Review Article

Immunotoxic effects of environmental pollutants in marine mammals

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ARTICLE INFO

ABSTRACT

Due to their marine ecology and life-history, marine mammals accumulate some of the highest levels of environmental contaminants of all wildlife. Given the increasing prevalence and severity of diseases in marine wildlife, it is imperative to understand how pollutants affect the immune system and consequently disease susceptibility. Advancements and adaptations of analytical techniques have facilitated marine mammal immunotoxicology research. Field studies, captive-feeding experiments and in vitro laboratory studies with marine mammals have associated exposure to environmental pollutants, most notable polychlorinated biphenyls (PCBs), organochlorine pesticides and heavy metals, to alterations of both the innate and adaptive arms of immune systems, which include aspects of cellular and humoral immunity. For marine mammals, reported immunotoxicology endpoints fell into several major categories: immune tissue histopathology, haematology/circulating immune cell populations, functional immune assays (lymphocyte proliferation, phagocytosis, respiratory burst, and natural killer cell activity), immunoglobulin production, and cytokine gene expression. Lymphocyte proliferation is by far the most commonly used immune assay, with studies using different organic pollutants and metals predominantly reporting immunosuppressive effects despite the many differences in study design and animal life history. Using combined field and laboratory data, we determined effect threshold levels for suppression of lymphocyte proliferation to be between <0.001–10 ppm for PCBs, 0.002–1.3 ppm for Hg, 0.009–0.06 for MeHg, and 0.1–2.4 for cadmium in polar bears and several pinniped and cetacean species. Similarly, thresholds for suppression of phagocytosis were 0.6–1.4 and 0.08–1.9 ppm for PCBs and mercury, respectively. Although data are lacking for many important immune endpoints and mechanisms of specific immune alterations are not well understood, this review revealed a systemic suppression of immune function in marine mammals exposed to environmental contaminants. Exposure to immunotoxic contaminants may have significant population level consequences as a contributing factor to increasing anthropogenic stress in wildlife and infectious disease outbreaks.

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1. Introduction

1.1. Immunotoxicology and risk assessment

The field of toxicology grew due to concerns about adverse effects of chemical substances on humans, then gradual attention was given to domestic animals and wildlife (Newman and Unger, 2003). Traditional toxicity assessments focused on endpoints relating to morbidity or mortality, but it was not until the 1970s that specific interactions of xenobiotics with elements of the immune system were discovered. The first review of immunotoxicology by Vos (1977) was fundamental in establishing that exposure to a broad spectrum of xenobiotics can alter lymphoid tissues and cell populations as well as the function of immune responses, subsequently affecting the health of exposed animals. The immunotoxicity of chemicals can now be assessed via their effects in three broad categories: histopathology of lymphoid tissues, changes in immune function and/or frequency and characteristics of immune cell populations, and changes in host resistance to infectious pathogens (Kimber and Dearman, 2002). In a comprehensive study of the immunotoxicity of over 50 chemicals in mice (*Mus musculus*), the U.S. National Toxicology Program (NTP) established strong correlations between changes in a battery of immune endpoints and altered host resistance (Luster et al., 1992, 1993). These results confirmed the utility and sensitivity of immune endpoints as indicators of toxic insult.

Knowledge of the nature and magnitude of potential adverse health risks from exposure to immunotoxic contaminants is invaluable for the generation of management or conservation plans in highly exposed populations. The assessment of risk combines exposure and relevant dose–response data to estimate the potential of adverse effects (Luster et al., 1994), and while much information is available on exposure and tissue levels of contaminants in marine mammals and other wildlife, dose–response data are lacking, particularly for immunotoxicity. Establishment of threshold effect levels, lowest observable effect levels and/or EC50 for various contaminants are needed for relevant immune endpoints in order to assess potential hazards. These are often difficult to establish for marine mammals due to ethical and logistical constraints related to field and experimental work. Furthermore, there is additional need and challenges to extrapolate effects at the molecular, cellular and individual level to the population level, which is most relevant for management and conservation.

1.2. The marine mammal immune system

The ultimate function of the immune system is to protect against infectious diseases, which may be caused by invading parasites, viruses, bacteria or other microorganisms, and also to respond to aberrant macromolecules such as cancerous cells (Abbas et al., 2012). The immune system is comprised of a complex network of tissues, cells and molecules that work in a concerted effort to resist infections (Fig.ure 1). The immune response to invading pathogens consists of two separate, but interconnected functional systems: innate/non-specific immunity and adaptive/specific immunity; the most important difference between the arms of the immune system is the specificity and memory response of adaptive immunity. Together, the innate and adaptive arms provide immediate and long-term protection from infectious pathogens.

Research over the decades has revealed few differences between the immune system of marine and more highly studied terrestrial mammals, such that much of our understanding of marine mammals comes from rodent and human immunology. The innate immune system consists of various cells and biochemical mechanisms in place to protect the host within minutes and hours of exposure to antigenic stimuli (Fig. 1). Immune cells in marine mammals have been characterized using cross-reactive and species specific monoclonal and polyclonal antibodies against cell surface antigens, including various Cluster of Differentiation (CD) markers, major histocompatibility complex (MHC), and other surface proteins (De Guise, 2004; Ross and De Guise, 2007). Phagocytic cells involved in the rapid destruction of invading pathogens, such as neutrophils, macrophages and dendritic cells, were characterized first in several cetacean species (De Guise et al., 2004; Jaber et al., 2003a,b; Kawashima et al., 2004) and quantitative assays to measure phagocyte function were also developed (De Guise et al., 1995a; Noda et al., 2003). The function of natural killer (NK) cells, specialized lymphocytes involved in the killing of virus infected and tumour cells, has been described in harbour seals (Ross et al.,
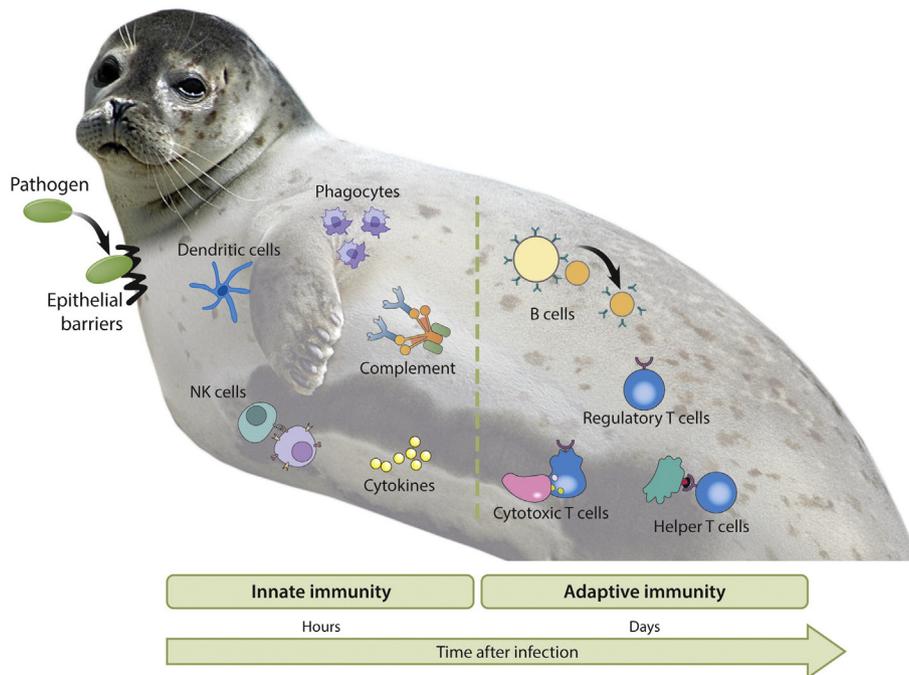

Fig. 1. Cells and molecules of the mammalian innate and adaptive immune system. The function of the immune system is to combat invading pathogens or cancerous cells and this functionality relies on the interaction of a number of innate and adaptive cells and secreted proteins.

1996c) and beluga (De Guise et al., 1997). The acute phase proteins such as C-reactive protein and haptoglobin as well as many cytokines have been quantified in circulation and characterized at the molecular level in marine mammals (see Beineke et al., 2010 and Ross and De Guise, 2007).

The acquired/adaptive immune system evolved to combat pathogens beyond the control of innate immunity. The principal cells involved in the adaptive immune response are lymphocytes, including B and T cells, and their secreted molecules (Fig. 1). These morphologically indistinguishable cell subsets have been identified via expression of surface antigens; T cells were recognized by CD2, and further subsets by CD4 and CD8, while B cells were recognized by CD19 and CD21 molecules (De Guise et al., 2002). Immunoglobulin subclasses have been purified and quantified in plasma and/or partially sequenced in many species of marine mammals (see review by Beineke et al., 2010). Together, B cells and their secreted antibodies form the humoral immune response which acts against extracellular pathogens, while different T cell subsets (helper, cytotoxic and regulatory cells) mediate cellular immunity via interactions with antigen-presenting cells (e.g. macrophages, dendritic cells). Ultimately, activated lymphocytes undergo massive clonal expansion and interact with innate immune cells, other lymphocytes, and a flurry of cytokines to undertake an impressive and complex immune response to eliminate pathogens (Mosmann and Sad, 1996).

1.3. Basis for marine mammal immunotoxicology

Worldwide reporting of diseases in marine organisms has increased over the past decades, raising concerns about the health of marine ecosystems (Gulland and Hall, 2007). Outbreaks typically occur near heavily urbanized and polluted coastal areas, and there are several significant cases of deteriorated marine mammal health, which highlight the influence of anthropogenic pollution on health and disease dynamics (Duignan et al., 2014). High persistent organic pollutant (POP) tissue burdens (eg. ΣPCBs: >50–100 μg/g lw) in Baltic grey seal (*Haliocherus grypus*) and ringed seal (*Pusa hispida*) populations in the 1970–80s were linked to leiomyoma, stenosis and occlusions of the uterus. In addition, pathological investigations revealed a high prevalence of lesions such as adrenocortical hyperplasia and osteoporosis later called the "Baltic Seal Disease Complex" (Helle, 1980; Helle et al., 1976; Olsson et al., 1994; Roos et al., 2012). In the same time period, the endangered beluga (*Delphinapterus leucas*) population in the St.-Lawrence Estuary was shown to have very high organohalogen tissue burdens (eg. ΣPCBs: 10–500 μg/g lw) and high prevalence of degenerative, infectious, neoplastic, hyperplastic and necrotic lesions (De Guise et al., 1995b; Martineau et al., 1994). Lastly, epizootic outbreaks of morbillivirus in 1988/89, 1990/91 and 2002 in harbour seals (*Phoca vitulina*) and stripped dolphins (*Stenella coeruleoalba*) in polluted European and Mediterranean waters resulted in the death of thousands of animals (Dietz et al., 1989; Duignan et al., 2014). Many environmental and chronic anthropogenic stressors likely contributed to overall immune health and cumulative evidence suggests that exposure to high concentrations of organohalogenated pollutants and mercury played a key role in reducing host resistance (Aguilar and Borrell, 1994; Hall et al., 1992, 2006; Jepson et al., 1999; Siebert et al., 1999).

Marine mammals are particularly vulnerable to the accumulation of environmental pollutants. In addition to inhabiting human polluted coastal waters, marine mammals are vulnerable to bioaccumulative pollutants due to their typical position at the top of long marine food chains. Bioaccumulative pollutants are often fat-soluble and resistant to metabolic break-down, thus they persist in the environment and progressively accumulate and magnify through the food web (Macdonald et al., 2002; Mackay and Fraser, 2000). As long-lived animals, marine mammals are exposed throughout their lifetime to complex mixtures of environmental pollutants, making them the most contaminated animals on the planet (Letcher et al., 2010; Muir et al., 1996; Ross et al.,

2000). Furthermore, many species of marine mammals undergo periods of energy deficiency and nutritional stress related to reproduction, migration or hibernation, which may lead to seasonal mobilization of lipid stores and associated pollutants, resulting in higher exposure to target organs (de Swart et al., 1995; Debier et al., 2006).

Controlled feeding experiments with laboratory rodents have provided the mechanistic understanding and causal evidence for the relationship between exposure to various environmental pollutants and immune modulation (Luebke et al., 2007; Luster et al., 1994; Vos et al., 1989). Though similar controlled conditions are nearly impossible to create for marine mammal studies, a "weight of evidence" approach coupling results from laboratory experiments to semi-controlled and free-ranging wildlife studies can offer useful and comprehensive knowledge about the adverse effects of immunotoxic pollutants in marine mammals (Ross, 2000). The following sections will examine the published marine mammal literature in detail for each immune endpoint and draw conclusions based on this "weight of evidence" approach.

There exists a number of comprehensive reviews of immunotoxicological effects and methodologies for laboratory animals and wildlife (Luster et al., 1987; Vos et al., 1989; Luebke et al., 1997; Tryphonas et al., 2004) and for specific pollutants (Corsini et al., 2014; Sweet and Zelikoff, 2010). Though several excellent reviews exist for immunology and immunotoxicology in marine mammals, these are either dated (Ross et al., 1996a,b) or topically review the subject (e.g. Beineke et al., 2010; De Guise, 2004; Ross and De Guise, 2007); there is a lack of a comprehensive overview of immunotoxicity studies in marine mammals. Here, we review the extensive and growing literature on the effects of environmental pollutants on the immune system of marine mammals. The aim of this paper is to provide an updated overview of existing data and to critically examine and extricate data on immune specific endpoints in multiple marine mammal species. This analysis is undertaken in order to integrate and extrapolate immunotoxic effects from multiple studies to better understand effect thresholds and dose–response relationships of environmental chemicals in marine mammals, which serves as the basis for population effect assessments.

2. Immunotoxicology of marine mammals

Field studies, captive-feeding experiments and in vitro laboratory studies reporting contaminant-mediated alterations of the immune system in marine mammals are summarized in Tables 1–5, Figs. 2–4 and in supplementary tables. We review over 50 published reports where some measure of immunity is associated with pollutants. The number of reports examining cetaceans, pinnipeds and polar bears were 18, 29 and 4, respectively. These included 34 studies linking to PCBs, 24 to other organic pollutants (organochlorines, PBDEs, PAHs, dioxin and butyltins; many shared PCBs), 9 to mercury, 11 to other heavy metals, and 3 to perfluorinated compounds (PFCs). Contaminant effects in marine mammals were detected for the following immune endpoints: immune tissue histopathology, haematology/circulating immune cell populations, lymphocyte proliferation, phagocytosis, respiratory burst, natural killer cell activity, immunoglobulin production and cytokine gene expression.

We used ISI Web of Knowledge/Web of Science, ScienceDirect and Google Scholar to locate peer-reviewed scientific articles using key words to focus the search on marine mammal species and immune effects such as marine mammals, seal, whale, polar bear, contaminants, PCB, mercury, immunological effects, immunotoxic, lymphocyte proliferation, etc. Although we attempted to be systematic within the scope of the review, we acknowledge that this may not be an exhaustive representation of all the material that may exist in the scientific literature.

Where available, raw data was extracted manually from the published articles in order to perform further meta-analyses. Lymphocyte proliferation and phagocytosis were the endpoints with the most data that could be analysed statistically. To compare among studies, these immune endpoints values were transformed to 'percent of control'

Table 1
Marine mammal haematological alterations associated with exposure to environmental contaminants.

Species	Area	Pollutant (µg/l ww)	Observed alterations	Reference
Harbour seal	Wadden Sea	PCB: 7109-15062[a]	↑ Concentration granulocytes ↑ Concentration lymphocytes ↑ Concentration basophils ↓ Concentration monocytes	de Swart et al. (1994), Reijnders (1988)
	North Sea	NA	↑ Concentration monocytes	Weirup et al. (2013)
	Wadden Sea	Metals: 1-4000[b]	↑ Concentration lymphocytes ↓ Percent macrophages	Kakuschke et al. (2011)
	NE Pacific	PCB: 0.4–7.0[c]	↓ Concentration lymphocytes ↓ Percent lymphocytes	Mos et al. (2006)
Northern fur seal	Alaska	PCB: 20.6 ± 6.9[c]	↑ Concentration neutrophils ↑ Concentration leukocytes ↑ Concentration haptoglobin	Beckmen et al. (2003)
Stellar sea lion	Alaska	NA	↑ Concentration haptoglobin	Zenteno-Savin et al. (1997)
Bottlenose dolphin	Florida	Hg: 0.01–3.0	↑ Concentration neutrophils ↑ Concentration band cells ↓ Concentration lymphocytes ↓ Concentration eosinophils ↓ Concentration platelets	Schaefer et al. (2011)
	South Carolina	PFC: 500-9000[d]	↑ Concentration lymphocytes ↓ Concentration eosinophils ↓ Concentration monocytes ↓↑ Concentration α-globulin	Fair et al. (2013)

[a] PCB concentrations represent pooled blood samples from control and exposed groups.
[b] Metals include Ag, Al, Be, Cd, Ni, Pb, Ti, and Zn.
[c] Concentration in µg/g blubber lw.
[d] $\sum$ PFC include PFCA, PFSA, PFOS, PFOA and PFDA.

(e.g. no exposure); where controls were not available, the intercept of the relationship between the contaminant and the proliferative/phagocytic response was used. For the few cases in the *in vivo* exposure literature for lymphocyte function where contaminant concentrations were reported in serum, they were converted to blubber levels using the blood-blubber conversion factor in Yordy et al. (2010). *In vitro* data often included single congener exposures and these were not modified. Dose–response relationships were analysed using the DRC package in R (Ritz and Streibig, 2005). Parameters such as age class, wild or captive, in vitro or *in vivo*, mitogen type, exposure time, contaminant levels and contaminant type were recorded for each study. Lymphocyte proliferation was the only parameter with enough data for in-depth analysis and multiple regression models combined with Akaike Information Criteria were used to determine which parameters best explained lymphocyte proliferation (not enough data for similar analysis with phagocytosis). All statistics were done using R (R Core Team, 2014).

2.1. Lymphoid tissue histopathology

Tissues of the immune system consist of those that generate naïve lymphocytes, the primary or generative immune organs (bone marrow and thymus), and those that initiate a specific adaptive response, the secondary or peripheral immune organs (lymph nodes, spleen and mucosa-associated lymphatic tissues) (Moser and Leo, 2010). *In vivo* laboratory studies have revealed that effects on primary and secondary lymphoid organs have important consequences on various aspects of the immune system; for instance, altered or arrested maturation of immune cells in the thymus and bone marrow can lead to significant changes in cellular and humoral immune responses (Luebke et al., 2007; Silkworth and Antrim, 1985; Suh et al., 2002; Thurmond et al., 2000). Thus, effects on the immune endpoints discussed in subsequent sections may stem originally from toxic insults on lymphoid tissues.

Morphological changes in lymphoid tissues in connection to pollutant exposure have been reported in only one study on polar bears (*Ursus maritimus*) and one on harbour porpoises (*Phocoena phocoena*). Thymic and spleen dysfunction, characterized by interfollicular fibrosis, cortical lymphoid depletion, loss of the corticomedullary junction, and splenic lymphocyte depletion, correlated with blubber concentrations of organohalogen contaminants (eg. ΣPCB: 1–26 µg/g lw) in harbour porpoises stranded or by-caught in northern European waters (North Sea, Baltic Sea, coastal Iceland and Norway) (Beineke et al., 2005). Histological examination of lymph nodes, spleen and thymus in 82 polar bears from East Greenland revealed a weak positive relationship between the amount of secondary follicles in spleen and lymph nodes

Table 2
Adverse effect concentrations (ppm) for lymphocyte proliferation in marine mammals. Effect levels were calculated using the dose–response package 'DRC' in R (Ritz and Streibig, 2005). See Figs. 2 and 3 captions for details of the data used in this analysis.

	PCB		Hg		MeHg		Cd	
	Threshold[a]	EC50	Threshold[a]	EC50	Threshold[a]	EC50	Threshold[a]	EC50
All marine mammals	0.022 ± 0.019	51.8 ± 14.6	0.0048 ± 0.011	0.20 ± 0.20	0.0098 ± 0.0054	0.067 ± 0.18	0.19 ± 0.13	1.53 ± 0.45
Polar bears	<0.01[b]	133.7 ± 250.4	na	na	na	na	na	na
Seals	<0.01[c]	68.7 ± 32.2	0.0084 ± 0.020	0.46 ± 0.50	0.026 ± 0.025	0.12 ± 0.039	0.33 ± 0.15	1.21 ± 0.29
Harbour seal	2.08 ± 1.07	4.67 ± 1.76	0.0023 ± 0.0094	0.23 ± 0.31	0.062 ± 0.13	0.17 ± 0.12	0.39 ± 0.85	1.83 ± 1.94
Grey seal	0.17 ± 0.12	60.6 ± 15.1	1.30 ± 0.91	2.49 ± 0.90	0.032 ± 0.028	0.12 ± 0.032	0.39 ± 0.23	1.11 ± 0.23
Ringed seal	0.092 ± 0.14	16.1 ± 1.36	na	na	na	na	na	na
Cetaceans	5.42 ± 2.15	48.4 ± 9.26	0.047 ± 0.059	0.36 ± 0.21	0.016 ± 0.0049	0.039 ± 0.0059	0.21 ± 0.45	5.64 ± 5.05
Beluga	10.2 ± 4.25	21.3 ± 2.06	0.067 ± 0.094	0.32 ± 0.18	0.016 ± 0.0049	0.039 ± 0.0059	0.32 ± 4.42	1.15 ± 10.0
Bottlenose dolphin	0.14 ± 0.25	30.5 ± 13.5	0.21 ± 0.065	0.81 ± 0.092	na	na	2.44 ± 0.38	10.6 ± 0.63

[a] Calculated as the effective concentration giving a one percent response (EC1).
[b] $4.15 \times 10^{-6} \pm 5.26 \times 10^{-5}$.
[c] $8.32 \times 10^{-3} \pm 1.11 \times 10^{-2}$.

Table 3
Modulation of respiratory burst in marine mammal leukocytes by exposure to environmental pollutants.

Species	Location	Study type	Pollutant (μg/ml ww)	Effect on respiratory burst	Reference
Harbour seal	NE Pacific	*in vivo*	PCB: 0–7[a]	Stimulation	Mos et al. (2006)
	Captive (aquarium)	*in vitro*	PCB: 0–20[b]	Suppression	Levin et al. (2004)
	St Andrews	*in vitro*	PCB: 0–0.03[c]	Mixed[d]	Hammond et al. (2005)
	St Lawrence	*in vitro*	PBDE: 0–6	Stimulation	Frouin et al. (2010b)
Grey seal	St Andrews	*in vitro*	PCB: 0–0.03[c]	No effect	Hammond et al. (2005)
Beluga	Captive (aquarium)	*in vitro*	PCB: 0–20[b]	Stimulation	Levin et al. (2004)
Bottlenose dolphin	Captive (aquarium)	in vitro	PCB: 0–20[b]	Stimulation	Levin et al. (2004)
Killer whale	Captive (aquarium)	*in vitro*	PCB: 0–20[b]	Stimulation	Levin et al. (2004)

[a] PCB measured in μg/g blubber lw.
[b] PCB congeners tested separately.
[c] Used 1:1 mixture of Aroclor 1242 and 1260.
[d] Stimulation at 0.003 μg/g and suppression at 0.03 μg/g.

and tissue levels of organochlorine contaminants (eg. ΣPCB: 1–20 μg/g lw; Kirkegaard et al., 2005). Histopathology of lymphoid organs, including thymic atrophy in particular, is one of the most widely accepted immunotoxic effects of dioxins and other similar pollutants in exposed laboratory animals (Vos et al., 1989; Vos and Van Loveren, 1994). Therefore, despite the minimal published reports on the topic in marine mammals, the similarity of pathological observations with laboratory animals supports a universal detrimental effect of organohalogen contaminants on lymphoid tissues.

2.2. Circulating immune cells & haematology

Haematology is used in clinical health assessments of wildlife, providing useful information if baseline species-specific levels and variations are known (Boughton et al., 2011; Luebke et al., 2007). Haematological analyses can provide information on the number and relative proportion of white blood cells in circulation. Reference baseline haematology and biochemistry from free-ranging and captive marine mammals are reported comprehensively in the Marine Mammal Medicine Handbook (Dierauf and Gulland, 2001). There were 12 studies using clinical blood parameters as measures of immune toxicity in marine mammals, and those showing a link to contaminant exposure are summarized in Table 1. Three studies unable to associate changes with pollutants were not included in Table 1 in order to conserve space, and these were Schumacher et al. (1995); Hall et al. (1997) and Nyman et al. (2003).

The absolute number of granulocytes was correlated with tissue contaminant levels in four of the nine studies shown in Table 1. The number of total granulocytes, neutrophils and basophils were positively correlated with PCBs in harbour and northern fur seals (*Callorhinus ursinus*) (Beckmen et al., 2003; de Swart et al., 1994) and neutrophils and band cells were positively correlated with Hg in bottlenose dolphins (*Tursiops truncatus*) (Schaefer et al., 2011). However, mixed results were found for circulating lymphocytes and monocytes in many species, while eosinophils negatively correlated with Hg and PFCs in bottlenose dolphins (Table 1). Pollutant-mediated changes in circulating blood cells can be interpreted several ways and are notably confounded by natural fluctuations with age/development, infections and stress (Beckmen et al., 2003; Beineke et al., 2010; de Swart et al.,

1996; Fonfara et al., 2007; Hall et al., 1997; Kakuschke et al., 2011; Mos et al., 2006). The difficulty in predicting the direction and magnitude of effect of pollutants on immune cells and the lack of specificity of altered haematological parameters limit the use of this endpoint as a standalone indicator of immunotoxicity in marine mammals.

Haptoglobin is an acute phase response protein that helps mediate the innate immune response (Moser and Leo, 2010). Many factors can cause increased haptoglobin levels in circulation, including disease, trauma, stress and environmental pollutants. Elevated serum haptoglobin has been reported in PCB and PFC exposed harbour seals, northern fur seals and bottlenose dolphins (Beckmen et al., 2003; Fair et al., 2013; Zenteno-Savin et al., 1997). All three studies were unable to conclusively report that contaminants were the primary cause of the observed haptoglobin changes due to confounding variables in those stressed populations.

2.3. Lymphocyte proliferation

Lymphocytes are the active immune cells of adaptive immunity and functional assays have been developed to assess their ability to proliferate and thus mount a proper immune response. Mitogens, proteins which can non-specifically induce lymphocytes to proliferate, can be used to measure B and T cell activity and have been validated in marine mammals (De Guise et al., 1996a; de Swart et al., 1993); concanavalin A (ConA) and phytohemagglutinin (PHA) stimulate T cells, lipopolysaccharides from *Salmonella typhimurium* (LPS) stimulates B cells, and pokeweed mitogen (PWM) stimulates both B and T cells. Of all immune responses measured in immunotoxicology studies of marine mammals, lymphocyte proliferation is by far the most commonly used. We documented a total of 33 studies in marine mammals where lymphocyte proliferation was measured in relation to contaminant exposure (Supplementary Table 1).

Most studies of marine mammals reported a suppressive effect of contaminant exposure on lymphocyte proliferation (Supplementary Table 1). For those studies reporting mixed effects, the results were typically confounded by the use of several mitogens or for in vitro studies, through exposing with various chemical congeners. Generally, studies using several mitogens reported reduced T cell proliferation and minimal effect on B cell proliferation. Immuno-stimulation by

Table 4
Modulation of natural killer (NK) cell activity in marine mammals by exposure to environmental pollutants.

Species	Location	Study type	Pollutant (μg/ml)	Effect on NK activity	Reference
Harbour seal	Wadden Sea	*in vivo*	PCB: 7–15[a]	Suppression	Ross et al. (1996c)
	St Andrews	in vivo	PCB: 0–0.03[b]	No effect	Hammond et al. (2005)
Grey seal	St Andrews	in vitro	PCB: 0–0.03[b]	No effect	Hammond et al. (2005)
Bottlenose dolphin	South Carolina	in vivo	PFCs: 0.5–9	No effect	Fair et al. (2013)
	Captive (aquarium)	*in vitro*	PFOS: 0–5	No effect	Wirth et al. (2014b)
	Captive (aquarium)	*in vitro*	PBDE: 0–50	No effect	Wirth et al. (2014a)

[a] Captive feeding study of two groups of seals, with pooled plasma PCB in control and exposed seals of 7.1 and 15.1 μg/ml respectively.
[b] Used 1:1 mixture of Aroclor 1242 and 1260.

Table 5
Modulation of immunoglobulin production in marine mammals by exposure to environmental pollutants.

Species	Location	Study design	Pollutant (μg/ml)	Effect on immunoglobulins	reference
Harbour seal	Wadden Sea	Immunization with ovalbumin, tetanus toxoid, polio- and rabies-virus	PCB: 7–15[a]	↓ Ig vs. ovalbumin No effect vs. viruses	Ross et al. (1995)
Northern fur seal	Alaska	Circulating IgG + immunization with tetanus toxoid	PCB: 0–0.05	↓ Total serum Ig ↓ IG vs. tetanus toxoid	Beckmen et al. (2003)
Bottlenose dolphin	South Carolina	Circulating IgG	PFCs: 0.5–9	↑ Serum IgG	Fair et al. (2013)
Polar bear	Svalbard	Circulating IgG	PCB: 0–0.2	↓ Serum IgG	Bernhoft et al. (2000)
	Svalbard & Churchill	Circulating IgG + immunization with tetanus toxoid, influenza-, reo- and herpes-virus	PCB: 0–0.2	↓ Serum IgG ↓ Ig vs. influenza, reo- and herpes virus ↑ Ig vs. tetanus toxoid	Lie et al. (2004), Derocher et al. (2000)

[a] Captive feeding study of two groups of seals, with pooled plasma PCB in control and exposed seals of 7.1 and 15.1 μg/ml respectively.
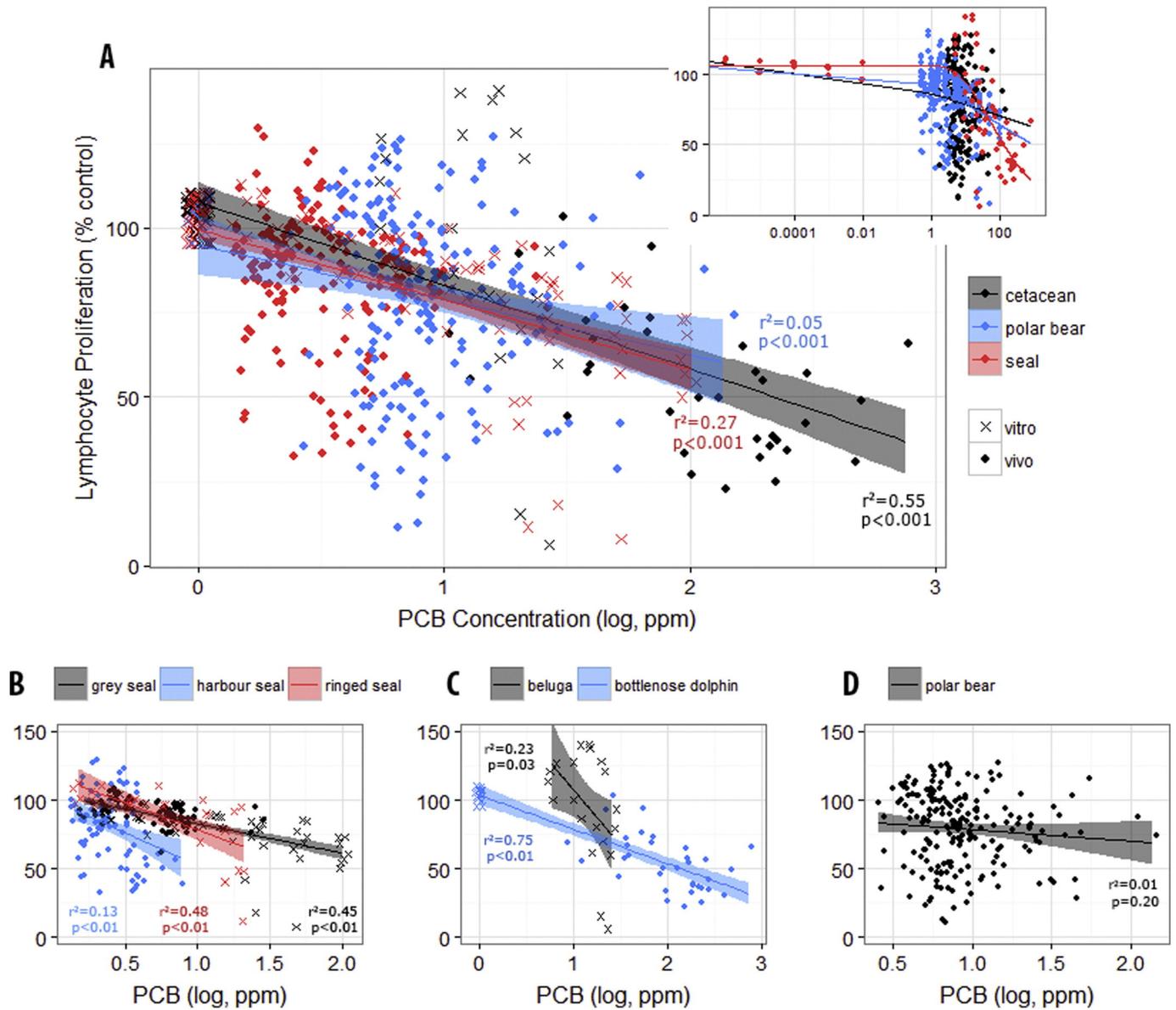

Fig. 2. PCB exposure reduces the lymphoproliferative response in marine mammals. Dose–response relationships are shown for major species groupings (A) as well as for individual species of seals (B), cetaceans (C) and polar bears (D). The inset in (A) shows the dose–response relationship on a logarithmic scale so as to visualize the sigmoidal curve over the broad range of PCB concentrations. As no data exists for effects at low concentrations in polar bears, results from Nakata et al. (2002) on cetaceans were used for concentrations <0.01 ppm in (A) to get the 'no effect' plateau; this was not done in (D). Data include results from *in vitro* (x; μg/ml) and in vivo (●; μg/g lw) exposure studies and include ∑ PCB for *in vivo* studies and ∑ PCB, Aroclors and individual congeners for *in vitro* studies. Shaded areas represent 95% confidence intervals. Figure is the result of a meta-analysis of raw data from all available published literature (see summary in Supplementary Table 1).
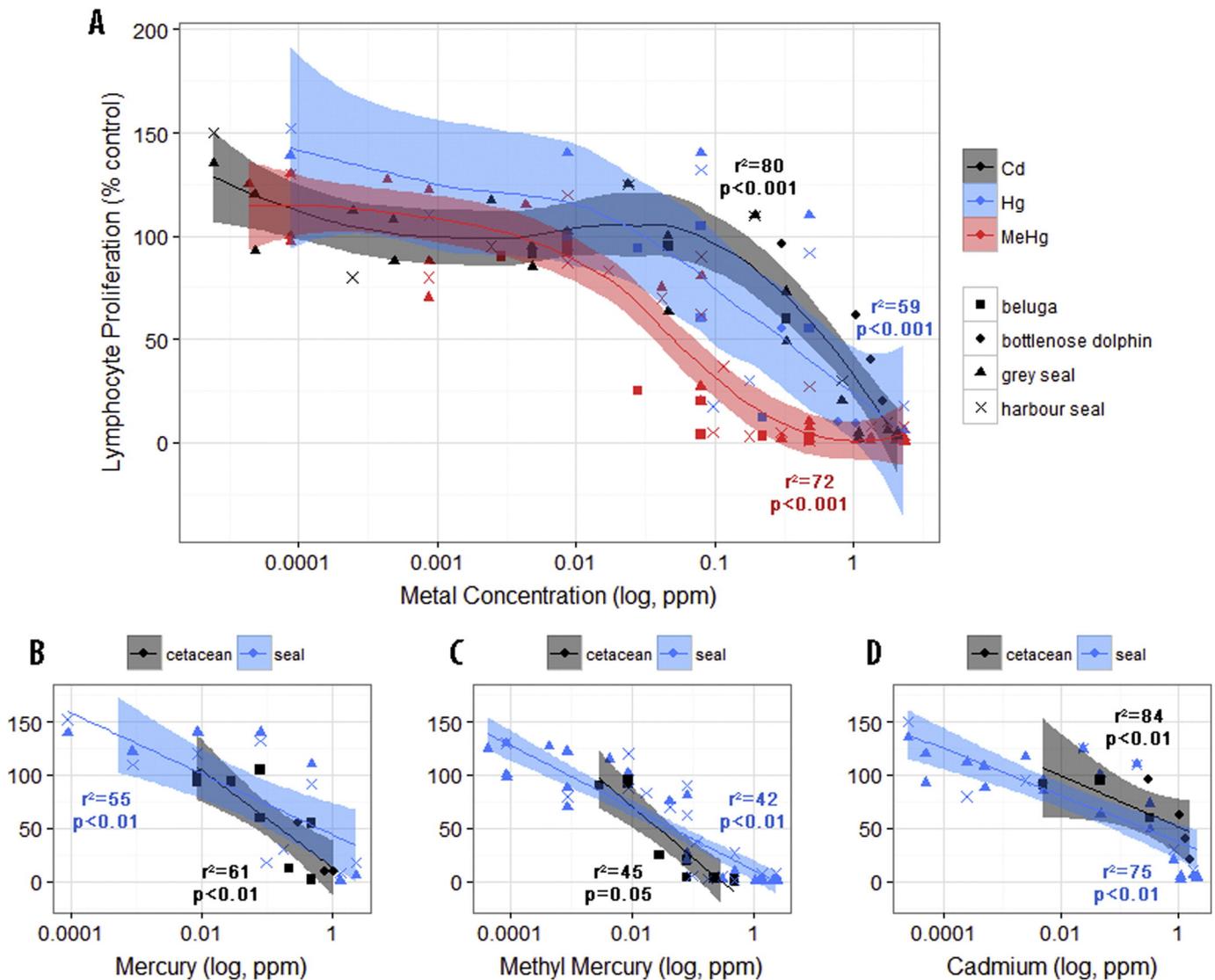
Fig. 3. Exposure to heavy metals (ppm; µg/ml) reduces the lymphoproliferative response in marine mammals. Dose–response relationships are compared between different metals in (A) and between species groupings for each metal in (B–D). Data for metals include only *in vitro* studies as these were the only ones available. Shaded areas represent 95% confidence intervals. Figure is the result of a meta-analysis of raw data from all available published literature (see summary in Supplementary Table 1).

environmental exposure in free-ranging marine mammals has been found in two studies of harbour seals (PCBs: Levin et al., 2005; metals: Kakuschke et al., 2005) and one study of bottlenose dolphins (PFCs: Fair et al., 2013). The positive correlation found for PFCs and metals was corroborated by *in vitro* studies on the same animals, and immuno-stimulation was detected *in vitro* for certain PCB congeners in various captive marine mammal species (Mori et al., 2006, 2008). These results suggested that certain pollutants may stimulate immune responses and/or produce a hormesis effect at low doses (i.e. biphasic response). Nonetheless, the weight of evidence from this review clearly revealed that PCBs and Hg reduced the proliferative ability of lymphocytes *in vitro* and in vivo, a cause-effect relationship supported by ample controlled laboratory evidence from rodent studies (eg. Luster et al., 1994).

A meta-analysis of all available published data showed a striking dose–response relationship for the effects of PCBs and heavy metals on lymphocyte proliferation for marine mammal groups and species (Figs. 2 & 3). The strength and continuity of the dose–response relationships revealed a collective and universal response to pollutant exposure in marine mammals, despite the many differences in study design, congener mixture exposures, and animal life history existing between studies (i.e. *in vitro* vs *in vivo*, $\sum$ PCB vs Aroclor vs individual congeners,

captive vs wild, etc) (Fig. 2A). Polar bear was the only species where the dose–response relationship was not very strong ($r^2 < 0.06$) due to confounding factors such as age, sex, condition and body mass influencing immune responses in wild polar bears (Derocher et al., 2000; Lie et al., 2005). Using the full dataset, multiple linear regression analysis including contaminant, methodological and biological factors found that PCB concentration was the primary predictor of lymphocyte proliferation (41.4% of the model variance). The effect threshold and EC50 of PCBs on lymphocyte proliferation for marine mammals (combined species data) was 0.022 ± 0.019 and 51.8 ± 14.6 ppm (µg/g lw), and species specific values ranged from <0.0001–10.2 and 4.7–134 ppm, respectively (Table 2). Effect threshold levels and EC50's for metals were typically lower than PCB values, with Hg and MeHg being lower than Cd (Table 2).

Though pollutant concentrations were the primary predictor of lymphocyte proliferation, multiple linear regression modelling found pollutant type (total, congeners, or specific metal), marine mammal group (whales, seals, polar bears) and species, and lymphocyte subgroup to be significant variables in the dose–response relationships (p < 0.001). Model results corroborate those from individual studies, which show that the range of effect on lymphocyte function is different for various PCB congeners, metals and other pollutants (De Guise et al.,
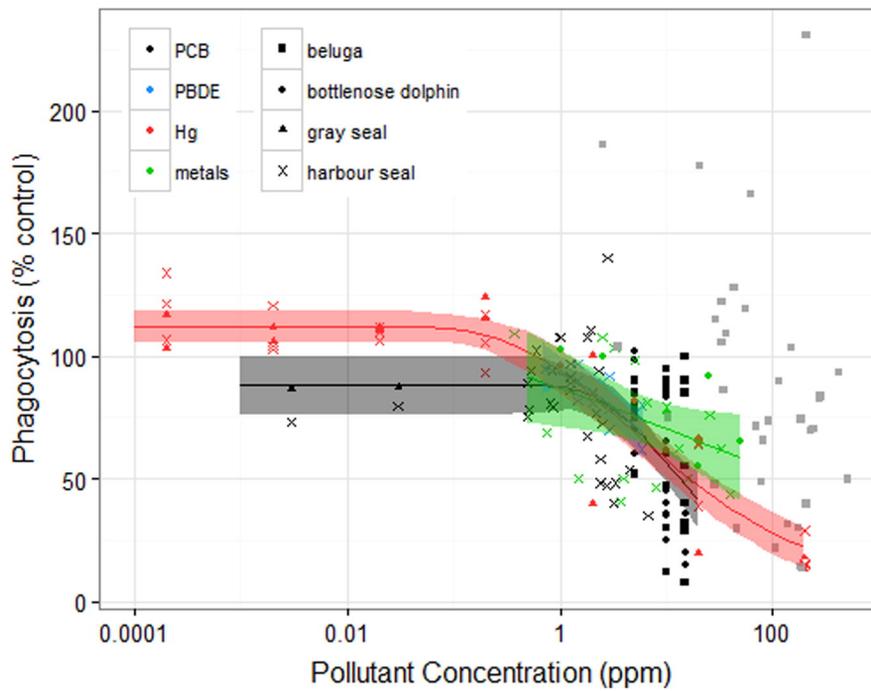
Fig. 4. PCB exposure reduces phagocytosis in marine mammals. Dose–response relationships are shown for major pollutant classes and species. PCB class includes $\sum$ PCB, Aroclors and individual congeners; PBDEs include only BDE-47, Hg includes Hg and MeHg; metals include Al, Cd, V, As, Se and Zn. All but two studies represent *in vitro* pollutant exposures; *in vivo* studies include one of harbour seals (Mos et al., 2006) and one of bottlenose dolphins (Schwacke et al., 2012). Grey squares are total PCBs in bottlenose dolphin from Schwacke et al. (2012) which fell well outside the dose–response curve of all other PCB studies. Figure is the result of a meta-analysis of raw data from all available published literature (see summary in Supplementary Table 2).

1996b, 1998; Frouin et al., 2010a,c, 2012; Lahvis et al., 1995; Mori et al., 2006, 2008; Neale et al., 2002; Pellissó et al., 2008). Species-specific responses to pollutants have also been reported previously in marine mammals, suggesting some species may be more vulnerable to pollutant exposure (Dufresne et al., 2010; Hammond et al., 2005; Mori et al., 2006, 2008). Lastly, the model substantiates the greater sensitivity of T cells compared to B cells to pollutant exposure observed in the literature (de Swart et al., 1994; Mos et al., 2006; Schwacke et al., 2012; Sørmo et al., 2009).

Interestingly, linear modelling of the meta-analysis data suggested that experiment type – *in vitro* vs. *in vivo* – was not of primary importance in the dose–response relationship. The complete overlap of *in vitro* and *in vivo* data for lymphocyte proliferation vs. pollutant concentrations further supported this and ANCOVA revealed a non-significant interaction of experiment type (i.e. *in vitro* vs. *in vivo*) with the dose–response relationship (p = 0.072, Supplementary Fig. 1). This is a particularly important finding as *in vitro* studies are conducted not only to pinpoint specific cellular immune effects, but also to predict real life responses. The problem with *in vitro* tests is that they do not incorporate whole-organism level processes such as various cell interactions and messenger molecules as well as chemical exposure, absorption, metabolism and excretion (Ross and De Guise, 2007). Nonetheless, many studies have demonstrated effective extrapolation of *in vitro* studies (e.g. Luebke et al., 1997) and our present results provide further support for the use of *in vitro* dose–response data in wildlife risk assessment.

2.4. Phagocytosis & respiratory burst

Neutrophils are the most abundant white blood cell in marine mammals and play an important role in inflammatory responses as the first line of defence against invading microorganisms, especially bacteria (De Guise et al., 1995a). As a type of phagocytic cell, neutrophils engulf pathogens through phagocytosis and destroy phagocytized material via biochemical processes of the respiratory burst. Phagocytosis can be measured by the leukocyte incorporation of fluorescent latex beads and quantified using flow cytometry and fluorescence. Respiratory

burst activity can also be analysed by fluorescence flow cytometry, but uses specific probes to measure the production of reactive oxygen species, including H_2O_2 (De Guise et al., 1995a). We documented 11 studies covering four marine mammal species (harbour seal, grey seal, beluga and bottlenose dolphin) that directly related *in vitro* or *in vivo* phagocytosis to contaminant exposure (Supplementary Table 2). There were only four studies reporting effects on respiratory burst and those included five marine mammal species (harbour seal, grey seal, beluga, bottlenose dolphin and killer whale) and focused largely of PCBs (Table 3).

The vast majority of studies showed that contaminant exposure acted to suppress phagocytosis in marine mammal neutrophils; this relationship held true for PCBs, PBDEs, heavy metals and polyaromatic hydrocarbons (PAHs) (Supplementary Table 2). The effective concentration causing 50% reduction in lymphocyte function (EC50) calculated using available raw data from published studies are shown in Supplementary Table 2; EC50 for PCBs was typically 5–12 μg/g ww (exception of dolphins in Schwacke et al., 2012), MeHg is more potent than Hg and together EC50s ranged from 4 to 30 μg/g ww, and other metals range from 1 to 100 μg/g ww. Well defined and overlapping dose–response relationships were apparent when plotting available data for each pollutant class (Fig. 4). Limited data prevented species specific dose–response curves, however when pooled together these resulted in consistent relationships over a large range of exposure concentrations. The effect threshold and EC50 of PCBs on phagocytosis for marine mammals (combined species data) was 1.05 ± 0.82 and 10.2 ± 2.55 ppm (μg/g ww), with seals (threshold: 0.65 ± 0.44 ppm, EC50: 3.7 ± 13.1 ppm) more sensitive to PCBs than cetaceans (threshold: 1.1 ± 0.7 ppm, EC50: 8.2 ± 1.1 ppm). PBDE data were only available for harbour seals and threshold and EC50 values were 0.34 ± 0.81 and 8.53 ± 3.69 ppm, respectively. Lastly, the effect thresholds for mercury on all marine mammals and seals and cetaceans separately were 0.098 ± 0.066, 0.082 ± 0.059 and 1.88 ± 36.16 ppm, respectively; EC50 values for the same groups were 4.49 ± 1.26, 3.94 ± 1.25 and 10.42 ± 10.00.

For PCBs, there were reportedly clear differences in species susceptibility to phagocytosis as well as differences in the effect of congeners.

Hammond et al. (2005) found phagocytosis in harbour seals was significantly reduced (20–30%) after exposure of up to 0.03 µg/ml of mixed Aroclors, while no effect was found for grey seals; similarly, Levin et al. (2004) reported more marked effects for bottlenose dolphins than belugas exposed to PCBs. Exposure studies with several congeners suggested that responses were mediated by non-coplanar PCBs and therefor effects were likely aryl hydrocarbon receptor (AhR) independent; interactions between congeners in mixtures was also observed resulting in synergistic and antagonistic effects (Levin et al., 2004). Though the mechanism of pollutant effect on phagocytosis remains unclear, differences in species and congener sensitivity suggest a receptor mediated pathway. Binding to cell surface receptors may block recognition pathways or interfere with transcription mechanisms critical for effective phagocytosis (Hammond et al., 2005). The effects of non-planar PCBs and mercury on phagocytosis are mediated in part by changes in calcium mobilization, cell membrane fluidity and permeability, and via interactions with metallothioneins (Lalancette et al., 2003; Pillet et al., 2000). Ultimately, modulation of phagocytosis may compromise the immune systems crucial first line of defence and increase an animal's susceptibility to infectious disease.

The influence of contaminants on leukocyte respiratory burst was mostly stimulatory, though suppression and biphasic (i.e. hormesis) effects have also been shown (Table 3). As with phagocytosis, differences existed in species sensitivity to PCB exposure for respiratory burst (Hammond et al., 2005; Levin et al., 2007); however, unlike phagocytosis, both planar and non-planar PCBs mediated respiratory burst activity, suggesting the potential for AhR-dependent and independent effects (Levin et al., 2007). Thus, despite the intimate link between these immune functions, contaminants may act through different mechanisms to disrupt leukocyte activity. Modulation of the respiratory burst may occur by way of several pathways; a) disruption of cellular calcium homeostasis via interaction with phospholipase C/protein kinase C; b) activation of NADPH oxidase via phospholipase A_2, which stimulates production of ROS; c) activation of tyrosine kinase activity and generation of ROS; and d) disruption of superoxide dismutase and scavenging of ROS (Fischer et al., 1998). A hormesis effect is possible where stimulation occurs at low exposures but over time cells in sensitive species may become over-stimulated and unable to mount a proper response. Nonetheless, disruption of any kind in respiratory burst function can have negative consequences; excess ROS production can cause cell and tissue injury and deplete antioxidant defences, while deficient ROS production may impair phagocyte ability to kill ingested pathogens (Levin et al., 2007).

2.5. NK cell activity

Natural killer (NK) cells are a heterogeneous population of large granular lymphocytes whose function is primarily against virus infected cells and tumour cells (Ross et al., 1996c). NK cells are critical in the early phase of viral infections and as non-specific defenders against the spread of pathogens; their cytotoxic activity results from the release of granule content near infected cells, allowing the released proteins (e.g. perforins and proteases) to induce apoptosis (Luebke et al., 2007). NK activity is measured by either the ^{51}Cr release assay or flow cytometry. In the Cr assay, target tumour cells radiolabelled with ^{51}Cr are incubated with PBMCs and the cytotoxic effect is measured as the release of radioisotopes resulting from the killing of target cells. The flow cytometry assay uses fluorescence to measure membrane integrity of target cells after incubation with NK cells (De Guise et al., 1997).

Despite the importance of NK cells in the immune response against viral infections, only five studies have been published on contaminant effects on NK activity in marine mammals (Table 4). Ross et al. (1996c) were the first and only to show reduced NK cell activity in exposed animals in their long-term captive feeding experiment of 22 harbour seals fed either polluted Baltic herring or relatively 'clean' Atlantic herring. In the other four studies NK activity from harbour seals, grey seals and bottlenose dolphins was not influenced by PCBs (Hammond et al., 2005), PBDEs (Wirth et al., 2014a) or PFCs (Fair et al., 2013; Wirth et al., 2014b). The lack of effect on NK function in these studies may be due to low exposure levels, low sensitivity of marine mammal NK cells to certain pollutants, or differences between in vivo and in vitro studies. PCB exposures in grey and harbour seals by Hammond et al. (2005) were orders of magnitude lower than those that elicited effects in the Baltic study; PCB thresholds thus likely lie between 0.03 and 15 µg/g lw. PFOS and PBDE exposure levels for bottlenose dolphin NK assays were selected as effects were observed in that range in a previous study in mice by the same researchers, suggesting dolphins are much less sensitive in this endpoint (Wirth et al., 2014a,b). Further investigations are needed to cover a wider range of exposure levels and obtain a better understanding of the effect thresholds for NK cell activity in marine mammals.

2.6. Antibody production

Antibodies are secreted into circulation where they bind microbial antigens to neutralize infectivity and promote critical effector functions, including phagocytosis, NK cell activity and complement activation. Antibodies are divided into several immunoglobulin (Ig) isotypes which differ in their structure, distribution in the body, and function; these have been characterized and quantified in many species of marine mammal (De Guise, 2004; Ross and De Guise, 2007). New-borns are limited to antibodies from the mother through placental transfer (variable between species and placenta type) and then lactation, and circulating immunoglobulins then increase with age as individuals are progressively exposed to new environmental microbes (Ross et al., 1994). Marine mammal studies of immunoglobulins have looked at general circulating levels of IgG, the most abundant isotype in circulation, and specific antibodies after immunization with different viruses or antigens. A summary of the studies and their results is shown in Table 5.

There were only six studies that investigated the influence of contaminant exposure on humoral immunity and most revealed

Table 6
Altered cytokine gene expression in marine mammals exposed to environmental contaminants.

Species	Location	Cytokine analysed	Pollutant (µg/ml)	Study type	Effect on gene expression	Reference
Harbour seal	North Sea	IL-2, IL-4, TGF-β	MeHg: 0–0.2	in vitro	↓ IL-2 & TGF-β ↑ IL-4	Das et al. (2008)
	North Sea	IL-2, IL-4, TGF-β	MeHg: 0–0.5	in vitro	No effect	Kakuschke et al. (2009)
	California	IL-1β, IL-2	PCB169: 7.2 BaP: 5	in vitro	↓ IL-1 & IL-2	Neale et al. (2005)
Grey seal	North Sea	IL-2, IL-4	Hg, Cd, Be, Ni[a]	in vitro	↑ IL-2 & IL-4 (Hg) ↓ IL-2 & IL-4 (Be, Ni) No effect (Cd)	Kakuschke et al. (2006)
Ringed seal	Baltic Sea	IL-1β	PCB: 3–170[b]	in vivo	↑ IL-1β	Routti et al. (2010)
	Labrador	IL-1β	PCB: 0.1–2.5[b]	in vivo	↑ IL-1β	Brown et al. (2014)

[a] 96-well plate coated with 50 µg per well for each metal.
[b] PCB concentration in liver in µg/g lw.

suppressed production of antibodies (Table 5). Circulating levels of antibodies (total or IgG) were reduced in more PCB exposed fur seal pups and polar bears, while they correlated positively with PFC concentrations in bottlenose dolphins. The opposite effects between studies may be linked to mechanistic or dose-related differences in the effect of PFCs and PCBs or a result from confounding biological factors, such as age and natural exposure to pathogens. As serum levels of IgG or total Ig are not specific to any pathogen, their modulation by pollutants may indicate an overall reduced production of antibodies.

More complex studies of humoral immunity in marine mammals revealed that POPs were associated with reduced production of specific antibodies after exposure to viral antigens through immunizations (Table 5). After immunization with tetanus toxoid, influenza, reovirus and herpes-virus, Lie et al. (2004) re-captured 56 wild polar bears from Canada and Svalbard and determined that PCB concentrations in the bears were negatively associated with the ability to produce antibodies specific to those viruses. In contrast, the harbour seal captive feeding experiment did not find a difference between exposed and control groups (n = 22) after exposure to tetanus toxoid, rabies virus and poliovirus; they did however measure lower antibody titres after ovalbumin immunization in the exposed group (de Swart et al., 1996). In a captive feeding experiment with sledge dogs used as proxies for polar bears, Sonne et al. (2010) reported reduced antibody response to tetanus toxoid and influenza virus 4–12 weeks after immunization. Antibody production is a complex process involving multiple immunological compartments and lymphoid organs and all phases of the humoral immune response, including antigen recognition and presentation, lymphocyte activation and differentiation, and cell cooperation (Beckmen et al., 2003). Therefore, many endpoints may be modulated by pollutants. Mixed responses between studies limits the predictive power of antibody modulation as an indicator for host-resistance in marine mammals, but further studies should be conducted to reduce confounding factors and identify threshold levels for pollutant-mediated effects on antibody production.

2.7. Cytokines

The immune system is a complex network of individual cells, cell aggregations, and lymphoid tissues and organs, and thus a coordinated immune response requires efficient communication within this network; cytokines fulfil that important role (Kidd, 2003). Cytokines include interferons (IFNs), interleukins (ILs) and various growth and stimulating factors (eg. Colony Stimulating Factor (CSF), Tumour Necrosis Factor (TNF)). Through their interactions with surface receptors of various cells, cytokines function to modulate lymphocyte proliferation and differentiation, lymphoid development, cell trafficking, and inflammation. Furthermore, cytokines can be divided into pro-inflammatory (IL-1, IL-6, IL-8 and TNF) and anti-inflammatory (IL-4, IL-10, and IL-13) and their secretion pattern can be associated with predominantly cellular and humoral responses (Abbas et al., 2012).

The analysis of cytokines in marine mammal effect studies is a relatively new field and studies have only appeared over the past few years (e.g. Lehnert et al., 2014; Weirup et al., 2013). We found six studies that related contaminant exposure to cytokines in marine mammals, and all used a genomic approach to study gene expression of specific cytokines (Table 6). In vivo IL-1 expression was positively associated with PCBs in ringed seals from Labrador and northern Europe (Brown et al., 2014; Routti et al., 2010) while in vitro exposure to CB-169 and benzo-a-pyrene suppressed IL-1 expression in California harbour seals (Neale et al., 2005). IL-1 is a pro-inflammatory cytokine associated with macrophage function among other roles, and has been shown to respond to dioxins through the AhR (Monteiro et al., 2008). The cause of discrepancy between studies is unclear, but may relate to complex differences between in vitro and *in vivo* cytokine profiles and signalling and the overall cytokine balance in animals related to physiological or potential sub-clinical infections.

The few existing studies in marine mammals support a model of contaminant-mediated modulation of lymphocyte function through suppressed IL-2 and stimulated IL-4 expression (Table 6). IL-2 is an important cytokine for T cell proliferation, NK cell activation and antibody production in B cells. IL-4 is anti-inflammatory and promotes T cell differentiation into Th2 cells (Das et al., 2008; Lehnert et al., 2014; Weirup et al., 2013). Neale et al. (2005) showed reduced IL-2 expression despite increased expression of the tyrosine kinase genes Fyn and Lck, which stimulate T cell receptor signalling, which suggested the contaminant effect occurred during signal transduction. Conclusions about contaminant effects on cytokines are further complicated by the large inter-individual and temporal variability in cytokine expression during exposures (Das et al., 2008; Fonfara et al., 2007; Kakuschke et al., 2009; Lehnert et al., 2014; Weirup et al., 2013). Nonetheless, cytokine profiling is an exciting new field in marine mammal immunology and toxicology and further studies will shed light into the mechanisms of cytokine disruption by contaminants in marine mammals.

3. Summary and conclusion

Marine mammals worldwide are exposed to the highest levels of environmental contaminants of all wildlife. The present review revealed that persistent organic pollutants and heavy metals, with PCBs and Hg most often reported, are associated with alterations of both the innate and adaptive arms of the immune system, and including cellular and humoral effects (Fig. 5). Because of abundant data, we now have a better understanding of how contaminants affect certain immune endpoints in marine mammals, such as lymphocyte proliferation and phagocytosis, but a lack of data on NK cell activity, respiratory burst, immunoglobulin production and cytokines is preventing similar understanding for these systems, especially as various diseases and chronic stress also influence the immune system. Nonetheless, our review shows a clear picture emerging regarding the directionality of contaminant effects; contaminant exposure is systemically suppressing immune function in marine mammals. Furthermore, immune responses are energetically costly and the immune system is intimately tied to the reproductive and endocrine system, such that pollutant-mediated immune effects may extend to other physiological systems with potentially costs for fitness and reproduction, and ultimately, population growth (McDade, 2003; Sonne, 2010).

The challenge in all ecotoxicology research is linking an observable effect in individuals to a specific cause, such as exposure to contaminants. Marine mammal science is particularly challenging given the logistical and ethical constraints of studying mammals that spend much or all their time at sea, in often remote areas accessible only at certain times of the year and only during certain periods of the animals life-cycle. As with most other biomarkers of effect, immune function varies in relation to many biological and ecological factors, including age, sex, condition and exposure to pathogens, among others (Dupont et al., 2013; Kakuschke et al., 2008a,b; Luebke et al., 2007; Ross, 2002; Ross et al., 1994; Tryphonas et al., 2004). It is only through minimizing and/or understanding these confounding factors as well as other anthropogenic stressors that we may accurately assess the relationship between pollutants and immune responses of free-ranging marine mammals and other wildlife. In vitro assays have provided controlled experimental conditions in which to test specific toxic effects and have advanced our understanding of the immunotoxic potential of contaminants, including hints on the mechanisms involved. The lack of differences between in vitro and in vivo effects on lymphocyte proliferation in this study strongly supports the relevance of *in vitro* assays to predict effects *in vivo*. Further work is needed however to link *in vitro* results to *in vivo* effects, and more importantly to population-level consequences.

Several reports suggest that the incidence of infectious diseases in the marine environment, including marine mammals specifically, has increased over the past decades (Gulland and Hall, 2007; Harvell,
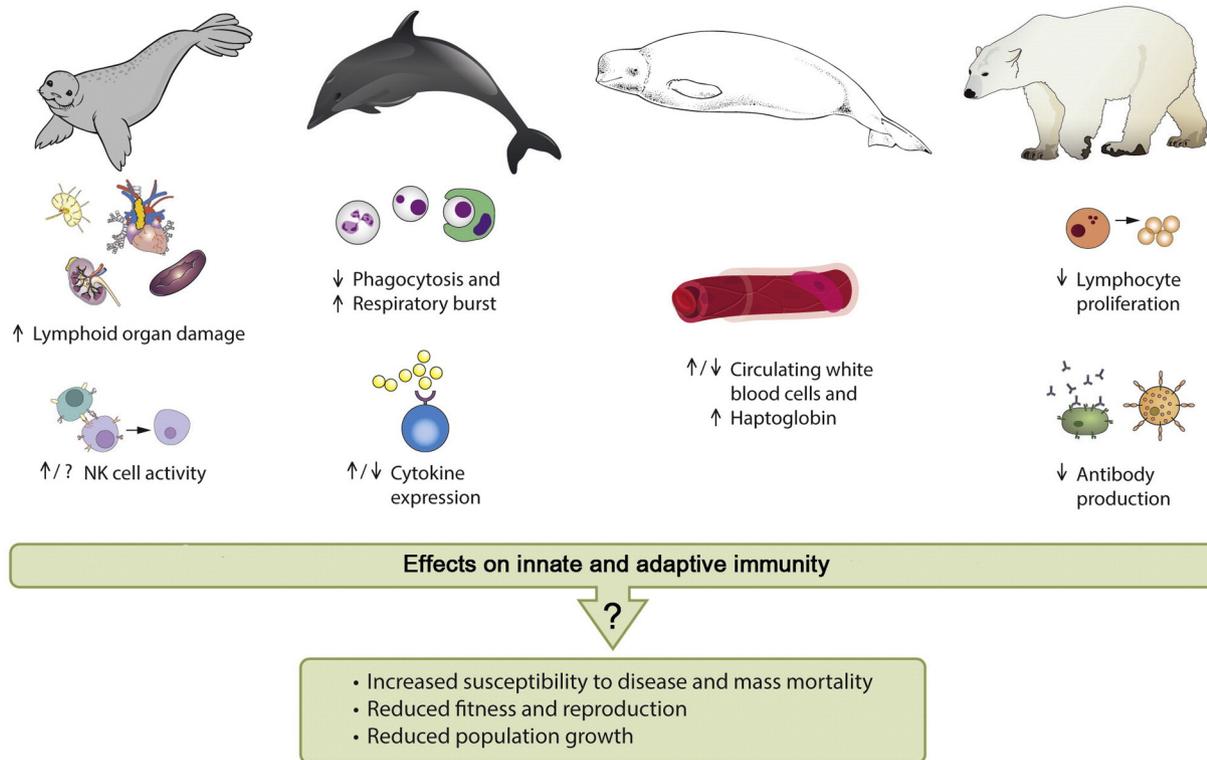
Fig. 5. Summary of documented contaminant-mediated effects on the immune system of marine mammals. The weight of evidence in the marine mammal immunotoxicology literature suggests that environmental pollutants modulate both innate and adaptive immunity, including aspects of cellular and humoral responses. Although still unclear, effects on the immune system may have consequences on intimately tied reproductive and endocrine systems, potentially leading to reduced fitness and population growth.

1999; Van Bressem et al., 2009). Interactions between populations and pathogens are nothing new, but this equilibrium is increasingly disturbed by environmental degradation (e.g. biological and chemical pollution, habitat destruction and nutritional stress, climate change, etc) potentially leading to increased stress, immune suppression and reduced host-resistance to disease (Beineke et al., 2010; Jenssen et al., 2015; Van Bressem et al., 2009; Weirup et al., 2013). Indeed, many studies have associated increased prevalence and severity of disease, including death, to contaminant exposure in heavily polluted marine mammal populations (Bennett et al., 2001; Hall et al., 2006; Jepson et al., 1999; Kannan et al., 2006; Martineau et al., 1994; Siebert et al., 2009; Wunschmann et al., 2001). Considering contaminant-mediated immune suppression has been quantitatively linked to disease host-resistance in controlled studies (Luster et al., 1992, 1993), immune assays represent one of the most relevant indicators to detect subtle and/or sub-clinical effects of contaminants on human and wildlife health. Most importantly, contaminant-mediated alterations of immune function may have population level consequences if marine mammals increasingly succumb to infection. Given evidence of the rise of marine diseases and the immunotoxic potential of POPs and heavy metals, further research and risk assessment of immune modulation in wildlife populations and humans is more important now than ever.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2015.10.007.

References

Abbas, A.K., Lichtman, A.H., Pillai, S., 2012. Basic Immunology: Functions and Disorders on the Immune System. Fourth edition. Elsevier Inc., Philidelphia.

Aguilar, A., Borrell, A., 1994. Abnormally high polychlorinated biphenyl levels in striped dolphins (Stenella coeruleoalba) affected by the 1990–1992 Mediterranean epizootic. Sci. Total Environ. 154, 237–247.

Beckmen, K.B., Blake, J.E., Ylitalo, G.M., Stott, J.L., O'Hara, T.M., 2003. Organochlorine contaminant exposure and associations with hematological and humoral immune functional assays with dam age as a factor in free-ranging northern fur seal pups (Callorhinus ursinus). Mar. Pollut. Bull. 46, 594–606.

Beineke, A., Siebert, U., Mclachlan, M., Bruhn, R., Thron, K., Failing, K., Muller, G., Baumgartner, W., 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.

Beineke, A., Siebert, U., Wohlsein, P., Baumgärtner, W., 2010. Immunology of whales and dolphins. Vet. Immunol. Immunopathol. 133, 81–94.

Bennett, P.M., Jepson, P.D., Law, R.J., Jones, B.R., Kuiken, T., Baker, J.R., Rogan, E., Kirkwood, J.K., 2001. Exposure to heavy metals and infectious disease mortality in harbour porpoises from England and Wales. Environ. Pollut. 112, 33–40.

Bernhoft, A., Skaare, J., Wiig, O., Derocher, A., Larsen, H., 2000. Possible immunotoxic effects of organochlorines in polar bears (Ursus Maritimus) at Svalbard. J. Toxic. Environ. Health A 59, 561–574.

Boughton, R.K., Joop, G., Armitage, S.a.O., 2011. Outdoor immunology: methodological considerations for ecologists. Funct. Ecol. 25, 81–100.

Brown, T.M., Ross, P.S., Reimer, K.J., Veldhoen, N., Danger, N.J., Fisk, A.T., Helbing, C.C., 2014. PCB related effects thresholds as derived through gene transcript profiles in locally contaminated ringed seals (Pusa hispida). Environ. Sci. Technol. 48, 12952–12961.

Corsini, E., Luebke, R.W., Germolec, D.R., DeWitt, J.C., 2014. Perfluorinated compounds: emerging POPs with potential immunotoxicity. Toxicol. Lett. 230, 263–270.

Das, K., Siebert, U., Gillet, A., Dupont, A., Di-Poï, C., Fonfara, S., Mazzucchelli, G., De Pauw, E., De Pauw-Gillet, M.-C., 2008. Mercury immune toxicity in harbour seals: links to in vitro toxicity. Environ. Heal. 7, 52.

De Guise, S., 2004. Immunotoxicology in marine mammals. In: Tryphonas, H., Fournier, M., Blakley, B.R., Smits, J.E.G., Brousseau, P. (Eds.), Investigative Immunotoxicology. Taylor & Francis, New York, USA.

De Guise, S., Flipo, D., Boehm, J.R., Martineau, D., Béland, P., Fournier, M., 1995a. 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., 1995b. Possible Mechanisms of Action of Environmental Contaminants on St. Lawrence beluga whales (Deiphinapterus leucas). Environ. Health Perspect. 103, 73–77.

De Guise, S., Bernier, J., Dufresne, M.M., Martineau, D., Pierre, B., Fournier, M., 1996a. Immune functions in beluga whales (Delphinapterus leucas): Evaluation of mitogen-induced blastic transformation of lymphocytes from peripheral blood, spleen and thymus. Vet. Immunol. Immunopathol. 50, 117–126.

De Guise, S., Bernier, J., Martineau, D., Beland, P., Fournier, M., 1996b. Effects on in vitro exposure of beluga whale splenocytes and thymocytes to heavy metals. Environ. Toxicol. Chem. 15, 1357–1364.

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., Beland, P., Fournier, M., 1998. Effects of in vitro exposure of beluga whale leukocytes to selected organochlorines. J. Toxic. Environ. Health A 55, 479–493.

De Guise, S., Erickson, K., Blanchard, M., DiMolfetto, L., Lepper, H.D., Wang, J., Stott, J.L., Ferrick, D.a., 2002. Monoclonal antibodies to lymphocyte surface antigens for cetacean homologues to CD2, CD19 and CD21. Vet. Immunol. Immunopathol. 84, 209–221.

De Guise, S., Erickson, K., Blanchard, M., DiMolfetto, L., Lepper, H.D., Stott, J.L., Ferrick, D.a., 2004. Characterization of F21.A, a monoclonal antibody that recognize a leucocyte surface antigen for killer whale homologue to β-2 integrin. Vet. Immunol. Immunopathol. 97, 195–206.

De Swart, R.L., Kluten, R.M., Huizing, C.J., Vedder, L.J., Reijnders, P.J., Visser, I.K., UytdeHaag, F.G., Osterhaus, A.D., 1993. Mitogen and antigen induced B and T 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., Loveren, H.V., Vos, J.G., Reijnders, P.J.H., Osterhaus, A.D.M.E., 1994. Impairment of immune function in harbor seals (Phoca vitulina) feeding on fish from polluted waters. Ambio 23, 155–159.

De Swart, R.L., Ross, P.S., Timmerman, H.H., Hijman, W.C., de Ruiter, E.M., Liem, A.K.D., Brouwer, A., van Loveren, H., Reijnders, P.J.H., Vos, J.G., Osterhaus, A.D.M.E., 1995. Short term fasting does not aggravate immunosuppression in harbour seals. Chemosphere 31, 4289–4306.

De Swart, R.L., Ross, P.S., Vos, J.G., Osterhaus, A.D., 1996. Impaired immunity in harbour seals (Phoca vitulina) exposed to bioaccumulated environmental contaminants: review of a long-term feeding study. Environ. Health Perspect. (104 Suppl.), 823–828.

Debier, C., Chalon, C., LeBoeuf, B.J., DeTillesse, T., Larondelle, Y., Thomé, J.-P., 2006. Mobilization of PCBs from blubber to blood in northern elephant seals (Mirounga angustirostris) during the post-weaning fast. Aquat. Toxicol. 80, 149–157.

Derocher, A., Larsen, H.J., J, S., Wiig, Ø., 2000. Immune response and chlorinated environmental pollutants in polar bears. Final Rep. to Eff. Program. Nor. Polar Institute, Tromso Norw., pp. 1–24.

Dierauf, L., Gulland, M., 2001. CRC Handbook of Marine Mammal Medicine. 2nd ed. CRC Press.

Dietz, R., Heide-jorgensen, M.P., Harkonen, T., 1989. Mass deaths of harbor seals (Phoca vitulina) in Europe. Ambio 18, 258–264.

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. Mar. Pollut. Bull. 60, 344–349.

Duignan, P., Van Bressem, M.-F., Baker, J., Barbieri, M., Colegrove, K., De Guise, S., de Swart, R., Di Guardo, G., Dobson, A., Duprex, W., Early, G., Fauquier, D., Goldstein, T., Goodman, S., Grenfell, B., Groch, K., Gulland, F., Hall, A., Jensen, B., Lamy, K., Matassa, K., Mazzariol, S., Morris, S., Nielsen, O., Rotstein, D., Rowles, T., Saliki, J., Siebert, U., Waltzek, T., Wellehan, J., 2014. Phocine distemper virus: current knowledge and future directions. Viruses 6, 5093–5134.

Dupont, A., Siebert, U., Covaci, A., Weijs, L., Eppe, G., Debier, C., De Pauw-Gillet, M.-C., Das, K., 2013. Relationships between in vitro lymphoproliferative responses and levels of contaminants in blood of free-ranging adult harbour seals (Phoca vitulina) from the North Sea. Aquat. Toxicol. 142-143, 210–220.

Fair, P.A., Romano, T., Schaefer, A.M., Reif, J.S., Bossart, G.D., Houde, M., Muir, D., Adams, J., Rice, C., Hulsey, T.C., Peden-Adams, M., 2013. Associations between perfluoroalkyl compounds and immune and clinical chemistry parameters in highly exposed bottlenose dolphins (Tursiops truncatus). Environ. Toxicol. Chem. 32, 736–746.

Fischer, L.J., Seegal, R.F., Ganey, P.E., Pessah, I.N., Kodavanti, P.R., 1998. Symposium overview: toxicity of non-coplanar PCBs. Toxicol. Sci. 41, 49–61.

Fonfara, S., Siebert, U., Prange, A., 2007. Cytokines and acute phase proteins as markers for infection in harbor porpoises (Phocoena phocoena). Mar. Mamm. Sci. 23, 931–942.

Frouin, H., Fortier, M., Fournier, M., 2010a. Toxic effects of various pollutants in 11B7501 lymphoma B cell line from harbour seal (Phoca vitulina). Toxicology 270, 66–76.

Frouin, H., Lebeuf, M., Hammill, M., Masson, S., Fournier, M., 2010b. Effects of individual polybrominated diphenyl ether (PBDE) congeners on harbour seal immune cells in vitro. Mar. Pollut. Bull. 60, 291–298.

Frouin, H., Ménard, L., Measures, L., Brousseau, P., Fournier, M., 2010c. T lymphocyte-proliferative responses of a grey seal (<I> Halichoerus grypus </I>) exposed to heavy metals and PCBs <I> in vitro </I>. Aquat. Mamm. 36, 365–371.

Frouin, H., Loseto, L.L., Stern, G.A., Haulena, M., Ross, P.S., 2012. Mercury toxicity in beluga whale lymphocytes: limited effects of selenium protection. Aquat. Toxicol. 109, 185–193.

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

Hall, A.J., Law, R.J., Wells, D.E., Harwood, J., Ross, H.M., Kennedy, S., Allchin, C.R., Campbell, L.A., Pomeroy, P.P., 1992. Organochlorine levels in common seals (Phoca vitulina) which were victims and survivors of the 1988 phocine distemper epizootic. Sci. Total Environ. 115, 145–162.

Hall, A., Pomeroy, P., Green, N., Jones, K., Harwood, J., 1997. Infection, haematology and biochemistry in grey seal pups exposed to chlorinated biphenyls. Mar. Environ. Res. 43, 81–98.

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

Hammond, J.A., Hall, A.J., Dyrynda, E.A., 2005. Comparison of polychlorinated biphenyl (PCB) induced effects on innate immune functions in harbour and grey seals. Aquat. Toxicol. 74, 126–138.

Harvell, C.D., 1999. Emerging Marine Diseases—Climate Links and Anthropogenic Factors. Science 285 (80), 1505–1510.

Helle, E., 1980. Lowered reproductive capacity in female ringed seals (Pusa hispida) in the Bothnian Bay, Northern Baltic Sea, with special reference to uterine occlusions. Ann. Zool. Fenn.

Helle, E., Olsson, M., Jensen, S., 1976. PCB levels correlated with pathological changes in seal uteri. Ambio 5, 261–262.

Jaber, J.R., Fernández, A., Herráez, P., Espinosa De Los Monteros, A., Ramírez, G.A., García, P.M., Fernández, T., Arbelo, M., Pérez, J., 2003a. Cross-reactivity of human and bovine antibodies in striped dolphin paraffin wax-embedded tissues. Vet. Immunol. Immunopathol. 96, 65–72.

Jaber, J.R., Pérez, J., Arbelo, M., Herráez, P., Espinosa De Los Monteros, A., Rodríguez, F., Fernández, T., Fernández, A., 2003b. Immunophenotypic characterization of hepatic inflammatory cell infiltrates in common dolphins (Delphinus delphis). J. Comp. Pathol. 129, 226–230.

Jenssen, B.M., Villanger, G.D., Gabrielsen, K.M., Bytingsvik, J., Bechshoft, T., Ciesielski, T.M., Sonne, C., Dietz, R., 2015. Anthropogenic flank attack on polar bears: interacting consequences of climate warming and pollutant exposure. Front. Ecol. Evol. 3.

Jepson, P.D., Bennett, P.M., Allchin, C.R., Law, R.J., Kuiken, T., Baker, J.R., Rogand, E., James, K.K., 1999. Investigating potential associations between chronic exposure to polychlorinated biphenyls and infectious disease mortality in harbour porpoises from England and Wales. Sci. Total Environ. 7, 339–348.

Kakuschke, A., Valentine-Thon, E., Griesel, S., Fonfara, S., Siebert, U., Prange, A., 2005. Immunological impact of metals in harbor seals (Phoca vitulina) of the North Sea. Environ. Sci. Technol. 39, 7568–7575.

Kakuschke, A., Valentine-Thon, E., Fonfara, S., Griesel, S., Siebert, U., Prange, A., 2006. Metal sensitivity of marine mammals: a case study of a gray seal (Halichoerus grypus). Mar. Mamm. Sci. 22, 985–996.

Kakuschke, A., Valentine-Thon, E., Fonfara, S., Griesel, S., Rosenberger, T., Siebert, U., Prange, A., 2008a. Metal-induced impairment of the cellular immunity of newborn harbor seals (Phoca vitulina). Arch. Environ. Contam. Toxicol. 55, 129–136.

Kakuschke, A., Valentine-Thon, E., Griesel, S., Rosenberger, T., Mundry, R., Siebert, U., Prange, A., 2008b. Blood metal levels and metal-influenced immune functions of harbour seals in captivity. Mar. Pollut. Bull. 56, 764–769.

Kakuschke, A., Valentine-Thon, E., Fonfara, S., Kramer, K., Prange, A., 2009. Effects of methyl-, phenyl-, ethylmercury and mercurychloride on immune cells of harbor seals (Phoca vitulina). J. Environ. Sci. 21, 1716–1721.

Kakuschke, A., Valentine-Thon, E., Griesel, S., Fonfara, S., Siebert, U., Prange, A., 2011. Are metal-induced hypersensitivities in harbor seals associated with liver function? Mar. Pollut. Bull. 62, 1891–1894.

Kannan, K., Perrotta, E., Thomas, N.J., 2006. Association between perfluorinated compounds and pathological conditions in Southern Sea otters. Environ. Sci. Technol. 40, 4943–4948.

Kawashima, M., Nakanishi, M., Kuwamura, M., Takeya, M., Yamate, J., 2004. Distributive and phagocytic characteristics of hepatic macrophages in five cetaceans belonging to Delphinidae and Ziphiidae. J. Vet. Med. Sci. 66, 671–680.

Kidd, P., 2003. Th1/Th2 balance : the hypothesis, its limitations, and implications for health and disease. Altern. Med. Rev. 8, 223–246.

Kimber, I., Dearman, R.J., 2002. Immune responses: adverse versus non-adverse effects. Toxicol. Pathol. 30, 54–58.

Kirkegaard, M., Sonne, C., Leifsson, P.S., Dietz, R., Born, E.W., Muir, D.C.G., Letcher, R.J., 2005. Histology of selected immunological organs in polar bear (Ursus maritimus) from East Greenland in relation to concentrations of organohalogen contaminants. Sci. Total Environ. 341, 119–132.

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 (Tursiops truncatus) are associated with increased concentrations of PCBs and DDT in peripheral blood. Environ. Health Perspect. 103, 67–72.

Lalancette, A., Morin, Y., Measures, L., Fournier, M., 2003. Contrasting changes of sensitivity by lymphocytes and neutrophils to mercury in developing grey seals. Dev. Comp. Immunol. 27, 735–747.

Lehnert, K., Müller, S., Weirup, L., Ronnenberg, K., Pawliczka, I., Rosenberger, T., Siebert, U., 2014. Molecular biomarkers in grey seals (Halichoerus grypus) to evaluate pollutant exposure, health and immune status. Mar. Pollut. Bull.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent

organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995–3043.

Levin, M., Morsey, B., Mori, C., Guise, S., 2004. Specific non-coplanar PCB-mediated modulation of bottlenose dolphin and beluga whale phagocytosis upon in vitro exposure. J. Toxic. Environ. Health A 67, 1517–1535.

Levin, M., De Guise, S., Ross, P.S., 2005. Association between lymphocyte proliferation and polychlorinated biphenyls in free-ranging harbor seal (*Phoca vitulina*) pups from British Columbia, Canada. Environ. Toxicol. Chem. 24, 1247–1252.

Levin, M., Morsey, B., De Guise, S., 2007. Modulation of the respiratory burst by organochlorine mixtures in marine mammals, humans, and mice. J. Toxic. Environ. Health A 70, 73–83.

Lie, E., Larsen, H.J.S., Larsen, S., Johansen, G.M., Derocher, A.E., Lunn, N.J., Norstrom, R.J., Wiig, Ø., Skaare, J.U., 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. Toxic. Environ. Health A 67, 555–582.

Lie, E., Larsen, H.J.S., Larsen, S., Johansen, G.M., Derocher, A.E., Lunn, N.J., Norstrom, R.J., Wiig, O., Skaare, J.U., 2005. Does high organochlorine (OC) exposure impair the resistance to infection in polar bears (*Ursus maritimus*)? part II: possible effect of OCs on mitogen- and antigen-induced lymphocyte proliferation. J. Toxic. Environ. Health A 68, 457–484.

Luebke, R.W., Hodson, P.V., Faisal, M., Ross, P.S., Grasman, K.A., Zelikoff, J., 1997. Aquatic pollution-induced immunotoxicity in wildlife species. Fundam. Appl. Toxicol. 37, 1–15.

Luebke, R., House, R., Kimber, I., 2007. Immunotoxicology and Immunopharmacology. Third edition. CRC Press, Florida.

Luster, M.I., Blank, J.A., Dean, J.H., 1987. Molecular and cellular basis of chemically induced immunotoxicity. Annu. Rev. Pharmacol. Toxicol. 27, 23–49.

Luster, M.I., Portier, C., Pait, D., White, K., Gennings, C., Munson, A.E., Rosenthal, G.J., 1992. Risk assessment in Immunotoxicology: I. Sensitivity and Predictability of immune tests. Fundam. Appl. Toxicol. 18, 200–210.

Luster, M., Portier, C., Pait, D.G., Rosenthal, G., Germolec, D., Corsini, E., Blaylock, B., Pollock, P., Kouchi, Y., Craig, W., White, K., Munson, A., Comment, C., 1993. Risk assessment in immunotoxicology: II. Relationships between immune and host resistance tests. Fundam. Appl. Toxicol.

Luster, M.I., Portier, C., Pait, D., Germoleca, D.R., 1994. The use of animal tests in risk assessment for immunotoxicology. Toxicol. in Vitro 8, 945–950.

Macdonald, R.W., Mackay, D., Hickie, B.E., 2002. Contaminant amplification in the environment. Environ. Sci. Technol. 36, 457–462.

Mackay, D., Fraser, A., 2000. Bioaccumulation of persistent organic chemicals: mechanisms and models. Environ. Pollut. 110, 375–391.

Martineau, D., De Guise, S., Fournier, M., Shugart, L., Girard, C., Lagacé, A., Béland, P., 1994. Pathology and toxicology of beluga whales from the St. Lawrence Estuary, Quebec, Canada. Past, present and future. Sci. Total Environ. 154, 201–215.

McDade, T.W., 2003. Life history theory and the immune system: steps toward a human ecological immunology. Am. J. Phys. Anthropol. (Suppl. 37), 100–125.

Monteiro, P., Gilot, D., Ferrec, E.L., Rauch, C., Lagadic-gossmann, D., Fardel, O., 2008. Dioxin-mediated up-regulation of aryl hydrocarbon receptor target genes is dependent on the calcium/calmodulin/CaMKI alpha pathway. Mol. Pharmacol. 73, 769–777.

Mori, C., Morsey, B., Levin, M., Nambiar, P.R., De Guise, S., 2006. Immunomodulatory effects of in vitro exposure to organochlorines on T-cell proliferation in marine mammals and mice. J. Toxic. Environ. Health A 69, 283–302.

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. J. Toxic. Environ. Health A 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 harbor seals. Environ. Toxicol. Chem. 25, 3110–3117.

Moser, M., Leo, O., 2010. Key concepts in immunology. Vaccine 28, 2–13.

Mosmann, T.R., Sad, S., 1996. The expanding universe of T-cell subsets : Thl, Th2 and more. Immunol. Today.

Muir, D.C.G., Ford, C.A., Rosenberg, B., Norstrom, R.J., Simon, M., Béland, P., 1996. Persistent organochlorines in beluga whales (*Delphinapterus leucas*) from the St Lawrence River estuary-I. Concentrations and patterns of specific PCBs, chlorinated pesticides and polychlorinated dibenzo-p-dioxins and dibenzofurans. Environ. Pollut. 93, 219–234.

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. Environ. Pollut. 120, 245–253.

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

Neale, J.C.C., Kenny, T.P., Tjeerdema, R.S., Gershwin, M.E., 2005. PAH- and PCB-induced alterations of protein tyrosine kinase and cytokine gene transcription in harbor seal (*Phoca vitulina*) PBMC. Clin. Dev. Immunol. 12, 91–97.

Newman, M.C., Unger, M.A., 2003. Fundamentals of Ecotoxicology. Second edition. CRC Press, Florida.

Noda, K., Aoki, M., Akiyoshi, H., Asaki, H., Shimada, T., Ohashi, F., 2003. Evaluation of the polymorphonuclear cell functions of bottlenose dolphins. J. Vet. Med. Sci. 65, 727–729.

Nyman, M., Bergknut, M., Fant, M.L., Raunio, H., Jestoi, M., Bengs, C., Murk, A., Koistinen, J., Bäckman, C., Pelkonen, O., Tysklind, M., Hirvi, T., Helle, E., 2003. Contaminant exposure and effects in Baltic ringed and grey seals as assessed by biomarkers. Mar. Environ. Res. 55, 73–99.

Olsson, M., Karlsson, B., Ahnland, E., 1994. Diseases and environmental contaminants in seals from the Baltic and the Swedish West Coast. Sci. Total Environ. 154, 217–227.

Pellissó, S.C., Muñoz, M.J., Carballo, M., Sánchez-Vizcaíno, J.M., 2008. Determination of the immunotoxic potential of heavy metals on the functional activity of bottlenose dolphin leukocytes in vitro. Vet. Immunol. Immunopathol. 121, 189–198.

Pillet, S., Lesage, V., Hammill, M., Cyr, D.G., Bouquegneau, J., Fournier, M., 2000. In vitro exposure of seal peripheral blood leukocytes to different metals reveal a sex-dependent effect of zinc on phagocytic activity. Mar. Pollut. Bull. 40, 921–927.

R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (URL http://www.R-project.org/ [WWW Document]).

Reijnders, P.J.H., 1988. Ecotoxicology perspectives in marine mammalogy: research principles and goals for a conservation policy. Mar. Mamm. Sci. 4, 91–102.

Ritz, C., Streibig, J.C., 2005. Bioassay Analysis using R. J. Stat. Softw. 12 (5).

Roos, A.M., Bäcklin, B.M.V.M., Helander, B.O., Rigét, F.F., Eriksson, U.C., 2012. Improved reproductive success in otters (*Lutra lutra*), grey seals (*Halichoerus grypus*) and sea eagles (*Haliaeetus albicilla*) from Sweden in relation to concentrations of organochlorine contaminants. Environ. Pollut. 170, 268–275.

Ross, P.S., 2000. Marine mammals as sentinels in ecological risk assessment. Hum. Ecol. Risk. Assess. 6, 29–46.

Ross, P.S., 2002. The role of immunotoxic environmental contaminants in facilitating the emergence of infectious diseases in marine mammals. Hum. Ecol. Risk. Assess. 8, 277–292.

Ross, P.S., De Guise, S., 2007. Marine mammal immunotoxicology. In: Luebke, R., House, R., Kimber, I. (Eds.), Immunotoxicology and Immunopharmacology, 3rd ed. CRC Press, Taylor & Francis Group, Boca Raton, FL.

Ross, P.S., de Swart, R.L., Visser, I.K., Vedder, L.J., Murk, W., Bowen, W.D., Osterhaus, A.D., 1994. Relative immunocompetence of the newborn harbour seal, *Phoca vitulina*. Vet. Immunol. Immunopathol. 42, 331–348.

Ross, P.S., Swart, R.L.D., Reijnders, P.J.H., van Loveren, H., Vos, J.G., Osterhaus, A.D.M.E., 1995. Contaminant-related suppression of delayed-type hypersensitivity and antibody responses in harbor seals fed herring from the Baltic Sea. Environ. Health Perspect. 103, 162–167.

Ross, P.S., De Swart, R.L., Van Loveren, H., Osterhaus, A.D.M.E., Vos, J.G., 1996a. The immunotoxicity of environmental contaminants to marine wildlife: a review. Annu. Rev. Fish Dis. 6, 151–165.

Ross, P.S., DeSwart, R., Addison, R., Van Loveren, H., Vos, J., Osterhaus, A., 1996b. Contaminant-induced immunotoxicity in harbour seals: wildlife at risk? Toxicology 112, 157–169.

Ross, P.S., DeSwart, R.L., Timmerman, H.H., Reijnders, P.J.H., Vos, J.G., Van Loveren, H., Osterhaus, A.D., 1996c. Suppression of natural killer cell activity in harbour seals (*Phoca vitulina*) fed Baltic Sea herring. Aquat. Toxicol. 34, 71–84.

Ross, P., Ellis, G.M., Ikonomou, M.G., Barrett-Lennard, L.G., Addison, R.F., 2000. High PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca*: effects of age, sex and dietary preference. Mar. Pollut. Bull. 40, 504–515.

Routti, H., Arukwe, A., Jenssen, B.M., Letcher, R.J., Nyman, M., Bäckman, C., Gabrielsen, G.W., 2010. Comparative endocrine disruptive effects of contaminants in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 152, 306–312.

Schaefer, A.M., Stavros, H.-C.W., Bossart, G.D., Fair, P.A., Goldstein, J.D., Reif, J.S., 2011. Associations between mercury and hepatic, renal, endocrine, and hematological parameters in Atlantic bottlenose dolphins (*Tursiops truncatus*) along the eastern coast of Florida and South Carolina. Arch. Environ. Contam. Toxicol. 61, 688–695.

Schumacher, U., Heidemann, G., Skírnisson, K., Schumacher, W., Pickering, R.M., 1995. Impact of captivity and contamination level on blood parameters of harbour seals (*Phoca vitulina*). Comp. Biochem. Physiol. A 112, 455–462.

Schwacke, L.H., Zolman, E.S., Balmer, B.C., De Guise, S., George, R.C., Hoguet, J., Hohn, A.A., Kucklick, J.R., Lamb, S., Levin, M., Litz, J.A., McFee, W.E., Place, N.J., Townsend, F.I., Wells, R.S., Rowles, T.K., 2012. Anaemia, hypothyroidism and immune suppression associated with polychlorinated biphenyl exposure in bottlenose dolphins (*Tursiops truncatus*). Proc. R. Soc. B 279, 48–57.

Siebert, U., Joiris, C., Holsbeek, L., Benke, H., Failing, K., Frese, K., Petzinger, E., 1999. Potential relation between mercury concentrations and necropsy findings in cetaceans from German waters of the North and Baltic Seas. Mar. Pollut. Bull. 38, 285–295.

Siebert, U., Prenger-Berninghoff, E., Weiss, R., 2009. Regional differences in bacterial flora in harbour porpoises from the North Atlantic: environmental effects? J. Appl. Microbiol. 106, 329–337.

Silkworth, J., Antrim, L., 1985. Relationship between Ah receptor-mediated polychlorinated biphenyl (PCB)-induced humoral immunosuppression and thymic atrophy. J. Pharmacol. Exp. Ther. 235, 606–611.

Sonne, C., 2010. Health effects from long-range transported contaminants in Arctic top predators: an integrated review based on studies of polar bears and relevant model species. Environ. Int. 36, 461–491.

Sonne, C., Larsen, H.J.S., Kirkegaard, M., Letcher, R.J., Dietz, R., 2010. Trans-generational and neonatal humoral immune responses in West Greenland sledge dogs (*Canis familiaris*) exposed to organohalogenated environmental contaminants. Sci. Total Environ. 408, 5801–5807.

Sørmo, E.G., Larsen, H.J.S., Johansen, G.M., Skaare, J.U., Jenssen, B.M., 2009. Immunotoxicity of polychlorinated biphenyls (PCB) in free-ranging gray seal pups with special emphasis on dioxin-like congeners. J. Toxic. Environ. Health A 72, 266–276.

Suh, J., Jeon, Y.J., Kim, H.M., Kang, J.S., Kaminski, N.E., Yang, K.-H., 2002. Aryl hydrocarbon receptor-dependent inhibition of AP-1 activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin in activated B cells. Toxicol. Appl. Pharmacol. 181, 116–123.

Sweet, L.I., Zelikoff, J.T., 2010. Toxicology and immunotoxicology of mercury: a comparative review in fish and humans. J. Toxicol. Environ. Health. B Crit. Rev. 4, 161–205.

Thurmond, T.S., Staples, J.E., Silverstone, A.E., Gasiewicz, T.A., 2000. The aryl hydrocarbon receptor has a role in the in vivo maturation of murine bone marrow B lymphocytes

and their response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 165, 227–236.

Tryphonas, H., Fournier, M., Blakley, B., Smits, J., Brousseau, P., 2004. Investigative Immunotoxicology. Taylor & Francis, New York, USA.

Van Bressem, M.-F., Raga, J.A., Di Guardo, G., Jepson, P.D., Duignan, P.J., Siebert, U., Barrett, T., Santos, M.C.D.O., Moreno, I.B., Siciliano, S., Aguilar, A., Van Waerebeek, K., 2009. Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. Dis. Aquat. Org. 86, 143–157.

Vos, J.G., 1977. Immune suppression as related to toxicology. Crit. Rev. Toxicol. 5, 67–101.

Vos, J.G., Van Loveren, H., 1994. Developments of immunotoxicology methods in the rat and applications to the study of environmental pollutants. Toxicol. in Vitro.

Vos, J., Loveren, H.V., Wester, P., Vethaak, D., 1989. Toxic effects of environmental chemicals on the immune system. TiPS 10, 289–292.

Weirup, L., Müller, S., Ronnenberg, K., Rosenberger, T., Siebert, U., Lehnert, K., 2013. Immune-relevant and new xenobiotic molecular biomarkers to assess anthropogenic stress in seals. Mar. Environ. Res. 92, 43–51.

Wirth, J.R., Peden-Adams, M.M., White, N.D., Bossart, G.D., Fair, P., 2014a. In vitro exposure of DE-71, a penta-PBDE mixture, on immune endpoints in bottlenose dolphins (tursiops truncatus) and B6C3F1 mice. J. Appl. Toxicol. 191–198.

Wirth, J.R., Peden-Adams, M.M., White, N.D., Bossart, G.D., Fair, P.a., 2014b. In vitro PFOS exposure on immune endpoints in bottlenose dolphins (Tursiops truncatus) and mice. J. Appl. Toxicol. 34, 658–666.

Wunschmann, A., Siebert, U., Frese, K., Weiss, R., Lockyer, C., Heide-Jørgensen, M.P., Müller, G., Baumgärtner, W., 2001. Evidence of infectious diseases in harbour porpoises (Phocoena phocoena) hunted in the waters of Greenland and by-caught in the German North Sea and Baltic Sea. Vet. Rec. 148, 715–720.

Yordy, J.E., Wells, R.S., Balmer, B.C., Schwacke, L.H., Rowles, T.K., Kucklick, J.R., 2010. Partitioning of persistent organic pollutants between blubber and blood of wild bottlenose dolphins: implications for biomonitoring and health. Environ. Sci. Technol. 44, 4789–4795.

Zenteno-Savin, T., Castellini, M., Rea, L., Fadely, B., 1997. Plasma haptoglobin levels in threatened Alaskan pinniped populations. J. Wildl. Dis. 33, 64–71.