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Contaminant-induced immunotoxicity in harbour seals: wildlife at risk?

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Abstract

Persistent, lipophilic polyhalogenated aromatic hydrocarbons (PHAHs) accumulate readily in the aquatic food chain and are found in high concentrations in seals and other marine mammals. Recent mass mortalities among several marine mammal populations have been attributed to infection by morbilliviruses, but a contributing role for immunotoxic PHAHs, including the polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) was not ruled out. We addressed this issue by carrying out a semi-field study in which captive harbour seals were fed herring from either the relatively uncontaminated Atlantic Ocean or the contaminated Baltic Sea for 2 years. We present here an overview of results obtained during this study. An impairment of natural killer (NK) cell activity, in vitro T-lymphocyte function, antigen-specific in vitro lymphocyte proliferative responses, and in vivo delayed-type hypersensitivity and antibody responses to ovalbumin was observed in the seals fed the contaminated Baltic herring. Additional feeding studies in PVG rats using the same herring batches suggested that an effect at the level of the thymus may be responsible for changes in cellular immunity, that virus-specific immune reponses may be impaired, and that perinatal exposure to environmental contaminants represents a greater immunotoxic threat than exposure as a juvenile or adult. Together with the pattern of TCDD toxic equivalents of different PHAHs in the herring, these data indicate that present levels of PCBs in the aquatic food chain are immunotoxic to mammals. A review of contaminant levels in free-ranging harbour seals inhabiting polluted areas of Europe and North America suggests that many populations may be at risk to immunotoxicity. This could result in diminished host resistance and an increased incidence and severity of infectious disease.

Keywords: Seals; Immunotoxicology; Morbillivirus; PCBs; PCDDs; PCDFs

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1. Environmental contaminants and virus epizootics in marine mammals

Many of the polyhalogenated aromatic hydrocarbons (PHAHs) readily bioaccumulate in wildlife species occupying high trophic levels as a consequence of their chemical characteristics and persistence. Classes of particular biological concern include the polychlorinated biphenyls (PCBs), the polychlorinated dibenzo-*p*-dioxins (PCDDs), and the polychlorinated dibenzofurans (PCDFs). The widespread contamination of the environment with these compounds, coupled with laboratory evidence implicating them in toxic effects at relatively low levels, highlight the risk that these contaminants may present to free-ranging animals occupying high trophic levels. Such chemicals have long been implicated in a number of population-level effects in raptors, piscivorous birds and seals, including hormonal alterations (Brouwer et al., 1989; Bosveld and van den Berg, 1994), reproductive and developmental toxicities (Helle et al., 1976; Reijnders, 1986; Addison, 1989; Tillitt et al., 1992), and skeletal malformations (Fox et al., 1991; Bergman et al., 1992; Mortensen et al., 1992).

While PHAHs are well established immunotoxicants in laboratory animals (Vos and Luster, 1989), little attention was paid to this aspect of their potential toxicity to wildlife species until recent virus-induced mass mortalities occurred among marine mammals. However, earlier studies did note higher levels of PCBs and *p,p'*-DDE in premature pups than full-term pups of a population of California sea lions (*Zalophus californianus*) in which San Miguel Sea Lion Virus and *Leptospira pomona* were isolated (Delong et al., 1973; Gilmartin et al., 1976). Recently, decreased lymphocyte proliferative responses to T-dependent mitogens were found to be correlated with serum PCB and dichlorodiphenyltrichloro-ethane (DDT) levels in bottlenose dolphins (*Tursiops truncatus*) sampled along the west coast of Florida, USA, although a sample size of five animals precludes definitive conclusions (Lahvis et al., 1995). Epidemiological studies established that striped dolphins (*Stenella coeruleoalba*) that died during the course of the dolphin morbillivirus epizootic in the Mediterranean Sea in 1990–91 had significantly higher concentrations of organochlorine concentrations than those that survived (Aguilar and Borrell, 1994). Similarly, harbour seals (*Phoca vitulina*) that died during the 1988 phocid distemper virus-1 (PDV-1) epizootic in Europe had higher levels of organochlorines than those that survived (Hall et al., 1992). Establishing a causal link between environmental contaminant levels and the outcome of virus infection in these two latter cases proved to be impossible, since decreased blubber stores may have had a concentrating effect on organochlorine levels in the blubber of dead animals. However, the high levels of contaminants found in these and other (Martineau et al., 1987; Subramanian et al., 1987; De Guise et al., 1995; Loganathan et al., 1990) studies provide grounds for speculation about their potential immunotoxicity in free-ranging populations of marine mammals.

In 1988, an epizootic of then unknown etiology led to the deaths of approximately 20 000 harbour seals and several hundred grey seals (*Halichoerus grypus*) in Europe (Dietz et al., 1989). The plethora of disease symptoms in affected animals, including fever, cutaneous lesions, gastrointestinal dysfunction, nervous disorders, and respiratory distress (Visser et al., 1991) cast doubt on the likelihood of a single disease entity. This prompted speculation about the involvement of an immunosuppressive agent or a role for pollution. A previously unidentified morbillivirus was eventually identified as the causal agent (Osterhaus and Vedder, 1988), being subsequently characterized and named phocid distemper virus-1, or PDV-1 (Osterhaus and Vedder, 1988, 1989; Mahy et al., 1988). While an identical, or very similar, virus was later shown to have been enzootic among North American seal populations (Henderson et al., 1992; Ross et al., 1992), serological analysis of archival samples established that European harbour and grey seal populations had been seronegative prior to 1988 (Osterhaus et al., 1989). Different hypotheses attempted to explain the catastrophic nature of the PDV-1 epizootic in Europe in 1988, and population density, migratory movements, algal blooms,

climatological factors and environmental pollution were hypothesized to have played a role (Dietz et al., 1989; Lavigne and Schmitz, 1990).

Susceptibility to virus infections involves multiple and often interacting factors, with numerous contributing elements. Such a relationship, for example, has been observed in measles epidemics in humans, in which nutritional, demographic and sociological factors influence the outcome of the event to differing degrees (Clements and Cutts, 1995). In the case of animals occupying high trophic levels, elevated concentrations of immunotoxic xenobiotics accumulated through the food chain represent a potentially important factor to be considered.

2. Immunotoxicological studies in the harbour seal

Subsequent to the PDV-1-induced epizootic in seals in 1988, we addressed the specific question of whether contaminants found at environmental levels are immunotoxic to harbour seals. A semi-field experiment was carried out, in which immune function was compared in two groups of harbour seals that were fed herring originating from either a relatively uncontaminated area or a contaminated coastal area. We present here an overview of the results obtained during the course of this study, compare these to results obtained in a follow-up study using laboratory rats, and, on the basis of evidence in the literature, extrapolate our observations to free-ranging populations of harbour seals.

While the immune systems of different mammalian species are structurally and functionally similar, fundamental differences do exist, precluding a detailed characterization of immune function in less studied species, such as the harbour seal. In initial studies, we demonstrated that immune function tests could be successfully adapted for application to studies in seals and yield biologically relevant information (Ross et al., 1993, 1994). These studies represented the first major investigation of the developing immune system of the harbour seal. The most striking finding was the demonstration that newborn harbour seals are relatively immunocompetent compared to their carnivorous terrestrial counterparts. It was hypothesized that this reflects an adaptation to its birth into a relatively hostile environment and a short period of maternal care. The importance of colostral intake was illustrated by showing the efficient transfer of PDV-1 neutralizing antibodies to pups, which may be expected to provide temporary protection against this seal pathogen. Subsequent studies provided additional information on the potential of immune function tests in seals (De Swart et al., 1993) which we applied in the feeding experiment.

The immunotoxic effects of contaminants in the captive feeding study was facilitated by limiting all additional variables which may have affected immune function. The functioning of the immune system relects, in part, the dynamic interaction between its components and external antigens. This is influenced by numerous external and intrinsic factors, including age, sex, season, stress and reproductive status (Boctor et al., 1989; Chandra and Kumari, 1994; Irwin, 1994). Seals in our study had been caught as recently-weaned pups in a relatively uncontaminated area, and were allowed an acclimation period of one year prior to the commencement of the feeding study. Herring destined for human consumption was obtained from either the relatively clean

Table 1
Analysis of chemical residues in herring (ng/g lipid) unless otherwise indicated[a]. Values represent mean of three batches used.

Compound	Atlantic	Baltic
ΣPCB	875	4398
Mono-*ortho* PCB	31	246
Di-*ortho* PCB	23	112
Non-*ortho* PCB	0.78	3.38
ΣPCDD (2,3,7,8-substituted)	0.031	0.148
ΣPCDF (2,3,7,8-substituted)	0.060	0.406
ΣDDT	152	2155
HCB	21	88
ΣHCH	10.6	228
Dieldrin	123	768
ΣTEQ (ng/kg lipid)	42	426

[a]Adapted from De Swart et al., 1995b; Ross et al., 1996c.

Atlantic Ocean or the contaminated Baltic Sea, and was fed to the two groups of seals (Table 1). The estimated daily intakes of 2,3,7,8-TCDD toxic equivalents (TEQs) by the Baltic group of seals were up to ten times higher (three batches of herring for each group were used during the course of the feeding experiment) than those of the Atlantic group of seals (De Swart et al., 1994), and led to a blubber concentration of 286 ± 17 ng TEQ/kg lipid in the Baltic seals compared to 90 ± 6 ng/kg lipid in the Atlantic seals (Ross et al., 1995). During the course of the study, seals of both groups remained healthy and exhibited normal growth patterns.

During the course of the feeding experiment, blood was sampled from both groups of seals every 6–9 weeks with minimal capture stress and different parameters of immune function were assessed. Samples were processed together in a double-blind manner. An early indication of a contaminant-related effect upon immune function was observed within 4 to 6 months of the start of the feeding experiment, when the natural cytotoxic activity of peripheral blood mononuclear cells against the YAC-1 tumour target cell proved to be reduced in the Baltic group (De Swart et al., 1994; Ross et al., 1996a). We subsequently showed that the functional characteristics of these cells in harbour seals were similar to those of natural killer (NK) cells described for other mammals, and concluded that the system detected NK cell activity (Ross et al., 1996a). This was based upon the demonstration of the tumour cell-directed cytotoxicity, the interleukin 2 (IL-2) responsiveness of effector cells, and the inhibiting effect exerted by NK cell-specific anti-asialo antibodies. All of these findings are consistent with the functional characteristics of NK cells described for other mammals. Mitogen-induced T-lymphocyte proliferative responses began to decline somewhat later (6–10 months following the start of the feeding experiment) (De Swart et al., 1994, 1995c). This was the first indication of impaired T-lymphocyte responses: only responses induced by the mitogens concanavalin A (Con A), phytohaemagglutinin (PHA) and pokeweed mitogen (PWM) proved to be reduced. Responses to the B-cell mitogen lipopolysaccharide (LPS) were unaffected.

While the results of these non-specific tests of immune function indicated that contaminants in the Baltic herring were immunotoxic, further evidence was provided when impaired mixed lymphocyte reactions (MLR) and antigen-specific lymphocyte proliferative responses were observed in the Baltic group (De Swart et al., 1995c). Since the MLR reflects the capacity of T-lymphocytes to respond to non-self lymphocytes, it may be considered a good parameter for T-cell function. The impairment of in vitro antigen-specific proliferative responses to rabies virus and tetanus toxoid antigens confirmed that specific immune responses were impaired in the Baltic group of seals. Finally, impaired delayed type hypersensitivity (DTH) and serum antibody responses to the protein ovalbumin in vivo provided evidence that the immune system as a whole was less capable of responding to a foreign substance in the Baltic group (Ross et al., 1995). The DTH swelling was characterized by the infiltration of mononuclear cells in the skin and a peak in skin thickness 24 h after intradermal injection, as in DTH reactions observed in other animals.

In order to assess any possible additional immunotoxic risk that free-ranging seals might be exposed to during a fast-induced mobilization of lipophilic compounds, we subjected the study animals of both groups to an experimental fasting period. Following more than 2 years on their respective diets, the seals of the two groups were fasted for a period of 15 days, and blood sampled for a series of immune function tests (De Swart et al., 1995b). Despite a significant mobilization of PCBs, DDT and other contaminants from blubber during this period, no contaminant-related impairment in immune function could be detected. Interestingly, levels of *Ah*-binding PHAHs remained largely unaffected in the serum of fasting animals, which may explain the lack of immunotoxic effects observed. Alternatively, the time course of a possible immunotoxic action at the level of leukocyte precursors could have led to immune function effects at a time point subsequent to our schedule of samplings. Our results

suggest that while chronic exposure to environmental concentrations of dietary contaminants pose an immunotoxic risk to harbour seals, a relatively short fast does not appear to exacerbate this phenomenon.

The impaired immune responses observed in seals fed the Baltic Sea herring are consistent with the effects observed in studies of laboratory animals exposed to TCDD-like compounds (Vos and Luster, 1989). An additional indication that PCB-like compounds led to a toxic effect in the seals of the Baltic group was the observed decline in vitamin A levels (De Swart et al., 1994). The negative effects of PCBs and their metabolites on serum vitamin A levels has been demonstrated in studies of laboratory rats (Brouwer et al., 1986). Recent studies have found one-time doses of TCDD to impair NK cell responsiveness during virus infection in SPF rats, but not basal or spontaneous NK cell activity (Selgrade et al., 1992; Yang et al., 1994). Differences in species used, contaminant profiles to which study animals were exposed, or the non-SPF status of the seals in our study, may explain why the spontaneous NK cell activity of the Baltic group of seals was reduced. The impaired T-lymphocyte function in seals of the Baltic group may reflect an immunotoxic effect in the thymus, since this lymphoid organ has been demonstrated to be highly sensitive to the actions of TCDD and related compounds in laboratory animals (De Heer et al., 1994).

Impaired DTH responses have also been observed in other immunotoxicological studies (Vos et al., 1973; Lundberg et al., 1992). While this in vivo test of immune function provides an indication of the memory-based ability of the immune system to mount an overall response to a foreign antigen, results of this test are considered to largely reflect T-helper cell function. Positive correlations between in vivo DTH reactions and the in vitro T-cell dependent mitogen-induced lymphocyte proliferative responses support the concept of a mediating role for T-lymphocytes in the DTH responses of seals in our study (Ross et al., 1995), and provide additional evidence of an immunotoxic effect which targeted T-lymphocytes or their precursors.

Our studies provided evidence that contaminants accumulated in the Baltic Sea food chain were immunotoxic to captive harbour seals. However, it remains difficult to translate the observed immunosuppression in the Baltic group of seals to an increase in susceptibility to infectious disease. Challenging seals in our study with a live virus would clearly not be ethically or legally acceptable. In addition, recreating a situation which would encompass the multiple variables involved in the PDV-1 epizootic in a controlled situation is virtually impossible.

3. Chronic feeding studies in the rat: mimicking the seal study

In an attempt to expand on our observations in seals, we conducted two feeding studies in laboratory rats. The wider availability of specific reagents for the determination of lymphocyte subpopulations and immunoglobulins represent a major advantage of studies using rats. In addition, the rat has been widely used in toxicological studies and extra information can be obtained by an evaluation of lymphoid tissue characteristics and by applying host resistance tests (Van Loveren and Vos, 1989; Van Loveren, 1995).

In the first of these studies, similar daily doses of the Baltic herring contaminants used in the seal study did not appreciably alter immune function in adult PVG rats fed freeze-dried herring from the Baltic Sea for 4 months (Ross et al., 1996c). However, rat cytomegalovirus (RCMV) titers were higher in the salivary glands of rats in the Baltic group, suggesting that contaminants may have affected the outcome of this virus infection without our being able to identify an immunological basis for this observation. Since immune function parameters were clearly impaired in the seals, we concluded that the harbour seal may be more sensitive than the rat to the immunotoxic effects of the contaminants in the Baltic Sea herring. While similar diets were used in both the seal and rat studies, differences

in lifespan, the half-life of TCDD-like PHAHs in the two species, and nutritional requirements may also have contributed to the observed differences in species sensitivity to the immunotoxic contaminants in the Baltic Sea herring. The relative insensitivity of the adult rat to the effects of TCDD-like compounds has been shown in other studies (Kerkvliet et al., 1990; Smialowicz et al., 1994), and perinatal exposure has been suggested to be a prerequisite to low level TCDD-induced immunotoxicity in rats (Vos and Moore, 1974).

For these reasons, we carried out a second experiment in which pregnant female PVG rats were dosed with the same Atlantic and Baltic Sea herring contaminant mixtures, and immune function was assessed in their pups (Ross et al., 1996b). In order to eliminate any possible dietary influence on immune function other than lipophilic contaminant levels, oil was extracted from the two herring batches and administered orally to pregnant females on a daily basis. A positive control group was exposed to Atlantic herring oil spiked with TCDD. Exposure began on day 6 of gestation and continued through birth until the pups were weaned. Rat pups exposed perinatally to the Baltic herring contaminant mixture had impaired cellular immune responses, with this being most pronounced at an early age. Effects on non-specific immune function parameters were characterized by impaired mitogen-induced T-lymphocyte proliferative responses and thymus-related effects, suggesting that developing thymocytes or their precursors were targeted. RCMV-directed immune responses including virus-associated NK responsiveness and specific antibody responses were impaired in both the Baltic and TCDD groups, while RCMV-specific T-lymphocyte proliferative responses were affected in the TCDD group. Functional immune responses in the youngest rat pups of the Baltic group fell consistently between the negative control group (Atlantic) and the positive control group (TCDD), but these differences became less apparent with time. Following infection, RCMV titers were similar in the salivary glands of all groups of experimentally-infected rats at the time of necropsy, likely reflecting the observed recovery of immune function with time. The 24-day half-life of TCDD in rats (Rose et al., 1976) would lead to rapidly diminishing contaminant burdens in the growing pups, essentially resulting in a removal of the source of immunotoxicity in the study animals. The reversibility of TCDD-induced thymus atrophy has been observed previously (Van Loveren et al., 1991).

Our observations of elevated RCMV titers in the salivary glands of adult rats but not rat pups suggest that the outcome of this virus infection may be affected by the contaminants in the Baltic herring, but also underline the complexity of the immune response. However, predictive relationships between immunosuppression and susceptibility to infection are difficult to establish (Luster et al., 1992). A selective impairment of immune function parameters as a result of genetic deficiencies (Shellam et al., 1981; Biron et al., 1989), medication use (Descotes, 1986) or exposure to PHAHs (Vos et al., 1991) can affect the outcome of virus infections. Immune alterations or host resistance effects were detected in rats exposed either in their diet as adults or as pups exposed perinatally, suggesting that the contaminants in the Baltic Sea herring were immunotoxic in these laboratory animals.

The more pronounced immunosuppression observed in the harbour seal study as compared to the rat studies may be related to differences in the experimental design, although it would appear that seals are more sensitive than rats to the immunotoxic action of contaminants in Baltic Sea herring. These observations are consistent with the demonstrated relative insensitivity of rats to the immunotoxic effects of TCDD (Vos et al., 1973; Smialowicz et al., 1994). The rat pups exposed perinatally exhibited immune alterations which were similar to those in the Baltic seals, providing a basis for comparison of these two species. The reductions in rat thymus weights, thymus and spleen T-cell numbers and T-cell function observed in perinatally-exposed rats, suggest that the impaired T-lymphocyte responses in the harbour seals of the Baltic group is related to an effect on thymus function or its thymocyte precursor cells. The observation that rat pups exposed perinatally are more sensitive to

the immunotoxic actions of the Baltic Sea herring contaminants than adult rats, provides a basis to speculate that free-ranging harbour seals exposed perinatally are at more risk than our studies of captive juveniles may suggest.

4. Free-ranging seal populations at risk?

PHAH-induced immunotoxicity has been shown to be largely mediated by the aryl hydrocarbon (*Ah*) receptor (Silkworth and Antrim, 1985; Kerkvliet et al., 1990). While it is not possible to rule out a contribution of non-*Ah* mediated compounds to the observed immunotoxicity in the Baltic group of seals, the TCDD-like PHAHs represent highly immunotoxic contaminants found at high concentrations in the Baltic Sea herring used in this study. The dose-related pattern of impaired immune functions observed among groups of perinatally-exposed rat pups (Atlantic > Baltic > TCDD) lends support to the concept of an *Ah*-receptor-mediated basis for Baltic herring-induced immunotoxicity. Toxic Equivalent Factors (TEFs) used in the estimation of the relative toxicity of the PHAH mixtures to harbour seals are largely based on studies carried out in laboratory rats, and as such, provide a general indication of toxicity.

While 2,3,7,8-TCDD remains the most potent *Ah*-related immunotoxicant among the 419 possible PCB, PCDD and PCDF congeners, chemical residue analysis of Baltic Sea herring suggested that the dioxins presented a limited immunotoxic risk in the diet of the harbour seals (Fig. 1). In fact, three PCB congeners (IUPAC numbers 126, 118 and 156), together with one PCDF congener (2,3,4,7,8-PeCDF), accounted for 80% of the total TEQ burden in the Baltic Sea herring, while the contribution of all PCDDs was less than 10% (results not shown). The PHAH profile in the blubber of harbour seals fed this fish for 2 years suggested that PCBs accounted for the great majority of the toxicity, with PCDDs and PCDFs contributing only slightly to the total TEQ. Such data confirm the results of others, who have found the TCDD-like PCBs (Tanabe et al., 1987; Kannan et al., 1989) and PCDFs (Ono et al., 1987) to represent a greater toxic potential than PCDDs in marine mammals. The diminishing contribution of PCDDs to the total TEQ profile from the herring to the seals in our studies suggests that harbour seals may be able to preferentially metabolize the planar PCDDs. Similar observations of the limited potential for bioaccumulation of toxic PCDDs in seals (Tanabe et al., 1989; Bergek et al., 1992) further illustrate the threat that PCBs pose to seals in the marine environment.

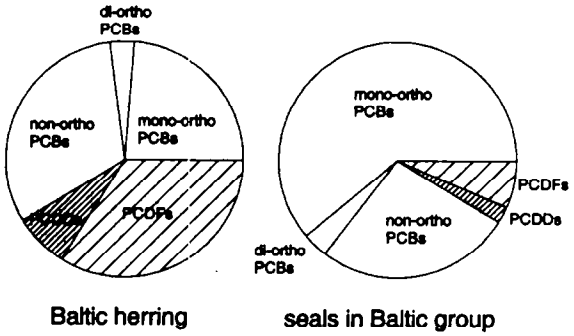

Fig. 1. Relative contributions of PCBs, PCDDs and PCDFs to the total TCDD toxic equivalents (TEQs) in Baltic Sea herring lipid and blubber of harbour seals fed this herring for 2 years.

In an attempt to extrapolate the results obtained in our studies to the environment, we have reviewed the literature on PCB burdens in free-ranging harbour seals. Differences in techniques to measure PHAH concentrations among laboratories, and during the course of time, render such an exercise difficult to carry out with a high degree of certainty. Contaminant concentrations are also affected by age, sex and condition (Hutchinson and Simmonds, 1994; for review see Addison, 1989), biasing reported results as a consequence of sampling strategy. Geographical ratios of specific congeners to ΣPCB will also vary, the consequence being that even where the ΣPCB concentrations may be similar between two areas, the profile may be more toxic in one than the other. However, the limited congener-specific data and the lack of information on species-specific toxicity in the harbour seal preclude an extensive extrapolation that would be based on a toxic equivalent approach. Since ΣPCB has been routinely published for harbour

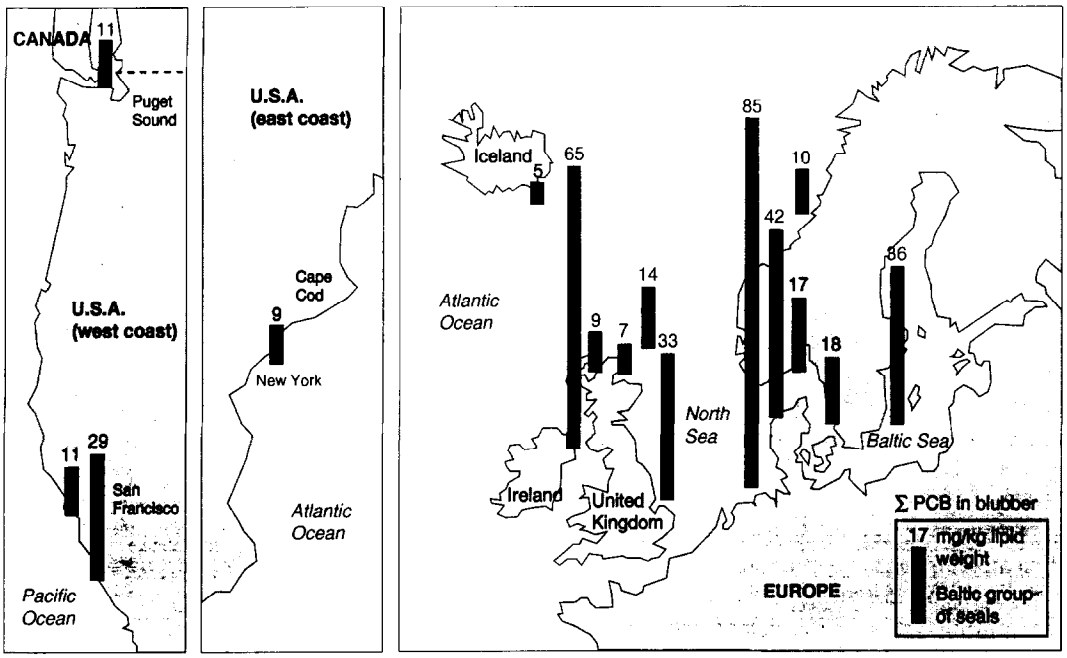

Fig. 2. Recent mean blubber levels of PCBs in harbour seals (*Phoca vitulina*) are higher in many areas of northern Europe and North America than in the immunosuppressed harbour seals of our captive feeding study (box insert at lower right). Data were compiled on the basis of ΣPCB in blubber expressed on a lipid weight basis, from young, live sampled seals where possible. Key to these data (as follows): location, full range PCB concentration mg/kg lipid weight in blubber, sample size, age class of animals, condition of animals, ΣPCB method. **Our study**: Atlantic group, 4–11, $n = 11$; Baltic group, 11–22, $n = 11$; juveniles, live, sum of 42 peaks (De Swart et al., 1995b). **USA**: San Francisco Bay, 2–29, $n = 14$; Monterey coast, 3–56, $n = 2$; unspecified age, live, Aroclor 1260 (Kopec and Harvey, 1995); Puget Sound, 2–23, $n = 8$, pups, dead, equivalent mixture of Aroclors 1016, 1221, 1254 and 1260 (S. Shaw, personal communication); Long Island, 3–15, $n = 9$, unspecified age, dead, sum of 21 peaks, wet weight data adapted using an estimated lipid content of 78% (Lake et al., 1995). **Iceland**: 1–13, $n = 7$, unspecified age and condition, Clophen A60 (Luckas et al., 1990). **United Kingdom**: N. Ireland, 1–169, $n = 11$; W. Scotland, 6–12, $n = 6$; Moray Firth, 1–13, $n = 16$; Orkney, 3–28, $n = 14$; The Wash, 13–83, $n = 6$; juveniles, live, Aroclor 1254 (Hall et al., 1992). **Germany** (Wadden Sea): 28–250, $n = 32$, unspecified age and condition, Clophen A60 (Luckas et al., 1990). **Norway**: west, 1–29, $n = 18$; south coast, 5–49, $n = 26$; unspecified age, dead or dying, Aroclor 1260 (Skaare et al., 1990). **Denmark** (Wadden Sea): 33–53, $n = 3$, adult, dead, sum of 31 peaks (Storr-Hansen and Spliid, 1993b). **Sweden**: Kattegat, 6–29, $n = 10$; Baltic Sea, 16–98, $n = 10$; juveniles, unspecified condition, Aroclor 1254 (Blomkvist et al., 1992).

seals, this value provides a relatively convenient indication of contaminant levels, and can help pinpoint marine mammal populations which may be most at risk to the effects of PHAH-related toxicity.

On the basis of recently published data on ΣPCBs for harbour seals, we have identified harbour seal populations in Europe and North America that have blubber concentrations of PCBs at or exceeding those in the captive harbour seals which had a contaminant-related impairment of immune function in our recent studies (Fig. 2). The available congener-specific data in which TEQ burdens are estimated confirm that the northwestern United Kingdom (Wells and Echarri, 1992), the Wadden Sea and the Kattegat areas (Storr-Hansen and Spliid, 1993a), and the Strait of Georgia in southwestern Canada (Addison et al., 1996) have harbour seal populations at risk for immunotoxicity, with blubber TEQ concentrations being at or above those observed in our Baltic group of seals (Ross et al., 1995). Since it is impossible to predict what effect such a degree of immunosuppression may

have on the susceptibility to infection, this analysis can only serve as a general predictive measure of environmental immunotoxicity. This, combined with the multitude of factors affecting the epidemiology of infectious disease outbreaks (e.g. population density, social behaviour, nutritional status) preclude any firm predictions regarding virus epizootics in these areas.

Taken together, our results demonstrate that current levels of persistent lipophilic contaminants present an immunotoxic risk to harbour seals inhabiting many coastal areas of Europe, including the Baltic Sea, the Wadden Sea, and the southeastern and western coasts of Great Britain. While recent data from North American harbour seals are limited, harbour seals inhabiting the New England coast and points along the west coast of the United States and Canada may also at risk to the immunotoxic actions of PHAHs. In retrospect, contaminants may well have affected the severity and extent of the 1988 PDV-1 epizootic in Europe, as well as recent virus epizootics in other marine mammal species. Interestingly, morbillivirus epizootics among different marine mammal species (for reviews see De Swart et al., 1995a; Osterhaus et al., 1995) have resulted in mass mortalities in populations shown to have very high body burdens of PHAHs, including bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico in 1987–88 (Lipscomb et al., 1994); striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea in 1990–91 (Aguilar and Borrell, 1994); and even in Baikal seals (*Phoca sibirica*) in Russia in 1987 (Nakata et al., 1995). While it is impossible to substantiate a causal link, such information, in conjunction with our results in captive harbour seals, does provide a basis for speculation and future research as to the link between contaminants, immunotoxicity and virus outbreaks.

The results obtained in these studies may also be of relevance for human health, since the Baltic Sea herring used was destined for human consumption. While direct interspecies comparisons are complicated by differences in, among others, the capacity to metabolize or accumulate immunotoxic PHAHs, length of nursing periods, feeding habits, and general health factors, they can serve to identify potential risks associated with the intake of dietary contaminants. Although some information is available on the intake and accumulation of PHAHs for humans, little is known about the effects of these. Certain human consumer groups, including fishermen and other fish consumers in industrialized nations (Svensson et al., 1991; Hovinga et al., 1992; Dewailly et al., 1994) and native Inuit in northern Canada (Dewailly et al., 1989, 1993a), have been found to have high daily intakes of TCDD-like PHAHs. In the latter case, an association was observed, and a causative link postulated, between PCB intake and an increased frequency of infectious disease in breast-fed babies (Dewailly et al., 1993c). This suggests that food chain-accumulated PHAHs may be immunotoxic to certain human consumer groups even in remote areas previously considered to be pristine. The observed immunotoxicity in laboratory rats and seals in our study occurred at daily intake levels that are in the same order of magnitude as those estimated for breast-fed Inuit infants (Dewailly et al., 1992, 1993c), whose mothers consume large

Table 2
Estimated daily intakes of dietary TCDD toxic equivalents accumulated in the aquatic food chain by animals in our studies and in breast-fed Canadian Inuit infants (ng/kg body weight)

Study	Exposure route	Daily intake	Ref.
Harbour seals	Atlantic herring	0.3–0.6	De Swart et al., 1994
Harbour seals	Baltic herring	1.2–5.6	De Swart et al., 1994
Adult PVG rats	Baltic herring	1.6	Ross et al., 1996c
Young PVG rats	Baltic herring oil	0.9	Ross et al., 1996b
Inuit infants	Breast milk	0.7	Dewailly et al., 1991, 1993b

quantities of fish and marine mammal products (Table 2).

Although PCB levels in the aquatic food chain declined following regulatory controls, they have largely stabilized since the 1980s (Olsson and Reutergardh, 1986; Loganathan et al., 1990; Hovinga et al., 1992). The continued cycling of persistent PHAH compounds suggests that the contamination of the aquatic food chain will remain of ecological concern well into the 21st century. Since perinatally-exposed animals exhibit more pronounced PHAH-induced immunosuppression and the lifespan of seals allows for long-lasting accumulation, free-ranging seals are likely to be more vulnerable to the immunotoxic effects of persistent PHAHs than our study seals. Such immunotoxicity may therefore predispose free-ranging seals in certain areas to an increased severity of infectious disease outbreaks in the decades to come.

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References

Addison, R.F. (1989) Organochlorines and marine mammal reproduction. Can. J. Fish. Aquat. Sci. 46, 360.

Addison, R.F., Ikonomou, M.G. and Smith, T.G. (1996) PCDD, PCDF and PCB in harbour seals (*Phoca vitulina*) from contaminated and "reference" sites in British Columbia. Environ. Sci. Technol. (in press).

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

Bergek, S., Bergqvist, P.-A., Hjelt, M., Olsson, M., Rappe, C., Roos, A. and Zook, D. (1992) Concentrations of PCDDs and PCDFs in seals from Swedish waters. Ambio 21, 553.

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

Biron, C.A., Byron, K.S. and Sullivan, J.L. (1989) Severe herpesvirus infections in an adult without natural killer cells. N. Engl. J. Med. 320, 1731.

Blomkvist, G., Roos, A., Jensen, S., Bignert, A. and Olsson, M. (1992) Concentrations of sDDT and PCB in seals from Swedish and Scottish waters. Ambio 21, 539.

Boctor, F.N., Charmy, R.A. and Cooper, E.L. (1989) Seasonal differences in the rhythmicity of human male and female lymphocyte blastogenic responses. Immunol. Invest. 18, 775.

Bosveld, A.T.C. and van den Berg, M. (1994) Effects of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) on fish-eating birds. Environ. Rev. 2, 147.

Brouwer, A., Van den Berg, K.J., Blaner, W.S. and Goodman, D.S. (1986) Transthyretin (prealbumin) binding of PCBs, a model for the mechanism of interference with vitamin A and thyroid hormone metabolism. Chlorinated Dioxins And Related Compounds 1985. 15, 1699.

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

Chandra, R.K. and Kumari, S. (1994) Nutrition and immunity: an overview. J. Nutr. 124, 1433S.

Clements, C.J. and Cutts, F.T. (1995) The epidemiology of measles: thirty years of vaccination. In: V. Ter Meulen and M.A. Billeter (Eds.), Measles Virus, Springer-Verlag, Berlin, pp. 13.

De Guise, S., Martineau, D., Béland, P. and Fournier, M. (1995) Possible mechanisms of action of environmental contaminants on St. Lawrence beluga whales (*Delphinapterus leucas*). Environ. Health Perspect. 103, 73.

De Heer, C., Verlaan, A.P.J., Penninks, A.H., Vos, J.G., Schuurman, H.-J. and Van Loveren, H. (1994) Time course of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced thymic atrophy in the Wistar rat. Toxicol. Appl. Pharmacol. 128, 97.

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. and Osterhaus, A.D.M.E. (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.

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. and Osterhaus, A.D.M.E. (1994) Impairment of immune function in harbor seals (*Phoca vitulina*) feeding on fish from polluted waters. Ambio 23, 155.

De Swart, R.L., Harder, T.C., Ross, P.S., Vos, H.W. and Osterhaus, A.D.M.E. (1995a) Morbilliviruses and morbillivirus diseases of marine mammals. Infect. Agent. Dis. 4, 125.

De Swart, R.L., Ross, P.S., Timmerman, H.H., Hijman, W.C., De Ruiter, E., Liem, A.K.D., Brouwer, A., Van Loveren, H., Reijnders, P.J.H., Vos, J.G. and Osterhaus, A.D.M.E. (1995b) Short-term fasting does not aggravate immunosuppression in harbour seals (*Phoca vitulina*) with high body burdens of organochlorines. Chemosphere 31, 4289.

De Swart, R.L., Ross, P.S., Timmerman, H.H., Vos, H.W., Reijnders, P.J.H., Vos, J.G. and Osterhaus, A.D.M.E. (1995c) Impaired cellular immune response in harbour seals (*Phoca vitulina*) feeding on environmentally contaminated herring. Clin. Exp. Immunol. 101, 480.

Delong, R.L., Gilmartin, W.G. and Simpson, J.G. (1973) Premature births in California sea lions: association with high organochlorine pollutant residue levels. Science 181, 1168.

Descotes, J. (1986) Immunotoxicology of Drugs and Chemicals, Elsevier, Amsterdam, pp. 1.

Dewailly, E., Nantel, A., Weber, J.-P. and Meyer, F. (1989) High levels of PCBs in breast milk of Inuit women from Arctic Québec. Bull. Environ. Contam. Toxicol. 43, 641.

Dewailly, E., Weber, J.-P., Gingras, S. and Laliberté, C. (1991) Co-planar PCBs in human milk in the province of Québec, Canada: are they more toxic than dioxin for breast-fed infants? Bull. Environ. Contam. Toxicol. 47, 491.

Dewailly, E., Nantel, A.J., Weber, J.P. and Meyer, F. (1992) Breast milk contamination by PCDDs, PCDFs, and PCBs in Arctic Québec: a preliminary assessment. Chemosphere 25, 1245.

Dewailly, E., Ayotte, P., Bruneau, S., Laliberté, C., Muir, D.C.G. and Norstrom, R.J. (1993a) Inuit exposure to organochlorines through the aquatic food chain in Arctic Québec. Environ. Health Perspect. 101, 618.

Dewailly, E., Bruneau, S., Ayotte, P., Laliberté, C., Gingras, S., Belanger, D. and Ferron, L. (1993b) Health status at birth of Inuit newborn prenatally exposed to organochlorines. Chemosphere 27, 359.

Dewailly, E., Bruneau, S., Laliberté, C., Belles-Iles, M., Weber, J.P., Ayotte, P. and Roy, R. (1993c) Breast milk contamination by PCBs and PCDDs/PCDFs in Arctic Québec: preliminary results on the immune status of Inuit infants. Dioxin 93, 403.

Dewailly, E., Ryan, J.J., Laliberte, C., Bruneau, S., Weber, J.-P., Gingras, S. and Carrier, G. (1994) Exposure of remote maritime populations to coplanar PCBs. Environ. Health Perspect. 102, 205.

Dietz, R., Heide-Jrgensen, M.-P. and Härkönen, T. (1989) Mass deaths of harbor seals (*Phoca vitulina*) in Europe. Ambio 18, 258.

Fox, G.A., Collins, B., Hayakawa, E., Weseloh, D.V., Ludwig, J.P., Kubiak, T.J. and Erdman, T.C. (1991) Reproductive outcomes in colonial fish-eating birds: a biomarker for developmental toxicants in Great Lakes food chains: spatial variation in the occurrence and prevalence of bill defects in young double-crested cormorants in the Great Lakes, 1979–1987. J. Great Lakes Res. 17, 158.

Gilmartin, W.G., Delong, R.L., Smith, A.W., Sweeney, J.C., De Lappe, B.W., Risebrough, R.W., Griner, L.A., Dailey, M.D. and Peakall, D.B. (1976) Premature parturition in the California sea lion. J. Wildl. Dis. 12, 104.

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

Helle, E., Olsson, M. and Jensen, S. (1976) DDT and PCB levels and reproduction in ringed seal from the Bothnian Bay. Ambio 5, 188.

Henderson, G., Trudgett, A., Lyons, C. and Ronald, K. (1992) Demonstration of antibodies in archival sera from Canadian seals reactive with a European isolate of phocine distemper virus. Sci. Total. Envir. 115, 93.

Hovinga, M.E., Sowers, M.F. and Humphrey, H.E.B. (1992) Historical changes in serum PCB and DDT levels in an environmentally-exposed cohort. Arch. Environ. Contam. Toxicol. 22, 362.

Hutchinson, J.D. and Simmonds, M.P. (1994) Organochlorine contamination in pinnipeds. Rev. Environ. Contam. Toxicol. 136, 123.

Irwin, M. (1994) Stress-induced immune suppression—role of brain corticotropin releasing hormone and autonomic nervous system mechanisms. Adv. Neuroimmunol. 4, 29.

Kannan, N., Tanabe, S., Ono, M. and Tatsukawa, R. (1989) Critical evaluation of polychlorinated biphenyl toxicity in terrestrial and marine mammals: increasing impact of non-ortho and mono-ortho coplanar polychlorinated biphenyls from land to ocean. Arch. Environ. Contam. Toxicol. 18, 850.

Kerkvliet, N.I., Baecher Steppan, L., Smith, B.B., Youngberg, J.A., Henderson, M.C. and Buhler, D.R. (1990) Role of the Ah locus in suppression of cytotoxic T lymphocyte activity by halogenated aromatic hydrocarbons (PCBs and TCDD): structure-activity relationships and effects in C57Bl/6 mice congenic at the Ah locus. Fundam. Appl. Toxicol. 14, 532.

Kopec, A.D. and Harvey, J.T. (1995) Toxic pollutants, health indices, and population dynamics of harbor seals in San Francisco Bay, 1989–1992, Moss Landing Marine Laboratories, Moss Landing, USA, pp. 138.

Lahvis, G.P., Wells, R.S., Kuehl, D.W., Stewart, J.L., Rhinehart, H.L. and 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.

Lake, C.A., Lake, J.L., Haebler, R., McKinney, R., Boothman, W.S. and Sadove, S.S. (1995) Contaminant levels in harbor seals from the northeastern United States. Arch. Environ. Contam. Toxicol. 29, 128.

Lavigne, D.M. and Schmitz, O.J. (1990) Global warming and increasing population densities: a prescription for seal plagues. Mar. Pollut. Bull. 21, 280.

Lipscomb, T.P., Schulman, F.Y., Moffett, D. and Kennedy, S. (1994) Morbilliviral disease in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the 1987–1988 epizootic. J. Wildl. Dis. 30, 567.

Loganathan, B.G., Tanabe, S., Tanaka, H., Watanabe, S., Miyazaki, N., Amano, M. and Tatsukawa, R. (1990) Comparison of organochlorine residue levels in the striped dolphin from western north Pacific, 1978–79 and 1986. Mar. Pollut. Bull. 21, 435.

Luckas, B., Vetter, W., Fischer, P., Heidemann, G. and Plötz, J. (1990) Characteristic chlorinated hydrocarbon patterns in the blubber of seals from different marine regions. Chemosphere 21, 13.

Lundberg, K., Dencker, L. and Gronvik, K.O. (1992) Effects of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) on the specific immune response to ovalbumin in the mouse. Chemosphere 25, 111.

Luster, M.I., Portier, C., Pait, D.G., White, K.L. Jr., Gennings, C., Munson, A.E. and Rosenthal, G.J. (1992) Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. Fundam. Appl. Toxicol. 18, 200.

Mahy, B.W.J., Barrett, T., Evans, S., Anderson, E.C. and Bostock, C.J. (1988) Characterization of a seal morbillivirus. Nature 336, 115.

Martineau, D., Béland, P., Desjardins, C. and Lagacé, A. (1987) Levels of organochlorine chemicals in tissues of beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Québec, Canada. Arch. Environ. Contam. Toxicol. 16, 137.

Mortensen, P., Bergman, A., Bignert, A., Hansen, H-J., Härknen, T. and Olsson, M. (1992) Prevalence of skull lesions in harbor seals (*Phoca vitulina*) in Swedish and Danish museum collections: 1835–1988. Ambio 21, 520.

Nakata, H., Tanabe, S., Tatsukawa, R., Amano, M., Miyazaki, N. and Petrov, E. (1995) Persistent organochlorine residues and their accumulation kinetics in Baikal seal (*Phoca sibirica*) from Lake Baikal, Russia. Environ. Sci. Technol. 29, 2877.

Olsson, M. and Reutergardh, L. (1986) DDT and PCB pollution trends in the Swedish aquatic environment. Ambio 15, 103.

Ono, M., Kannan, N., Wakimoto, T. and Tatsukawa, R. (1987) Dibenzofurans a greater global pollutant than dioxins? Mar. Pollut. Bull. 18, 640.

Osterhaus, A.D.M.E. and Vedder, E.J. (1988) Identification of virus causing recent seal deaths. Nature 335, 20.

Osterhaus, A.D.M.E. and Vedder, E.J. (1989) No simplification in the etiology of recent seal deaths. Ambio 18, 297.

Osterhaus, A.D.M.E., Groen, J., UytdeHaag, F.G.C.M., Visser, I.K.G., Vedder, E.J., Crowther, J. and Bostock, C.J. (1989) Morbillivirus infection in European seals before 1988. Vet. Rec. 125, 326.

Osterhaus, A.D.M.E., De Swart, R.L., Vos, H.W., Ross, P.S., Kenter, M.J.H. and Barrett, T. (1995) Morbillivirus infections of aquatic mammals: newly identified members of the genus. Vet. Microbiol. 44, 219.

Reijnders, P.J.H. (1986) Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 324, 456.

Rose, J.Q., Ramsey, J.C., Wentzler, T.H., Hummel, R.A. and Gehring, P.J. (1976) The fate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin following single and repeated oral doses to the rat. Toxicol. Appl. Pharmacol. 36, 209.

Ross, P.S., Visser, I.K.G., Broeders, H.W.J., Van de Bildt, M.W.G., Bowen, W.D. and Osterhaus, A.D.M.E. (1992) Antibodies to phocine distemper virus in Canadian seals. Vet. Rec. 130, 514.

Ross, P.S., Pohajdak, B., Bowen, W.D. and Addison, R.F. (1993) Immune function in free-ranging harbor seal (*Phoca vitulina*) mothers and their pups during lactation. J. Wildl. Dis. 29, 21.

Ross, P.S., De Swart, R.L., Visser, I.K.G., Murk, W., Bowen, W.D. and Osterhaus, A.D.M.E. (1994) Relative immunocompetence of the newborn harbour seal, *Phoca vitulina*. Vet. Immunol. Immunopathol. 42, 331.

Ross, P.S., De Swart, R.L., Reijnders, P.J.H., Van Loveren, H., Vos, J.G. and 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.

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

Ross, P.S., De Swart, R.L., Van der Vliet, H., Willemsen, L., De Klerk, A., Van Amerongen, G., Groen, J., Brouwer, A., Morse, D.C., Van Loveren, H., Osterhaus, A.D.M.E. and Vos, J.G. (1996b) Impaired cellular immune response in offspring of rats exposed during pregnancy and nursing to Baltic Sea herring oil or 2,3,7,8-TCDD. (Submitted for publication).

Ross, P.S., Van Loveren, H., De Swart, R.L., Van der Vliet, H., De Klerk, A., Timmerman, H.H., Van Binnendijk, R.S., Brouwer, A., Vos, J.G. and Osterhaus, A.D.M.E. (1996c) Host resistance to rat cytomegalovirus (RCMV) in adult PVG rats fed herring from the contaminated Baltic Sea. Arch. Toxicol. (in press).

Selgrade, M.J.K., Daniels, M.J. and Dean, J.H. (1992) Correlation between chemical suppression of natural killer cell activity in mice and susceptibility to cytomegalovirus: rationale for applying murine cytomegalovirus as a host resistance model and for interpreting immunotoxicity testing in terms of disease. J. Toxicol. Environ. Health 37, 123.

Shaw, S. Personal communication, Marine Environmental Research Institute, P.O. Box 179, Brooklin, ME, USA.

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

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

Skaare, J.U., Markussen, N.H., Norheim, G., Haugen, S. and Holt, G. (1990) Levels of polychlorinated biphenyls, organochlorine pesticides, mercury, cadmium, copper, selenium, arsenic, and zinc in the harbour seal, *Phoca vitulina*, in Norwegian waters. Environ. Pollut. 66, 309.

Smialowicz, R.J., Riddle, M.M., Williams, W.C. and Diliberto, J.J. (1994) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on humoral immunity and lymphocyte subpopulations — differences between mice and rats. Toxicol. Appl. Pharmacol. 124, 248.

Storr-Hansen, E. and Spliid, H. (1993a) Coplanar polychlorinated biphenyl congener levels and patterns and the identification of separate populations of harbor seals (*Phoca vitulina*) in Denmark. Arch. Environ. Contam. Toxicol. 24, 44.

Storr-Hansen, E. and Spliid, H. (1993b) Distribution patterns of polychlorinated biphenyl congeners in harbor seal (*Phoca vitulina*) tissues—statistical analysis. Arch. Environ. Contam. Toxicol. 25, 328.

Subramanian, A., Tanabe, S., Tatsukawa, R., Saito, S. and Miyazaki, N. (1987) Reduction in the testosterone levels by PCBs and DDE in Dall's porpoises of northwestern North Pacific. Mar. Pollut. Bull. 18, 643.

Svensson, B.G., Nilsson, A., Hansson, M., Rappe, C., Akesson, B. and Skerfving, S. (1991) Exposure to dioxins and dibenzofurans through the consumption of fish. N. Engl. J. Med. 324, 8.

Tanabe, S., Kannan, N., Subramanian, A., Watanabe, S. and Tatsukawa, R. (1987) Highly toxic coplanar PCBs: occurrence, source, persistency and toxic implications to wildlife and humans. Environ. Pollut. 47, 147.

Tanabe, S., Kannan, N., Ono, M. and Tatsukawa, R. (1989) Toxic threat to marine mammals: increasing toxic potential of non-ortho and mono-ortho coplanar PCBs from land to ocean. Chemosphere 18, 485.

Tillitt, D.E., Ankley, G.T., Giesy, J.P., Ludwig, J.P., Kuritamatsuba, H., Weseloh, D.V., Ross, P.S., Bishop, C.A., Sileo, L., Stromborg, K.L., Larson, J. and Kubiak, T.J. (1992) Polychlorinated biphenyl residues and egg mortality in double-crested cormorants from the Great Lakes. Environ. Toxicol. Chem. 11, 1281.

Van Loveren, H. (1995) Host resistance models. Hum. Exp. Toxicol. 14, 137.

Van Loveren, H. and Vos, J. G. (1989) Immunotoxicological considerations: a practical approach to immunotoxicity testing in the rat. In: A.D. Dayan and A.J. Paine (Eds.), Advances in Applied Toxicology, Taylor & Francis, London, pp. 143.

Van Loveren, H., Schuurman, H.J., Kampinga, J. and Vos, J.G. (1991) Reversibility of thymic atrophy induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and bis(tri-*n*-butyltin) oxide (TBTO). Int. J. Immunopharmacol. 13, 369.

Visser, I.K.G., Teppema, J.S. and Osterhaus, A.D.M.E. (1991) Virus infections of seals and other pinnipeds. Rev. Med. Microbiol. 2, 105.

Vos, J. G. and Luster, M. I. (1989) Immune alterations. In: R.D. Kimbrough and S. Jensen (Eds.), Halogenated Biphenyls, Terphenyls, Naphtalenes, Dibenzodioxins and Related Products, Elsevier, Amsterdam, pp. 295.

Vos, J.G. and Moore, J.A. (1974) Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Int. Arch. Allergy 47, 777.

Vos, J.G., Moore, J.A. and Zinkl, J.G. (1973) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the immune system of laboratory animals. Environ. Health Perspect. 5, 149.

Vos, J.G., Van Loveren, H. and Schuurman, H.J. (1991) Immunotoxicity of dioxin: immune function and host resistance in laboratory animals and humans. In: Banbury Report 35: Biological Basis for Risk Assessment of Dioxins and Related Compounds, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 79.

Wells, D.E. and Echarri, I. (1992) Determination of individual chlorobiphenyls (CBs), including non-ortho, and mono-ortho chloro substituted CBs in marine mammals from Scottish waters. Int. J. Environ. Anal. Chem. 47, 75.

Yang, Y.G., Lebrec, H. and Burleson, G.R. (1994) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on pulmonary influenza virus titer and natural killer (NK) activity in rats. Fundam. Appl. Toxicol. 23, 125.