, K. M., P. R. S. Kodavanti, E. C. Derr-Yellin, A. C. Casey and L. S. Kehn. 2000. PCBs, thyroid hormones, and ototoxicity in rats: Cross-fostering experiments demonstrate the impact of postnatal lactation exposure. Toxicological Sciences 57**:** 131-140.

De Guise, S. Lagace, A. Beland, P. 1994. Tumors in St. Lawrence beluga whales (Delphinapterus leucas). Vet. Pathol. 31: 444-449

De Guise, S. Flipo, D. Boehm, J. R. Martineau, D. Beland, P. Fournier, M. 1995. Immune functions in beluga whales (*Delphinapterus leucas*) : Evaluation of phagocytosis and respiratory burst with peripheral blood leukocytes using flow cytometry. Vet Immunol Immunopathol. 47 : 351-362.

De Guise, S. Martineau, D. Beland, P. Fournier, M. 1995. Possible Mechanisms of Action of Environmental Contaminants on St. Lawrence Beluga Whales (*Delphinapterus leucas*). Environmental Health Perspectives. 103 : 73 – 77

De Guise, S. Ross, P. S. Osterhaus, A. D. M. E. Martineau, D. Beland, P. Fournier, M. 1997. Immune functions in beluga whales (*Delphinapterus leucas*) : evaluation of natural killer cell activity. Vet Immunol Immunopathol. 58: 345-354

De Guise, S. Martineau, D. Béland, P. Fournier, M. 1998. Effects of in vitro exposure of beluga whale leukocytes to selected organochlorines. Journal of Toxicology and Environmental Health. 55 : 479-493.

De Swart, R.L. Kluten, R. M. G. Huizing, C. J. Vedder, L. J. Reijnders, P. J. H. Visser, I.K.G. UytdeHaag, F.G.C.M. Osterhaus, A.D.M.E. 1993. Mitogen and antigen induced T and B cell responses of peripheral blood mononuclear cells from the harbour seal (*Phoca vitulina*). Vet. Immunol. Immunopathol. 37: 217-230.

De Swart, R. L. Ross, P. S. Vedder, L. J. Timmerman, H. H. Heisterkamp, S. H. Van Loveren, H. Vos, J. G. Reijnders, P. J. H. Osterhaus, A. D. M. E. 1994. Impairment of immune function in harbor seals *(Phoca uitulina)* feeding on fish from polluted waters. Ambio 23, 155-159.

De Swart, R. L. Ross, P. S. Vedder, L .J. Boink, F. B. T. J. Reijnders, P. J. H. Mulder, P. G. H. Osterhaus, A. D. M. E. 1995. Haematology and clinical chemistry values of harbour seals (Phoca vitulina) fed environmentally contaminated herring remain within normal ranges. Can J Zool 73:2035-2043.

De Swart,R. L. Ross, P. S. Vos, J. G. Osterhaus, A. D. M. E. 1996. Impaired Immunity in Harbour Seals (*Phoca vitulina*) Exposed to Bioaccumulated Environmental Contaminants: Review of a Long-term Feeding Study. Environmental Health Perspectives. 104 : 823 – 828.

Dierauf, L. A. 1990. Handbook of Marine Mammal Medicine: Health, Disease and Rehabilitation. CRC Press Inc: Boca Raton, Florida.

Dufresne, M. M. Frouin, H. Pillet, S. Lesage, V. De Guise, S. Fournier, M. 2010. Comparative sensitivity of harbour and grey seals to several environmental contaminants using *in vitro* exposure. Marine Pollution Bulletin. 60 : 344-349.

Friend, M. Trainer, D. O. 1970. Polychlorinated biphenyl: interaction with duck hepatitis virus. Science. 170:1314-1316.

Frouin, H. Ménard, L. Measures, L. Brousseau, P. Fournier, M. 2010. T Lymphocyte-Proliferative Responses of a Grey Seal (*Halichoerus grypus*) Exposed to Heavy Metals and PCBs *in Vitro.* Aquatic Mammals. 36 : 365-371.

Gould, J. C., K. R. Cooper and C. G. Scanes. 1997. Effects of polychlorinated biphenyl mixtures and three specific congeners on growth and circulating growth-related hormones. General and Comparative Endocrinology 106**:** 221-230.

Gray, L. E., Jr., J. Ostby, R. Marshall and J. Andrews. 1993. Reproductive and thyroid effects of low-level polychlorinated biphenyl (Aroclor 1254) exposure. Fundam Appl Toxicol 20**:** 288-294.

Gulland, F. M. D. and A. J. Hall. 2007. Is marine mammal health deteriorating? Trends in the global reporting of marine mammal disease. EcoHealth 4**:** 135-150.

Hall, A.J., L. Schwacke, B. J. McConnell, T. K. Rowles. 2011. Assessing the population consequences of pollutant exposure to cetaceans using an individual based modelling framework. Paper SC/63/E5, International Whaling Commission.

Hall, A.J., B. J. McConnell, T. K. Rowles, A. Aguilar, A. Borrell, L. Schwacke, P.J.H. Reijnders and R.S. Wells. 2006a. Individual –based model framework to assess population consequences of polychlorinated biphenyl exposure in bottlenose dolphins. Environmental Health Perspectives 114(suppl 1):60–64

Hall, A. J. Hugunin, K. Deaville, R. Law, R. J. Allchin, C. R. Jepson, P. D. 2006b. The risk of infection from polychlorinated biphenyl exposure in the harbour porpoise (*Phocoena phocoena*): A case-control approach. Environmental Health Perspectives. 114: 704 – 711.

Hammond, J. A. Hall, A. J. Dyrynda, E. A. 2005. Comparison of polychlorinated biphenyl (PCB) induced effects on innate immune function in harbour and grey seals. Aquatic Toxicology. 74 : 126-138.

Imanishi, J. Nomura, H. Matsubara, M. Kita, M. Won, S-J. Mizutani, T. Kishida, T. 1980. Effect of polychlorinated biphenyl on viral infections in mice. Infection and Immunity. 29:275-277.

Keogh, M. J. Spoon, T. Ridgway, S. H. Jensen, E. Van Bonn, W. Romano, T. A. 2011. Simultaneous measurement of phagocytosis and respiratory burst of leukocytes in whole blood from

bottlenose dolphins (Tursiops truncatus) utilising flow cytometry. Veterinary Immunology and Immunopathology. 144 : 468 – 475.

Koller, L. D. Thigpen, J. E. 1973. Biphenyl-exposed rabbits. American Journal of Veterinary Research. 34 : 1605-1606.

Kuby, J. 1997. Immunology, Third edition. W.H. Freeman and Company: New York.

Lahvis, G. P. Wells, R. S. Kuehl, D. W. Stewart, J. L. Rhinehart, H. L. Via, C. S. 1995. Decreased lymphocyte responses in free-ranging bottlenose dolphins (Tursipos truncates) are associated with increased concentrations of PCBs and DDT in peripheral blood. Environmental Health Perspectives. 103 : 67-72.

Lalancette *et al.* 2003. – (Lalancette, A. Morin, Y. Measures, L. Fournier, M. 2003. Contrasting changes of sensitivity by lymphocytes and neutrophils to mercury in developing grey seals. Developmental and Comparative Immunology. 27 : 735-747.)

Levin, M. Morsey, B. De Guise, S. 2006. Modulation of the Respiratory Burst by Organochlorine mixtures in Marine Mammals, Humans and Mice. Journal of Toxicology and Environmental Health. 70: 73-83

Levin, M. Leibrecht, H. Mori, C. Jessup, D. De Guise, S. 2007. Immunomodulatory effects of organochlorine mixtures upon in vitro exposure of peripheral blood leukocytes differ between free-ranging and captive southern sea otters (Enhydra lutris). Veterinary Immunology and Immunopathology. 119 : 269-277.

Loose, L. D. Silkworth, J. B. Pittman, K. A. Benitz, K. F. Mueller, W. 1978. Impaired host resistance to endotoxin and malaria in polychlorinated biphenyl- and hexachlorobenzene-treated mice. Infection and Immunity. 20 : 30-35.

Luster, M. J. Portier, C. Gayla Pait, D. White, K. L. Gennings, C. Munson, A. E. Rosenthal, G. J. 1992. Risk Assessment in Immunotoxicology. I Sensitivity and Predictability of Immune Tests. Fundamental and Applied Toxicology. 18 : 200-210.

Luster, M. J. Portier, C. Gayla Pait, D. Rosenthal, G. J. Germolec, D. R. Corsini, E. Blaylock, B. L. Pollock, P. Kouchi, Y. Craig, W. White, K. L. Munson, A. E. Comment, C. E. 1993. Risk Assessment in Immunotoxicology. II Relationships between immune and host resistance tests. Fundamental and Applied Toxicology. 21 : 71-82.

Lie, E., H. J. Larsen, S. Larsen, G. M. Johansen, A. E. Derocher, N. J. Lunn, R. J. Norstrom, O. Wiig and J. U. Skaare. 2004. Does high organochlorine (OC) exposure impair the resistance to infection in polar bears (*Ursus maritimus*)? Part I: Effect of OCs on the humoral immunity. J Toxicol Environ Health A 67**:** 555-582.

Luebke, R. W., P. V. Hodson, M. Faisal, P. S. Ross, K. A. Grasman and J. Zelikoff. 1997. Aquatic pollution-induced immunotoxicity in wildlife species. Fundamental and Applied Toxicology 37**:** 1-15.

Mori, C. Morsey, B. Levin, M. Gorton, T. S. De Guise, S. 2008. Effects of Organochlorines, Individually and in Mixtures, on B-Cell Proliferation in Marine Mammals and Mice. Journal of Toxicology and Environmental Health. 71 : 266-275.

Mos, L. Morsey, B. Jeffries, S. J. Yunker, M. B. Raverty, S. de Guise, S. Ross, P. S. 2006. Chemical and biological pollution contribute to the immunological profiles of free-ranging harbour seals. Environmental Toxicology and Chemistry. 25 : 3110-3117.

Morse, D. C., H. B. W. M. Koeter, A. E. Smits Van Prooijen and A. Brouwer. 1992. Interference of polychlorinated biphenyls in thyroid hormone metabolism: Possible neurotoxic consequences in fetal and neonatal rats. Chemosphere 25**:** 165-168.

Murai, K., K. Okamura, H. Tsuji, E. Kajiwara, H. Watanabe, K. Akagi and M. Fujishima. 1987. Thyroid function in "yusho" patients exposed to polychlorinated biphenyls (PCB). Environ Res 44**:** 179-187.

Nakata, H. Sakakibara, A. Kanoh, M. Kudo, S. Watanabe, H. Nagai, N. Miyazaki, N. Asano, Y. Tanabe, S. 2002. Evaluation of mitogen-induced responses in marine mammal and human lymphocytes

by in-vitro exposure of butyltins and non-ortho coplanar PCBs. Environmental Pollution. 120 : 245-253.

National Cancer Institute: Cancer Therapy Evaluation Program, 2003. Common Terminology Criteria for Adverse Events, Version 3.0, DCTD, NCI, NIH, DHHS. (http://ctep.cancer.gov), Publish Date: August 9, 2006. Accessed on the 8th of November 2011.

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

Ojo-Amaize, E. A. Conley, E. J. Peter, J. B. 1994. Decreased Natural Killer Cell Activity is Associated with Severity of Chronic Immune Dysfunction Syndrome. Clinical Infectious Diseases. 18: 157-159.

Pillet, S. Lesage, V. Hammill, M. Cyr, D. G. Bouquegneua, J. M. Fournier, M. 2000. *In Vitro* Exposure of Seal Peripheral Blood Leukocytes to Different Metals Reveal a Sex-Dependent Effect of Zinc on Phagocytic Activity. Marine Pollution Bulletin. 40 : 921-927.

Roletto, J. 1993. Hematology and serum chemistry values for clinically healthy and sick pinnipeds. Journal of Zoo and Wildlife Medicine. 24 : 145 – 157.

Ross, P. S. De Swart, R. L. Timmerman, H. H. Vedder, L. J. Van Loveren, H. Vos, J. G. Reijnders, P. J. H. Osterhaus, A. D. M. E. 1995. Suppression of natural killer cell activity in harbour seals (*Phoca vitulina*) fed Baltic Sea herring. Aquat Toxicol 34:71-84.

Restum, J. C., S. J. Bursian, J. P. Giesy, J. A. Render, W. C. Helferich, E. B. Shipp, D. A. Verbrugge and R. J. Aulerich. 1998. Multigenerational study of the effects of consumption of PCB-contaminated carp from Saginaw Bay, Lake Huron, on mink. 1. Effects on mink reproduction, kit growth and survival, and selected biological parameters. Journal of Toxicology and Environmental Health-Part a-Current Issues 54**:** 343-375.

Ribas-Fito, N., M. Sala, M. Kogevinas and J. Sunyer. 2001. Polychlorinated biphenyls (PCBs) and neurological development in children: a systematic review. J Epidemiol Community Health 55**:** 537-546.

Ross, P., R. Deswart, R. Addison, H. Vanloveren, J. Vos and A. Osterhaus. 1996. Contaminant-induced immunotoxicity in harbour seals: Wildlife at risk? Toxicology 112**:** 157-169.

Routti, H., M. Nyman, B. M. Jenssen, C. Backman, J. Koistinen and G. W. Gabrielsen. 2008. Bone-related effects of contaminants in seals may be associated with vitamin D and thyroid hormones. Environmental Toxicology and Chemistry 27**:** 873-880.

Schwacke, L. H., E. S. Zolman, B. C. Balmer, S. De Guise, R. C. George, J. Hoguet, A. A. Hohn, J. R. Kucklick, S. Lamb, M. Levin, J. A. Litz, W. E. Mcfee, N. J. Place, F. I. Townsend, R. S. Wells and T. K. Rowles. 2011. Anaemia, hypothyroidism and immune suppression associated with polychlorinated biphenyl exposure in bottlenose dolphins (*Tursiops truncatus*). Proc Roy Soc B 279**:** 48-57.

Shellam, G.R. Allan, J. E. Papadimitriou, J. M. Bancroft, G. J. 1981. Increased susceptibility to cytomegalovirus infection in beige mutant mice. Proc. Natl. Acad. Sci. USA 78: 5104-5108.

Silkworth, J. B., L. Antrim and L. S. Kaminsky. 1984. Correlations between polychlorinated biphenyl immunotoxicity, the aromatic hydrocarbon locus, and liver microsomal enzyme induction in C57BL/6 and DBA/2 mice. Toxicol Appl Pharmacol 75**:** 156-165.

Talcott, P.A. Koller, L. D. Exon, J. H. 1985. The effect of lead and polychlorinated biphenyl exposure on rat natural killer cell cytotoxicity. Int. J. Immunopharmac. 7: 255-261.

Thomas, P. T. Hinsdill, R. D. 1978. Effect of polychlorinated biphenyls on the immune response of rhesus monkeys and mice. Toxicolology and Applied Pharmacology. 44:41-51

Thomas, P. T. and R. D. Hindsill. 1980. Perinatal PCB exposure and its effect on the immune system of young rabbits. Drug and Chemical Toxicology 3**:** 173-184.

Tryphonas, H. 2001. Approaches to detecting immunotoxic effects of environmental contaminants in humans. Environmental Health Perspectives 109**:** 877-884.

Wade, M. G., W. G. Foster, E. V. Younglai, A. Mcmahon, K. Leingartner, A. Yagminas, D. Blakey, M. Fournier, D. Desaulniers and C. L. Hughes. 2002. Effects of subchronic exposure to a complex mixture of persistent contaminants in male rats: Systemic, immune, and revoductive effects. Toxicological Sciences 67**:** 131-143.

Welsh, R. M. Brubaker, J. O. Vargas-Corte, M. O'Donnell, C. L. 1991. Natural Killer (NK) Cell Response to Virus Infections in Mice with Severe Combined Immunodeficiency. The Stimulation of NK Cells and the NK Cell-dependent Control of Virus Infections Occur Independently of T and B Cell Function. J. Exp. Med. 173: 1053-1063

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Appendix 1

Equation relating proportional changes in host resistance (i.e. survival probability) to proportional change in T-lymphocyte proliferation response to Con A (see Luster *et al*. 1993).

$$\frac{-(\exp(-a+b-\text{deltaConA} * b) - \exp(-a + b))}{(\exp(-a+b-\text{deltaConA} * b)+1)}$$

Appendix 2. Summary of marine mammal studies indicating some effect of PBDEs on immune function.

Harbour Porpoises	**Beineke *et al*. 2005.** **Investigations of the Potential Influence of Environmental Contaminants on the Thymus and Spleen of Harbor Porpoises *(Phocoena phocoena)*** ***Environ. Sci. Technol. 39. 3933-3938***	Harbor porpoises from the German North and Baltic Seas exhibit a higher incidence of bacterial infections compared to whales from less polluted arctic waters. The potential adverse effect of environmental contaminants such as polychlorinated biphenyls (PCBs) and heavy metals on the immune system and the health status of marine mammals is still discussed controversially. The aim of the present study was to investigate the possible influence of PCB, polybrominated diphenyl ether (PBDE), toxaphene, (*p*,*p*'-dichlorodiphenyl)trichlorethane (DDT), and (*p*,*p*'-dichlorodiphenyl)dichlorethene (DDE) on the immune system of harbor porpoises. Lymphoid organs are influenced by a variety of factors, and therefore special emphasis was given to separating the confounding effect of age, health status, nutritional state, geographical location, and sex from the effect of contaminant levels upon thymus and spleen. Contaminant analysis and detailed pathological examinations were conducted on 61 by-caught and stranded whales from the North and Baltic Seas and Icelandic and Norwegian waters. Stranded harbor porpoises were more severely diseased than by-caught animals. **Thymic atrophy and splenic depletion were significantly correlated to increased PCB and PBDE levels**. However, lymphoid depletion was also associated with emaciation and an impaired health status. The present report supports the hypothesis of a contaminant-induced immunosuppression, possibly contributing to disease susceptibility in harbor porpoises. However, further studies are needed to determine if lymphoid depletion is primarily contaminant-induced or secondary to disease and emaciation in this cetacean species.
Harbour Seal	**Frouin *et al.* 2010. Effects of individual polybrominated diphenyl ether (PBDE) congeners on harbour seal immune cells** *in vitro Marine Pollution Bulletin. 60. 291-298.*	Effects of polybrominated diphenyl ethers (PBDEs) on the immune system of marine mammals are poorly understood. One important innate immune function of granulocytes is the respiratory burst which generates reactive oxygen species (ROS) used to kill engulfed microorganisms. The present study investigates ***in vitro*** the effects of BDE-47, -99 and -153, on the formation of ROS, on intracellular level of thiols, on activity and efficiency of phagocytosis and on apoptosis in granulocytes of harbour seals. Compounds were tested at four different concentrations ranging from 1.5 to 12 µM. **Results showed that ROS levels, thiol levels and phagocytosis were all affected when harbour seal cells were exposed to the highest concentration (12 µM) of PBDE congeners.** Apoptosis was not affected by PBDEs. The observed effects were similar in adults, pups and in the 11B7501 cell line of harbour seals.
Sea Otters	**Kannan et al. 2007. A Comparative Analysis of Polybrominated Diphenyl Ethers and Polychlorinated Biphenyls in Southern**	Southern sea otters (Enhydra lutris nereis) from the California coast continue to exhibit a slower population regrowth rate than the population in Alaska. Infectious diseases have been identified as a frequent cause of death. Infectious diseases caused by varied pathogens including bacteria, fungi, and parasites were suggestive of compromised immunological health of mature animals in this population. To test the hypothesis that elevated exposure to

	Sea Otters that Died of Infectious Diseases and Noninfectious Causes. *Arch. Environ. Contam. Toxicol.* 53. 293–302	immunotoxic contaminants such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) contribute to disease susceptibility via immunosuppression, we determined concentrations of PBDEs and PCBs in livers of 80 adult female sea otters that died of infectious diseases, noninfectious causes, or emaciation. Concentrations of PBDEs and PCBs in sea otter livers varied widely (10–26,800 ng/g and 81–210,000 ng/g lipid weight, respectively). Concentrations of PBDEs in sea otters were some of the highest values reported for marine mammals so far. Although PCB concentrations in sea otters have declined during 1992–2002, the mean concentration was at the threshold at which adverse health effects are elicited. **Concentrations of PBDEs and PCBs were significantly correlated, suggesting co-exposure of these contaminants in sea otters.** No significant association was found between the concentrations of PBDEs and the health status of sea otters. Concentrations of PCBs were significantly higher in otters in the infectious disease category than in the noninfectious category, suggesting an association between elevated PCB concentrations and infectious diseases in Southern sea otters.
Melon-headed whales	**Kajiwara *et al*. 2007. Polybrominated diphenyl ethers (PBDEs) and organochlorines in melon-headed whales,** *Peponocephala electra***, mass stranded along the Japanese coasts: Maternal transfer and temporal trend.** *Environmental Pollution*. 156. 106-114.	Polybrominated diphenyl ethers (PBDEs) and organochlorine compounds (OCs) were determined in the blubber of 55 melon-headed whales (***Peponocephala electra***) mass stranded along the Japanese coasts since 1982. DDTs and PCBs were predominant in all the specimens investigated. In whales that died during the latest event in 2006, concentrations of PBDEs (190–510 ng/g lipid wt) were approximately two orders of magnitude lower than DDTs and PCBs, but comparable with HCHs and HCB. **Maternal transfer of PBDEs to offspring through the whole reproductive process was estimated to be 85% of the mother's body burden, while that occurring during gestation was much lower (2.6–3.5%).** Concentrations of PCBs, DDTs, and HCB were lower in melon-headed whales stranded after the year 2000 than those stranded in 1982, **whereas PBDE and CHL levels showed a temporal increase during the past 20 years,** suggesting that the peak of their usage and contamination occurred after the year 1982.
Harbour Seal	**Jennifer Neale, 2003** **FINAL REPORT: Contaminant-induced immune alterations in the Pacific harbor seal, *Phoca vitulina richardsi*, of the central coast and San Francisco Estuary UC Marine Council.** **http://escholarship.org/uc/item/1t41h8zj#page-1**	1) PCBs and DDE In Blood Of Free-ranging Harbor Seals (*Phoca Vitulina*)From Central California and Bristol Bay, Alaska 2) Contaminant Exposures and Health Correlates in Harbor Seals *(Phoca Vitulina)* of the San Francisco Bay, California 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; haematocrit and haemoglobin also were negatively correlated to 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 4) Molecular Cloning And Sequencing Of Tyrosine Kinases From The Harbor Seal (*Phoca Vitulina*)—in prep. 5) Maternal Transfer Of Persistent Organic Pollutants Via Lactation In Harbor Seals (*Phoca Vitulina*)—in prep.
Belugas	**Desforges et al. 2012. Transplacental transfer of polychlorinated biphenyls and polybrominated diphenyl ethers in arctic beluga whales (*Delphinapterus leucas*). Environmental Toxicology and Chemistry. 31. 296-300**	**This study found that arctic beluga whales (*Delphinapterus leucas*) transferred, on average, 11.4% (7.5 mg) and 11.1% (0.1 mg) of their polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) blubber burden to their near-term fetuses.** A single physicochemical parameter, log K_{OW}, largely explained this transplacental transfer for PCBs ($r^2 = 0.79$, $p < 0.00001$) and PBDEs ($r^2 = 0.37$, $p = 0.007$), with congeners having a log $K_{OW} < 6.5$ preferentially transferred to the fetus. Blubber concentrations of 257 ng/g lipid weight (lw) PCBs and 3.8 ng/g (lw) PBDEs in beluga fetuses highlights the exposure to endocrine-disrupting compounds during a critical developmental stage. The implications of detecting these levels of legacy PCBs and the flame retardant PBDEs in unborn arctic beluga are unclear.