Retinoids in marine mammals and their use as biomarkers of organochlorine compounds

A. BORRELL, V. TORNERO AND A. AGUILAR

Department of Animal Biology and GRUMM, Parc Científic de Barcelona, University of Barcelona, Barcelona, 08028, Spain
Contact e-mail: assump@bio.ub.es

ABSTRACT

Retinoids, also known as vitamin A, are non-endogenous molecules that are essential for a number of physiological processes in mammals. Imbalance of retinoids has been associated with reproductive impairment, embryonic mortality, growth retardation and bone deformities, pathologies in skin and the nervous system, and immune suppression. Mammals cannot produce retinoids so their primary source is dietary. They are absorbed by the small intestine and packaged as retinyl esters in chylomicrons, which enter the circulation and end up mostly in the liver and fatty tissues. Plasma retinoid levels are homeostatically regulated, so they remain constant despite variations in dietary supply or tissue stores. Therefore body depletion of retinoids cannot be reliably assessed through levels in blood, and should be evaluated through concentrations in depot tissues. In marine mammals, the main storage sites for retinoids are liver and blubber. Although not a universal rule, the concentration of retinoids often increases with age in both sexes because of progressive build-up of retinyl esters. In addition, sex often affects retinoid levels, but the nature and magnitude of this effect varies between species and populations. Taxonomic, life-style (particularly dietary) and climatic differences may explain dissimilarities in the effect of age and sex on retinoid levels. For this reason, retinoids can be used to distinguish populations or population components showing distinct dietary, behavioural, or other traits. Disease, particularly when affecting organs of physiological importance or inducing malnutrition, may affect retinoid tissue levels, so care should be taken when studying concentrations in stranded animals. Organochlorine compounds, particularly PCBs, dioxin (TCDDs) and DDTs, increase mobilisation of retinoids from hepatic and extrahepatic storage sites into serum, accompanied by enhanced degradation and elimination of retinoids through urine. In terrestrial mammals, this effect increases retinoid concentration. Conversely, in some species of marine mammals plasma retinoid levels have been reported to decrease when exposure to organochlorines increases, although the physiological mechanisms are unclear. However, given the homeostatic regulation of retinoids in blood, variation in plasma is expected to be less than that in liver or blubber. Because retinoid tissue levels vary in marine mammals even at moderate exposure to organochlorines, and original levels are restored when such exposure decreases or disappears, retinoids may be used as a biomarker of the impact of pollutants on populations. Further research is needed to validate their use, particularly in cetaceans.

KEYWORDS: MARINE MAMMALS; RETINOL; ORGANOCHLORINES; BIOMARKERS

INTRODUCTION

Retinoids, also known as vitamin A, are a family of essential molecules involved in a number of physiological functions in mammals. They are not produced endogenously and can only be acquired from external sources (Blomhoff, 1994); therefore, the capacity of the organism for regulation is limited (Green and Green, 1994). From an environmental perspective, retinoids have attracted attention because some xenobiotic compounds, particularly organochlorine compounds such as PCBs and dioxins, have been found to cause their depletion in mammalian body tissues. This effect may induce severe physiological dysfunction (e.g. Thompson, 1976; Brouwer *et al.*, 1989a; b; Jurek *et al.*, 1990; Håkansson *et al.*, 1991a; b; 1992; Ikegami *et al.*, 1991; Chu *et al.*, 1995; 1996; Kelley *et al.*, 2000). As a result of their physiological role and reactivity to certain chemicals, retinoids have been proposed as a biomarker for exposure to organochlorine and perhaps other pollutants (Peakall, 1992; Jensen *et al.*, 1995; Murk *et al.*, 1998). Given that marine mammals (particularly small predators such as seals, dolphins and porpoises) are subject to extremely high levels of organochlorine exposure, this type of pollution is a potential threat to the conservation of populations (Aguilar *et al.*, 1999; O'Shea and Aguilar, 2001).

Although the liver and other tissues of some large whales have long been recognised as a profitable source of retinoids (see below), information on these compounds in marine mammals is limited. This paper reviews current information on retinoid physiology, natural patterns of variation, and effect of organochlorine exposure in marine mammals. When no information is available, literature on terrestrial mammals is referred to.

CHEMICAL STRUCTURE OF RETINOIDS

Retinoid is a general term referring to a group of closely related compounds whose molecular structure consists of four isoprenoid units joined in a head-to-tail manner. This definition includes compounds such as retinol and retinol derivatives, retinal, retinyl palmitate and retinoic acid.

All-trans-retinol (vitamin A alcohol) is the parent vitamin A compound. It is a fat-soluble primary alcohol of low molecular weight (mw = 286). The aldehyde form, retinal, is found in the retina of the eye, and retinoic acid, a metabolite of vitamin A, is highly active in a number of physiological processes (Wolf, 1984).

PHYSIOLOGY OF RETINOIDS

Although retinoids can be toxic in high concentrations and the adverse effects of hypervitaminosis are documented in both man and animals (Armstrong *et al.*, 1994), in natural conditions the most frequent disorders and pathological effects are produced by low availability of the compound.

Retinoids play an important role in: vision (xerophthalmia and night blindness are both symptoms of its deficiency); the maintenance of the reproductive, endocrine and immune systems; growth and foetal development; and the regulation of the proliferation and differentiation of many cell types. Thus, imbalance of retinoids has been associated with a diversity of anomalies, including reproductive impairment, embryonic mortality, growth retardation and bone deformities, pathologies in skin and the nervous system, and immune suppression (Thompson, 1976; Peakall, 1992). Many of these effects are mediated by the action of retinoic

acid on gene expression (Blomhoff *et al.*, 1991). In addition, retinoids have a protective effect against the development of various cancers (Wolf, 1984).

For mammals, none of which can synthesise retinoids, vitamin A is an essential nutrient. Dietary retinoids are available from two sources: from plants in the form of provitamin A precursor compounds, namely β- (mainly), α- and γ-carotene and cryptoxanthin; and from animal tissues as long-chain retinyl esters.

Once in the digestive system, retinoids are absorbed by the small intestine and packaged as retinyl esters in chylomicrons, which enter the circulation and are taken mostly by the liver. In this organ, chylomicrons are metabolised and retinyl esters are processed for hepatic storage or for secretion as retinol bound to retinol binding protein or RBP (mw = 21000) (Blomhoff, 1994; Green and Green, 1994).

RBP delivers plasma retinoids to target tissues throughout the body (Soprano and Blaner, 1994). Over 95% of RBP-retinol circulates in the blood as a 1:1 molar complex with a second transport protein called transthyretin or TTR (mw = 54980), which also transports thyroid hormones TT4 (Blomhoff, 1994; Green and Green, 1994). It has been established that retinoids recycle among plasma, liver and extrahepatic tissues, since the plasma retinoid turnover is more than one order of magnitude greater than the utilisation rate. The vehicle for retinoid recycling is RBP (Blomhoff *et al.*, 1992; Sommer and West, 1996).

Plasma retinoid levels are constant despite great variation in dietary supply or in liver and extrahepatic tissues stores. Thus, it appears that plasma retinoid levels are homeostatically regulated, ensuring that retinoids are continuously available to vitamin A-dependent cells (Wolf, 1984). As a consequence, body depletion of retinoids cannot be assessed through circulating levels in blood, but should be evaluated through concentrations in depot tissues such as liver and fat. The excretion of retinoids in the urine does not appear to be affected by the retinoid status of the animal itself but by the amount of retinoids available through the diet (Raila *et al.*, 2000).

STORAGE OF RETINOIDS IN TISSUES

The comparative tissue distribution of retinoids in mammals has not been studied systematically. However, surveys available for terrestrial species usually point to the liver as the main storage site, with 50-80% of the body load commonly present in this organ. Extrahepatic tissues such as kidneys, adipose tissue, lung or testis, can also play a significant role in the storage and mobilisation of these compounds (Blaner and Olson, 1994). However, there are dissimilarities among species and/or taxonomic groups. For example, in the Canidae and Mustelidae families, retinoid concentrations in plasma are about 10-50 times higher than in other mammals; indeed, in many mammals such a high level would reflect hypervitaminosis A (Schweigert *et al.*, 1990; 1991b). Kidney retinoid concentration in canids is also high and far exceeds those in the liver; such low hepatic levels would normally be considered an indication of severe vitamin A deficiency in other mammals (Underwood, 1984; Schweigert and Buchholz, 1995). It should be pointed out that the urine of canids contains both retinol and retinyl esters (Schweigert *et al.*, 1991a), while that of human and rats only contains metabolic forms of retinoids, such as retinoic acid (Schweigert and Buchholz, 1995). Therefore, the high level of retinoids observed in the kidney of at least the canids can be associated with this particular form of excretion (Schweigert and Buchholz, 1995). As stated above, generally in terrestrial mammals, the concentration of retinoids in blood is kept constant homeostatically and it decreases only when storage tissues are severely depleted (Wolf, 1984; Blomhoff *et al.*, 1992).

Information on the distribution of retinoids in the body of marine mammals is limited to a few studies that report the concentration in selected tissues from the same individuals (Table 1). There are some data on concentrations in isolated tissues, but these cannot be compared between studies because of substantial variation at individual, population and species levels (see below). The information available suggests that, as is usual in terrestrial mammals, retinoids are extensively stored in the form of retinyl esters in the liver. Indeed, it has long been known that the liver of cetaceans is extremely rich in retinoids (Schmidt-Nielsen *et al.*, 1934), and the interest in obtaining this compound for commercial production of vitamin A led a number of researchers during the first half of the century to investigate its contents in the tissues of large whales (e.g. Klem, 1935; Wetlesen, 1938; Braekkan, 1948; Ishikawa *et al.*, 1948; 1951; Kaneko, 1948; Mori and Saiki, 1950; Tawara and Fukazawa, 1950a; b). A similar richness in hepatic retinoids was later confirmed in pinnipeds (Rodahl and Davies, 1949; Schweigert *et al.*, 1987; Ball *et al.*, 1992; Schweigert and Buchholz, 1995; Käkëla and Hyvärinen, 1997; Käkëla *et al.*, 1997).

However, in marine mammals, blubber is also a significant storage site of retinoids and the concentration of retinoids in the blubber of at least some marine mammals appears to be higher than in comparable fatty tissues of man and other terrestrial mammals (Schweigert *et al.*, 1987). Thermoregulatory and lipid storage needs render fatty tissues of marine mammals to be a substantial proportion of body mass, usually in the range 15-55% and, given the lipophilic nature of retinoids, this allows for massive accumulation of these compounds. The blubber/body mass ratio in marine mammals is inversely scaled, so smaller species tend to have a larger contribution of fatty tissues, and therefore larger relative retinoid stores, than larger species (Ryg *et al.*, 1990; 1993; Aguilar *et al.*, 1999). In grey seals (*Halichoerus grypus*), Schweigert *et al.* (1987) have estimated that blubber accounts for about 40% of total body reserves of retinoids. Borrell *et al.* (1999) found that blubber is also a significant site for retinoid deposition in harbour porpoises (*Phocoena phocoena*) from West Greenland.

Information on retinoid levels in tissues or body organs other than liver and blubber is fragmentary. Mori and Saiki (1950) reported concentrations in the intestine of sperm whales (*Physeter macrocephalus*), Iida *et al.* (1998) in muscle of Antarctic minke whales (*Balaenoptera acutorostrata*), Gregory *et al.* (1955) in the milk of blue whales (*B. musculus*), and Rosas and Lehti (1996) in the milk of Amazon river dolphins (*Inia geoffrensis)*. However, the sample size in these studies was extremely small, often limited to a single individual, and they offer no reliable insight into individual variation. Studies in harp seals (*Pagophilus groenlandicus*), grey seals and common seals (*Phoca vitulina*) indicate that other tissues such as kidneys, lung, retina, pancreas and spleen also have minor shares of the retinoid body content (Rodahl and Davies, 1949).

MAIN FACTORS AFFECTING VARIATION IN TISSUE CONCENTRATIONS

As stated above, retinoids are regulated within individual organisms. However biological traits (e.g. sex, age, diet and body condition, incidence of disease, occurrence of

Table 1

Distribution of retinoids (mean ± SD) in plasma (μg/ml) and other tissues (μg/g tissue) of marine mammals. Only surveys reporting concentrations in more than one tissue have been included (see text).

Species	Location	*n*	Age/Sex (M/F)	Liver	Blubber	Serum	Kidney	Lung	Reference
Harp seal (*Pagophilus groenlandicus*)	Newfoundland	1	Adult	720	3.6	-	1.8	0.9	Rodahl and Davis, 1949
Grey seal (*Halichoerus grypus*)	Pembrokeshire	1	Juvenile	465	1.074	-	4.725	0.75	Rodahl and Davis, 1949
Grey seal (*Halichoerus grypus*)	Sable Island	12	Adult M	502.6 ± 314.9	33.7 ± 10.9	0.26 ± 0.057	-	-	Schweigert *et al.*, 1987
Grey seal (*Halichoerus grypus*)	Sable Island	5	Adult F	264.9 ± 118.4	62.4 ± 3.7	0.41 ± 0.085	-	-	Schweigert *et al.*, 1987
Grey seal (*Halichoerus grypus*)	Sable Island	21	Juvenile	375.7 ± 320.6	21.9 ± 14.8	0.21 ± 0.068	-	-	Schweigert *et al.*, 1987
Grey seal (*Halichoerus grypus*)	Sable Island	6	Adult M	609 ± 395	45 ± 10	0.2 ± 0.1	8 ± 3 (all)	-	Schweigert and Buchholz, 1995
Harbour seal (*Phoca vitulina*)	Wash	1	Juvenile	27	Not detected	-	0.27	0.18	Rodahl and Davis, 1949
Ringed seal (*Pusa hispida*)	Baltic Sea	7-9	Adult	175.3 ± 32.6 (*n*=7)	21.6 ± 3.4 (*n*=9)	-	-	-	Käkelä *et al.*, 1997
Ringed seal (*Pusa hispida*)	Lake Ladoga	4	Juvenile	36.1 ± 7.6	3.1 ± 0.5	-	-	-	Käkelä *et al.*, 1997

lactation) and anthropogenic influences (e.g. environmental pollutants) have a substantial effect on tissue levels and body content of retinoids.

Age

The influence of ageing on retinoids status in terrestrial mammals has been widely studied. Many authors reported an increase in concentrations with age: e.g. liver and blood of rats (Blomhoff *et al.*, 1988); plasma of Florida panthers, *Felis concolor oryi* (Dunbar *et al.*, 1999) and man (Malvy *et al.*, 1993; Stephenson and Gildengorin, 2000), and in the kidney of dogs (Schweigert *et al.*, 1998). However, other surveys revealed either no trend in retinoid levels between age classes, or even decreasing ones. For example, Garry *et al.* (1987) found similar plasma retinoid levels in young and old humans, and Savage *et al.* (1999) reported that age did not affect plasma levels of retinoids in free-ranging African elephants (*Loxodonta africana*). A decrease in serum retinoids was observed by Succari *et al.* (1991) in humans and by Shrestha *et al.* (1998) in female Nepalese elephants (*Elephas maximus*).

Similarly, studies on pinnipeds and cetaceans (Table 2) do not produce consistent results. While many populations showed, both in the liver and in the blubber, an increasing trend in retinoid concentrations with age, others revealed no apparent trend or even a decreasing tendency with age. This variation could not be explained by inter-specific, inter-population or even inter-tissue differences. For example, the studies on ringed seals (*Pusa hispida*) from Lake Saimaa by Käkelä *et al.* (1997) showed a significant positive age-related trend in the blubber and a negative trend in the liver, while those conducted on the same species by the same research group and with an identical sample size ($n = 12$) in Spitsbergen showed the opposite result: a negative trend in the blubber and a positive trend in the liver, although in this case the correlation was non-significant (Table 2).

However, although a general, consistent pattern cannot be deduced from the information available, an increasing trend was the most common finding. This relationship appears to be the result of a decrease in the circulatory clearance of retinoids and other liposoluble compounds with age, coupled with an excess intake of retinoids via diet, which leads to a

Table 2

Age trends in retinoid concentrations observed in tissues of marine mammals. * = only females; ** = significative $p<0.05$; " = statistics not performed; ↑ = positive trend; ↓ = negative trend.

Species	Location	*n*	Liver	Blubber	Reference
Australian fur seal (*Arctocephalus forsteri*)	Australia	24*	↑**		Southcott *et al.* 1974
Grey seals (*Halichoerus grypus*)	Sable Island	65	↑ "	↑ "	Schweigert *et al.* 1987
Hooded seals (*Cystophora cristata*)	Newfoundland	60	↑ "		Rodahl and Davis, 1949
Harp seal (*Pagophilus groenlandicus*)	Newfoundland	145	↑ "		Rodahl and Davis, 1949
Ringed seals (*Pusa hispida*)	Lake Saimaa	12	↓**	↑**	Käkelä *et al.* 1997
Ringed seals (*Pusa hispida*)	Spitsbergen	12	↑	↓	Käkelä *et al.* 1997
Ringed seals (*Pusa hispida*)	Baltic Sea	9	↓	↑	Käkelä *et al.* 1997
Harbour porpoise (*Phocoena phocoena*)	Greenland	100		↑	Borrell *et al.*, 1999

build-up of retinyl ester concentrations with age (Maiani *et al.*, 1989; Krasinski *et al.*, 1990). Although it is not clear why some species or populations do not show this general trend, taxonomic, life-style (particularly dietary) and climatic differences may be responsible.

Sex

Information on sex-related variation in retinoids is even more sparse and less consistent than that for age. In terrestrial mammals, no gender-related differences were observed in circulating concentrations of retinoids in black rhinoceros, *Diceros bicornis* (Ghebremeskel *et al.*, 1988), serum levels in free-ranging African elephants (Savage *et al.*, 1999) or liver and serum concentration in humans (Raica *et al.*, 1972; Succari *et al.*, 1991). Conversely, circulating retinoid levels were reported to be higher in female Florida panthers (Dunbar *et al.*, 1999) but lower in females in some human populations (Krasinski *et al.*, 1989; Stephenson and Gildengorin, 2000). These inter-specific differences may be produced by dissimilarities in types of diet and source of retinoids.

In marine mammals, studies on pinnipeds have often suggested sex-related differences although these varied among tissues and species (Fig. 1). Levels of retinoids were found to be higher in the blubber of adult female grey seals (Schweigert *et al.*, 1987) and in the liver of adult female Australian fur seals (*Arctocephalus forsteri*) (Southcott *et al.*, 1974) than in the corresponding tissues of adult males. However, other surveys have shown the reverse trends. Thus, Rodahl and Davies (1949) found higher concentrations in the liver of male hooded and harp seals than in those of females, and Schweigert *et al.* (1987) found a similar difference in the liver of grey seals. In cetaceans, the only available survey refers to harbour porpoises, in which no significant differences were found between the blubber retinoid concentrations of males and females (Borrell *et al.*, 1999).

It has been suggested that mothers transfer retinoids to their calves during lactation (Simms and Ross, 2000), which would explain the lower levels in the liver of adult females (Schweigert *et al.*, 1987). Milk is a source of essential nutrients, including retinoids. Although studies are limited, marine mammals appear to have relatively higher levels of retinoids in their milk than terrestrial mammals. However, this appears to be due to the high lipid content of the milk in pinnipeds and cetaceans because, when concentrations are expressed as quantity per unit lipid, levels are of the same order of magnitude or even lower than in terrestrial mammals (Schweigert and Stobo, 1994; Debier *et al.*, 1999). Irrespective of this, during lactation, females of both cetaceans and pinnipeds mobilise a large proportion of their blubber reserves, including the blubber-associated retinoid stores. This explains why during lactation, unlike humans, marine mammals may have high levels of circulatory retinoids coupled with lowered stores of retinoids in the blubber and probably other tissues (Schweigert *et al.*, 1987). However, no explanation has been put forward to explain the higher concentrations of males reported in some studies.

Similarly to the age-related variation, it is likely that taxonomic, dietary and life-style dissimilarities between sexes are responsible for sex-related variations. Reproductive activity may be particularly significant in adult individuals because it often involves changes in hormone levels, behavioural traits and diet (see below).

Diet and nutritive condition

Since retinoids are incorporated via food, diet affects tissue levels. However, it is unknown, even in man and laboratory animals, whether body stores of retinoids change as a function of long-term intake of these compounds (Ascherio *et al.*, 1992; Booth *et al.*, 1997; Scrofano *et al.*, 1998). As mentioned above, retinoids in blood are homeostatically controlled when liver stores are sufficient and therefore they only respond to extreme situations, for which reason diet has not been observed to have an effect on them (Blaner and Olson, 1994).

In marine mammals, information on the influence of diet on retinoid status is limited to the study by Käkelä *et al.* (1997), who reported differences in liver and blubber levels between freshwater and marine ringed seals and attributed them to food quality. Differences in diet, as well as climatic or photoperiod dissimilarities may explain variations in retinoid levels between allopatrid populations of the same species. However, such differences may also occur between different components within a single population. For example, variation in diet associated with age, sex or

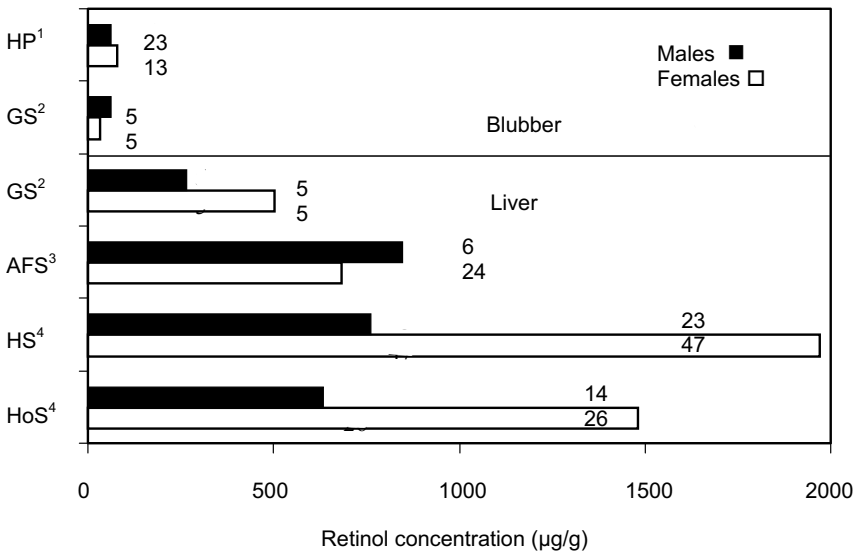

Fig. 1. Sex related variation in retinoid levels (µg/g) in liver and blubber of different marine mammal species. Key: HP = Harbour porpoise; GS = Grey seal; AFS = Australian fur seal; HS = Harp seal; HoS = Hooded seals. References: [1]Borrell *et al.*, 1999; [2]Schweigert *et al.*, 1987; [3]Soutcott *et al.*, 1974; [4]Rodahl and Davies, 1949.

reproductive condition has been reported for many cetaceans and pinnipeds (Seaman *et al.*, 1982; Perez and Mooney, 1986; Stewart and Murie, 1986; Bernard and Hohn, 1989; Recchia and Read, 1989; Rodhouse *et al.*, 1992; Smith and Read, 1992; Clarke *et al.*, 1993). Retinoids thus have the potential to be used to distinguish populations or population components with distinct dietary, behavioural or other traits, provided that the natural sources of variation are properly controlled.

The effect of nutritive condition on retinoid levels is difficult to assess. Neither Rodahl and Davies (1949) or Borrell *et al.* (1999), found any significant effect of condition on the retinoids present in the liver and blubber of hooded and harp seals, or in the blubber of harbour porpoises, respectively. Nevertheless, this conclusion should be treated with caution. The sample examined by Borrell *et al.* (1999) was mainly composed of healthy individuals. While in these conditions, retinoid tissue distribution may remain unaltered. It may require situations of food shortage, massive fat mobilisation (e.g. during migration in large baleen whales or during intensive lactation in some phocids), or starvation caused by disease or other condition, for retinoids to be significantly mobilised, redistributed or excreted. This may be particularly relevant for stranded cetaceans, often found in poor nutritive condition.

The tissue vitamin concentration reflects the essential amounts of these substances necessary for enzymatic and metabolic pathways, coupled with any excess picked up from the environment. The establishment of baseline values of retinoid concentrations is a requisite for the understanding of the chronic effects of toxicity and deficiency (Gelatt *et al.*, 1999).

Disease

Disease, particularly when it affects organs of physiological importance or induces malnutrition, may affect tissue levels of retinoids. However, the information available on this is restricted to humans. Patients suffering from acute or chronic diseases of the liver such as hepatitis, cirrhosis and hepatic tumours have markedly reduced serum levels of RBP, TTR and retinol. Those affected by significant renal disease also show disorders in RBP and retinoid transport, since the kidneys are a major site for RBP catabolism; thus, levels of retinoids increase when excretion is reduced as a consequence of renal tubular damage or reduced glomerular filtration rate of retinol RBP (Goodman, 1984). In addition, sub-normal serum concentrations of RBP and retinoids have been found in patients with a variety of cancers, but it is not clear whether this is a result of protein or energy denutrition (Soprano and Blaner, 1994).

No information is available on disease and retinoids in marine mammals. Given that disease may affect retinoid tissue levels, data from stranded animals in which disease is suspected should not be included in surveys of retinoid status.

EFFECT OF ORGANOCHLORINE POLLUTANTS ON RETINOIDS

Organochlorine compounds can alter retinoid metabolism. However, the biochemical pathway and intensity of the toxic effect appears to vary among species (Håkansson *et al.*, 1991a; Zile, 1992). In general, exposure to PCBs, dioxin (TCDDs) and DDTs leads to depletion of retinoid reserves in mammalian tissue due to increased mobilisation of retinoids from storage sites, especially the liver, and a subsequent increase in their degradation rate (Kelley *et al.*, 2000).

In terrestrial mammals (e.g. rats, otters, minks) feeding on a diet containing toxic organochlorine compounds, the retinol and retinyl ester concentrations in several body organs (liver, depot fat, intestine, lungs and adrenals) have been found to be lower in sample groups exposed to organochlorines than in non-polluted groups. (Brunström *et al.*, 1991; Håkansson *et al.*, 1992; Zile, 1992; Chu *et al.*, 1996; 1998; Murk *et al.*, 1998; Käkëla *et al.*, 1999; Nilsson *et al.*, 2000; Rolland, 2000; Simpson *et al.*, 2000). In contrast, the concentration of retinoids in kidney and, to a lesser extent, in serum, generally increased (Brouwer *et al.*, 1989a; Jurek *et al.*, 1990; Håkansson *et al.*, 1991a; b; Van Birgelen *et al.*, 1994a; b; Chu *et al.*, 1995; Nilsson *et al.*, 2000). This indicates that organochlorines increase mobilisation of retinoids from hepatic and extrahepatic storage sites into serum, accompanied by enhanced degradation and renal elimination of retinoids through urine (Kelley *et al.*, 1998; 2000). Studies on coplanar PCBs and TCDDs have shown that the toxic effect of these compounds is positively correlated with their ability to bind the Ah (arylhydrocarbon) receptor, causing the induction of cytochromes P-450 1A1 and 1A2 (Pelissier *et al.*, 1992; Brouwer, 1995). Thus, it appears that the mixed-function oxidases containing the cytochrome P450s are particularly active in metabolising retinoic acid (Roberts *et al.*, 1979; Ikegami *et al.*, 1991). Moreover, Roberts *et al.* (1992) reported that many rabbit liver cytochrome P-450 isoforms including 2A4, 1A2, 2E1, 2E2, 2C3, 2G1 can catalyse the 4-hydroxylation of both retinol and retinaldehyde. These findings indicate that the decrease in hepatic retinoids storage is related to the induction of cytochrome P-450 and retinoid metabolisation. In laboratory animals exposed to individual PCB congeners, the order of potency in causing reductions in the hepatic contents of retinoids was: PCB 126 > PCB 77 > PCB 153. This order of potency was found to be positively correlated with the ability of each congener to induce cytochrome P450 and with its toxicity measured as weight loss and thymic involution (Chen *et al.*, 1992; Håkansson *et al.*, 1994). In addition, exposure to organochlorines also inhibits the intestinal absorption of ingested vitamin A, thus exacerbating the imbalance produced by the previous effects (Bank *et al.*, 1989).

However, the retinoid depletive effect of these toxic organochlorines can not simply be extrapolated to all organochlorine forms or derivatives. For example, long-term (1 year) experiments conducted with mink fed with methylsulfonyl-PCBs, which are not very AhR-active, did not reveal any effect on retinoid concentrations in tissues (Lund *et al.*, 1999).

Given the evolutionary basis of the physiological processes involved, most of these effects can probably be extended to marine mammals. However, the specific pathways or dynamics may be somewhat different. Thus, most of the studies so far undertaken in three species of pinnipeds and the polar bear (Table 3) have shown a decrease in plasma retinoids when PCB or other organochlorine (OCs) loads increased (Brouwer *et al.*, 1989b; De Swart *et al.*, 1994; Jensen *et al.*, 1995; Beckmen *et al.*, 1997; Skaare *et al.*, 2001). These results originate from studies in both captive and wild populations. In experiments with captive seals, retinoid concentrations returned to normal when animals were fed with slightly contaminated fish (Brouwer *et al.*, 1989b). Unfortunately, only plasma was analysed, so the mechanisms of this decrease were unclear. Given the homeostatic regulation of retinoids in blood, variation in plasma is expected to be lower than in other tissues such as liver or blubber. The only exception (Table 3)

Table 3

Details of studies reporting observed effects of organochlorine pollutants on plasma retinol levels (1) or plasma retinoid levels (2) in marine mammals, including the polar bear.

Species	Location	*n*	Pollutant	Study type	Effect on concentration	References
Harbour seal (*Phoca vitulina*)	-	24	Organochlorines	Experimental	(1) Decrease	Brouwer *et al.*,1989
Harbour seal (*Phoca vitulina*)	-	22	Organochlorines	Experimental	(2) Decrease	De Swart *et al.*, 1994
Northern elephant seal (*Mirounga angustirostris*)	California	31	Organochlorines	Wild	(1) Decrease	Beckmen *et al.*, 1997
Grey seal (pups) (*Halichoerus grypus*)	Norway	51	PCBs	Wild	(2) Decrease	Jenssen *et al.*, 1995
Harbour seal (pups) (*Phoca vitulina*)	British Columbia/ Washington State	61	PCBs	Wild	(1) Decrease (between populations)	Simms *et al.*, 2000
Harbour seal (pups) (*Phoca vitulina*)	British Columbia/ Washington State	37	PCBs	Wild	(1) Increase (in non-nursing pups)	Simms *et al.*, 2000
Polar bear (*Ursus maritimus*)	Svalbard/ Russian Arctic	79	PCBs	Wild	(2) Decrease	Skaare *et al.*, 2001

appears to be the study by Simms *et al.* (2000), which showed that, although retinoid levels in more polluted populations of harbour seal pups were lower than those in a cleaner population, in non-nursing pups levels were positively correlated with organochlorine levels in the blubber. This correlation was explained by the mobilisation of hepatic stores of retinoids into blood and the disruption of the vitamin A transport complex following exposure to milk-derived pollutants, as previously observed in laboratory and terrestrial mammals.

In pinnipeds, hydroxylated PCBs, which are metabolites produced by phase I enzymes, have also been shown to disrupt retinoid transport complexes in plasma, reducing delivery of retinoids to target tissues (Brouwer *et al.*, 1989b; 1998; Ross and Troisi, 2001) as has been seen in terrestrial mammals.

Given that variation in retinoid tissue levels in marine mammals appears to occur even at moderate exposure to organochlorines (Håkansson *et al.*, 1992; Jensen *et al.*, 1995) and that original levels are restored when pollutants disappear or significantly decrease (Brouwer *et al.*, 1989b), retinoids are potentially sensitive biomarkers of organochlorine exposure. However, it is likely that this sensitivity is higher for retinoid reserve tissues, such as blubber, than for blood. In addition, retinoids play a critical role in reproduction and immune competence, two functions through which organochlorines have allegedly impacted marine mammal populations (e.g. see Reijnders *et al.*, 1999). Thus the identification of any potential imbalance of these compounds is relevant to the assessment of the pollutants impact on the involved populations. However, prior to the use of retinoids as biomarkers in ecotoxicological studies, further research is needed to clarify the dynamics of retinoids and their degradation pathways in the tissues of marine mammals, particularly cetaceans.

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REFERENCES

Aguilar, A., Borrell, A. and Pastor, T. 1999. Biological factors affecting variability of persistent pollutant levels in cetaceans. *J. Cetacean Res. Manage.* (special issue) 1:83-116.

Armstrong, R.B., Ashenfelter, K.O., Eckhoff, C., Levin, A.A. and Shapiro, S.S. 1994. General and reproductive toxicology of retinoids. pp. 545-72. *In:* M.B. Sporn, A.B. Roberts and D.S. Goodman (eds.) *The Retinoids: Biology, Chemistry and Medicine.* Raven Press Ltd, New York. 679pp.

Ascherio, A., Stampfer, M.J., Colditz, G.A., Rimm, E.B., Litin, L. and Willett, W.C. 1992. Correlations of vitamin A and E intakes with the plasma concentrations of carotenoids and tocopherols among American men and women. *J. Nutr.* 122(9):1792-801.

Ball, M.D., Nizzi, C.P., Furr, H.C., Olson, J.A. and Oftedal, O.T. 1992. Fatty-acyl esters of retinol (Vitamin A) in the liver of the harp seal (*Phoca groenlandica*), hooded seal (*Cystophora cristata*), and California sea lion (*Zalophus californianus*). *Biochem. Cell Biol.* 70:809-13.

Bank, P.A., Cullum, M.E., Jensen, R.K. and Zile, M.H. 1989. Effect of hexachlorobiphenyl on vitamin A homeostasis in the rat. *Biochem. Biophys. Acta* 990:306-14.

Beckmen, K.B., Lowenstine, L.J., Newman, J., Hill, J., Hanni, K. and Gerber, J. 1997. Clinical and pathological characterisation of northern elephant seal skin disease. *J. Wildl. Dis.* 33:438-49.

Bernard, H.J. and Hohn, A.A. 1989. Differences in feeding habits between pregnant and lactating spotted dolphins (*Stenella attenuata*). *J. Mammal.* 70(1):211-5.

Blaner, W.S. and Olson, J.A. 1994. Retinol and retinoic acid metabolism. pp. 229-55. *In:* M.B. Sporn, A.B. Roberts and D.S. Goodman (eds.) *The Retinoids: Biology, Chemistry and Medicine*. Raven Press Ltd, New York. 679pp.

Blomhoff, R. 1994. Overview of vitamin A metabolism and function. pp. 1-35. *In:* R. Blomhoff (ed.) *Vitamin A in Health and Disease*. Marcel Dekker, New York. 677pp.

Blomhoff, R., Berg, T. and Norum, K.R. 1988. Distribution of retinol in rat liver cells: effect of age, sex and nutritional status. *Br. J. Nutr.* 60:233-9.

Blomhoff, R., Green, M.H., Green, J.B., Berg, T. and Norum, K.R. 1991. Vitamin A metabolism: new perspectives on absorption, transport and storage. *Physiol. Rev.* 71(4):951-90.

Blomhoff, R., Green, M.H. and Norum, K.R. 1992. Vitamin A: Physiological and biochemical processing. *Annu. Rev. Nutr.* 12:37-57.

Booth, S.L., Tucker, K.L., McKeown, N.M., Davidson, K.W., Dallaal, G.E. and Sadowski, J.A. 1997. Relationships between dietary intakes and fasting plasma concentrations of fat-soluble vitamins in humans. *J. Nutr.* 127(4):587-92.

Borrell, A., Cantos, G., Aguilar, A., Lockyer, C., Brouwer, A., Heide-Jørgensen, M.P., Jensen, J. and Spenkelink, B. 1999. Patterns of variability of retinol levels in a harbour porpoise population from an unpolluted environment. *Mar. Ecol. Prog. Ser.* 185:85-92.

Braekkan, O.R. 1948. Vitamins in whale liver. *Sci. Rep. Mar. Biol. Res.* 32:1-25.

Brouwer, A. 1995. Metabolism of xenobiotics in laboratory animals and widllife species: potential impact on physiology and health. Paper SC/M95/P8 presented to the IWC Scientific Committee Workshop on Chemical Pollutants and Cetaceans, March 1995,

Bergen (unpublished). [Paper available from the Office of this Journal].

Brouwer, A., Håkansson, H., Kukler, A., Van den Berg, K.J. and Ahlborg, U.G. 1989a. Marked alterations in retinoid homeostasis of Sprague-Dawley rats induced by a single i.p dose of 10μg/kg of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicology* 58:267-83.

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

Brouwer, A., Morse, D.C., Lans, M.C., Schuur, A.G., Murk, A.J., Wehler, E.K., Bergman, A. and Visser, T.J. 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicol. Ind. Health* 14:59-84.

Brunström, B., Håkansson, H. and Lundberg, K. 1991. Effects of a technical PCB preparation and fractions thereof on ethoxyresorufin *O*-deethylase activity, vitamin A levels and thymic development in the mink (*Mustela vision*). *Pharmacol. Toxicol.* 69:421-6.

Chen, L.-C., Berberian, I., Koch, B., Mercier, M., Azais-Braesco, V., Glauert, H.P., Chow, C.K. and Robertson, L.W. 1992. Polychlorinated and polybrominated biphenyl congeners and retinoid levels in rat tissues: structure-activity relationships. *Toxicol. Appl. Pharmacol.* 114:47-55.

Chu, I., Villeneuve, D.C., Yagminas, A., Lecavalier, P., Håkansson, H., Ahlborg, U.G., Valli, V.E., Kennedy, S.W., Bergman, A., Seegal, R.F. and Feeley, M. 1995. Toxicity of PCB 77 (3,3',4,4'-tetrachlorobiphenyl) and PCB 118 (2,3',4,4',5-pentaclorobiphenyl) in the rat following subchronic dietary exposure. *Fundam. Appl. Toxicol.* 26(2):282-92.

Chu, I., Villeneuve, D.C., Yagminas, A., Lecavalier, P., Poon, R., Feeley, M., Kennedy, S.W., Seegal, R.F., Håkansson, H., Ahlborg, U.G., Valli, V.E. and Bergman, A. 1996. Toxicity of 2,2',4,4',5,5'-hexachlorobiphenyl in rats: Effects following 90-day oral exposure. *J. Appl. Toxicol.* 16(2):121-8.

Chu, I., Poon, R., Yagminas, A., Lecavalier, P., Håkansson, H., Valli, V.E., Kennedy, S.W., Bergman, A., Seegal, R.F. and Feeley, M. 1998. Subchronic toxicity of PCB 105 (2,3,3,4,4',-pentachlorobiphenyl) in rats. *J. Appl. Toxicol.* 18(4):285-92.

Clarke, M.R., Martins, H.R. and Pascoe, P. 1993. The diet of sperm whales (*Physeter macrocephalus* Linnaeus 1758) off the Azores. *Philos. Trans. R. Soc. Lond. B. (Biol. Sci.)* 339(1287):67-82.

Debier, C., Kovacs, K.M., Lydersen, C., Mignolet, E. and Larondelle, Y. 1999. Vitamin E and vitamin A contents, fatty acid profiles, and gross composition of harp and hooded seal milk through lactation. *Can. J. Zool.* 77:952-8.

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 harbour seals (*Phoca vitulina*) feeding on fish from polluted waters. *Ambio* 23:155-9.

Dunbar, M.R., Cunningham, M.W. and Linda, S.B. 1999. Vitamin A concentrations in serum and liver from Florida panthers. *J. Wildl. Dis.* 35(2):171-7.

Garry, P.J., Hunt, W.C., Bandrofchak, J.L., Vanderjagt, D. and Goodwin, J.S. 1987. Vitamin A intake and plasma retinol levels in healthy elderly men and women. *Am. J. Clin. Nutr.* 46:989-94.

Gelatt, T.S., Arendt, T., Murphy, M.S. and Siniff, D.B. 1999. Baseline levels of selected minerals and fat-soluble vitamins in Weddel seals (*Leptonychotes weddelli*) from Erebus Bay, McMurdo Sound, Antarctica. *Mar. Poll. Bull.* 38:1251-8.

Ghebremeskel, K., Williams, G., Lewist, J.C.M. and Du Toit, M. 1988. Serum alpha tocopherol, all-trans retinol, total lipids and cholesterol in the black rhinoceros (*Diceros bicornis*). *Comp. Biochem. Physiol. A* 91(2):343-6.

Goodman, D.S. 1984. Plasma retinol-binding protein. pp. 44-88. *In:* M.B. Sporn, A.B. Roberts and D.S. Goodman (eds.) Vol. 2. *The Retinoids*. Academic Press, Orlando, FL.

Green, M.H. and Green, J.B. 1994. Dynamics and control of plasma retinol. pp. 119-33. *In:* R. Blomhoff (ed.) *Vitamin A in Health and Disease*. Marcel Dekker, New York. 677pp.

Gregory, M.E., Kon, S.K., Rowland, S.J. and Thompson, S.Y. 1955. The composition of the milk of the blue whale. *J. Dairy Res.* 22:108-12.

Håkansson, H., Johansson, L., Manzoor, E. and Ahlborg, U.G. 1991a. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the vitamin A status of Hartley guinea pigs, Sprague-Dawley rats, C57B1/6 mice, DBA/2 mice and Golden Syrian hamsters. *J. Nutr. Sci. Vitaminol.* 37(2):117-38.

Håkansson, H., Manzoor, E. and Ahlborg, U.G. 1991b. Interaction between dietary vitamin A and single oral doses of 2,3,7,8-tetrachlorodebenzo-p-dioxin (TCDD) on the TCDD-induced toxicity and on the vitamin A status in the rat. *J. Nutr. Sci. Vitaminol.* 37(3):239-56.

Håkansson, H., Manzoor, E. and Ahlborg, U.G. 1992. Effects of technical PCB preparations and fractions thereof on vitamin A levels in the mink (*Mustela vison*). *Ambio* 21(8):588-90.

Håkansson, H., Manzoor, E., Trossvik, C., Ahlborg, U.G., Chu, I. and Villeneuve, D. 1994. Effect on tissue vitamin A levels in the rat following subchronic exposure to four individual PCB congeners (IUPAC 77, 118, 126 and 153). *Chemosphere* 29:2309-13.

Iida, H., Murata, Y., Matsumoto, G., Toda, S., Yamashita, Y. and Yokoyama, M. 1998. Chemical composition of the edible parts of minke whale *Balaenoptera acutorostrata*. *Bull. Nat. Res. Inst. Fish. Sci.* 11:27-36.

Ikegami, S., Tsuchihashi, F. and Nishide, E. 1991. Decrease in hepatic storage of vitamin A and induction of cytochrome P-450 by dietary organochlorine pesticides in rats. *J. Food Hyg. Soc. Japan* 32(1):1-7.

Ishikawa, S., Omote, Y. and Soma, Y. 1948. Analytical distillation of vitamin A in the whale liver oil. *Sci. Rep. Whales Res. Inst., Tokyo* 2:35-41.

Ishikawa, S., Omote, Y. and Okuda, H. 1951. Substances related to vitamin A in the whale liver oil. *Sci. Rep. Whales Res. Inst., Tokyo* 5:53-9.

Jenssen, B.M., Skaare, J.U., Woldstad, S., Nastad, A.T., Haugen, O., Kloven, B. and Sormo, E.G. 1995. Biomarkers in blood to assess effects of polychlorinated biphenyls in free-living grey seal pups. pp. 607-15. *In:* Blix (ed.) *Whales, Seals, Fish and Man: Proceedings of the International symposium on the Biology of Marine Mammals in the Northeast Atlantic. Tromso, Norway, 29 November –1 December 1994*.

Jurek, M.A., Powers, R.H., Gilbert, L.G. and Aust, S.D. 1990. The effect of TCDD on acyl coenzyme A:retinol acyltransferase activity and vitamin A accumulation in the kidney of male Sprague-Dawley rats. *J. Biochem. Toxicol.* 5(3):155-60.

Käkëla, R. and Hyvärinen, H. 1997. Fatty acid compositions and vitamin A and E status of endangered freshwater ringed seals (from Finland and Western Russia) differ from those of marine seals. *Rec. Res. Devel. Lipids Res.* 1:1-7.

Käkëla, R., Hyvärinen, H. and Käkëla, A. 1997. Vitamins A-1 (retinol), A-2 (3,4 didehydroretinol) and E (alpha-tocopherol) in the liver and blubber of lacustrine and marine ringed seals (*Phoca hispida* sp.). *Comp. Biochem. Physiol. B* 116:27-33.

Käkëla, R., Käkëla, A., Hyvärinen, H., Asikainen, J. and Dahl, S.K. 1999. Vitamins A_1, A_2 and E in minks exposed to polychlorinated biphenyls (*Aroclor 1242*) and copper, via diet based on freshwater or marine fish. *Environ. Toxicol. Chem.* 18:2595-9.

Kaneko, A. 1948. Molecular distillation of fin whale liver oil. *Sci. Rep. Whales Res. Inst., Tokyo* 2:46-50.

Kelley, S.K., Milsson, C.B., Green, M.H., Green, J.B. and Håkansson, H. 1998. Use of model-based compartmental analysis to study effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on vitamin A kinetics in rats. *Toxicol. Sci.* 44(1):1-13.

Kelley, S.K., Nilsson, C.B., Green, M.H., Green, J.B. and Håkansson, H. 2000. Mobilisation of vitamin A stores in rats after administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin: A kinetic analysis. *Toxicol. Sci.* 55(2):478-84.

Klem, A. 1935. Studies on the biochemistry of whale oils. *Hvalråd. Skr.* 11:49-108.

Krasinski, S.D., Russell, R.M. and Otradovec, C.L. 1989. Relationship of vitamin A and vitamin E intake to fasting plasma retinol, retinol binding protein, retinyl esters, carotene, alpha-tocopherol and cholesterol among elderly people and young adults: increased plasma retinyl esters among vitamin A supplement users. *Am. J. Clin. Nutr.* 49:112-20.

Krasinski, S.D., Cohn, J.S., Shaefer, E.J. and Russel, R.M. 1990. Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects: evidence for delayed plasma clearance of intestinal lipoproteins. *Clin. Invest.* 85:883-92.

Lund, B.O., Örberg, J., Bergman, Å., Larsson, C., Bergman, A., Bäcklin, B.M., Håkansson, H., Madej, A., Brouwer, A. and Brunström, B. 1999. Chronic and reproductive toxicity of a mixture of 15 methylsulfonyl-polychlorinated biphenyls and 3-methylsulfonyl-2,2-bis-(4-chlorophyenyl)-1,1-dichloroethene in mink (*Mustela vison*). *Environ. Toxicol. Chem.* 18:292-8.

Maiani, G., Mobarhan, S., Ceccanti, M., Ranaldi, L., Gettner, S. and Bowen, P. 1989. Beta-carotene serum responses in younger and elderly females. *Eur. J. Clin. Nutr.* 43:749-61.

Malvy, D.J.M., Buurtschy, B., Dostalova, L. and Amedee-Manesme, O. 1993. Serum retinol, beta-carotene, alpha-tocopherol and cholesterol in healthy French children. *Int. J. Epidemiol.* 22(2):237-46.

Mori, T. and Saiki, M. 1950. Properties of fats and oils contained in various parts of a sperm whale body. *Sci. Rep. Whales Res. Inst., Tokyo* 3:79-84.

Murk, A.J., Leonards, P.E.G., Van Hattum, B., Luit, R., Van de Weiden, M.E.J. and Smit, M. 1998. Application of biomarkers from exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). *Environ. Toxicol. Pharmacol.* 6:91-102.

Nilsson, C.B., Hoegberg, P., Trossvik, C., Azais-Braesco, V., Blaner, W.S., Fex, G., Harrison, E.H., Nau, H., Schmidt, C.K., Van Bennekum, A.M. and Håkansson, H. 2000. 2,3,7,8-tetrachrorodibenzo-p-dioxin increases serum and kidney retinoic acid levels and kidney retinol esterification in the rat. *Toxicol. Appl. Pharmacol.* 169(2):121-31.

O'Shea, T.J. and Aguilar, A. 2001. Cetacea and Sirenia. pp. 427-96. *In:* R.F. Shore and B.A. Rattner (eds.) *Ecotoxicology of Wild Mammals*. John Wiley and Sons Ltd, New York, NY. 730pp.

Peakall, D.B. 1992. *Animal Biomarkers as Pollution Indicators*. Chapman and Hall, London. 291pp.

Pelissier, M.A., Siess, M.H., Lhuissier, M., Grolier, P., Suschetet, M., Narbonnes, J.F., Albrecht, R. and Robertson, L.W. 1992. Effect of prototypic polychlorinated biphenyls on hepatic and renal vitamin contents and drug-metabolising enzymes in rats fed diets containing low or high levels of retinyl palmitate. *Chem. Res. Toxicol.* 30(8):723-9.

Perez, M.A. and Mooney, E.E. 1986. Increased food and energy consumption of lactating northern fur seals, *Callorhinus ursinus*. *Fish. Bull.* 84(2):371-81.

Raica, N., Scott, J., Lowry, L. and Sauberlich, H.E. 1972. Vitamin A concentration in human tissues collected from five areas in the United States. *Am. J. Clin. Nutr.* 25:291-6.

Raila, J., Bucholz, I., Auerle, H., Raila, G., Schoon, H.A. and Schwigert, F.J. 2000. The distribution of vitamin A and retinol-binding protein in the blood plasma, urine, liver and kidneys of carnivores. *Vet. Res.* 31:541-51.

Recchia, C.A. and Read, A.J. 1989. Stomach contents of harbour porpoises, *Phocoena phocoena* (L.), from the Bay of Fundy. *Can. J. Zool.* 67:2140-6.

Reijnders, P.J.H., Donovan, G.P., Aguilar, A. and BjØrge, A. 1999. Report of the Workshop on Chemical Pollution and Cetaceans, March 1995, Bergen, Norway. *J. Cetacean Res. Manage.* (special issue) 1:1-42.

Roberts, A.B., Nichols, M.D., Newton, D.L. and Sporn, M.B. 1979. In vitro metabolism of retinoic acid in hamster intestine and liver. *J. Biol. Chem.* 254:6296-302.

Roberts, E.S., Vaz, A.D.N. and Coon, M.J. 1992. Role of isozymes of rabbit microsomal cytochrome p450 in the metabolism of retinoic acid, retinol and retinal. *Mol. Pharmacol.* 41:427-33.

Rodahl, K. and Davies, A.W. 1949. Vitamin A in seals. *Biochemistry* 45:408-12.

Rodhouse, P.G., Arnbom, T.R., Fedak, M.A., Yeatman, J. and Murray, W.A. 1992. Cephalopod prey of the southern elephant seal, *Mirounga leonina* L. *Can. J. Zool.* 70:1007-15.

Rolland, R. 2000. A review of chemically-induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. *J. Wildl. Dis.* 36(4):615-35.

Rosas, F.C.W. and Lethi, K.K. 1996. Nutritional and mercury content of milk of the Amazon river dolphin, *Inia geoffrensis*. *Comp. Biochem. Physiol. A* 115(2):117-9.

Ross, P.S. and Troisi, G.M. 2001. Pinnipedia. pp. 371-426. *In:* R.F. Shore and B.A. Ratner (eds.) *Ecotoxicology of Wild Mammals*. John Wiley and Sons Ltd, New York, NY. 730pp.

Ryg, M., Lydersen, C., Markussen, N.H., Smith, T.G. and Oritsland, N.A. 1990. Estimating the blubber content of phocid seals. *Can. J. Fish. Aquat. Sci.* 47(6):1223-7.

Ryg, M., Lydersen, C., Knutsen, L.O., Bjorge, A., Smith, T.G. and Oritsland, N.A. 1993. Scaling of insulation in seals and whales. *J. Zool., Lond.* 230:193-206.

Savage, A., Leonnng, K.M., Grobler, D., Lehhnnhardt, J., Dierenfeld, E.S., Stevens, E.F. and Aebischer, C. 1999. Circulating levels of alpha-tocopherol and retinol in free-ranging African elephants (*Loxodonta africana*). *Zoo Biol.* 18:319-23.

Schmidt-Nielsen, S., Flood, A. and Stene, J. 1934. Ueber Grösse und Vitamingehalt der Leber verschiedener Tiere. *K. Nor. Vidensk. Selsk. For.* 7:81. [In Norwegian].

Schweigert, F.J. and Buchholz, Y. 1995. Vitamin A metabolism in carnivores with special reference to fur bearing animals. *Scientifur* 19(4):305-7.

Schweigert, F.J. and Stobo, W.T. 1994. Transfer of fat-soluble vitamins and PCBs from mother to pups in grey seals (*Halichoerus grypus*). *Comp. Biochem. Physiol.* 109:11-117.

Schweigert, F.J., Stobo, W.T. and Zucker, H. 1987. Vitamin A status in the grey seal (*Halichoerus grypus*) on Sable Island. *Int. J. Vitam. Nutr. Res.* 57:239-45.

Schweigert, F.J., Ryder, O.A., Rambeck, W.A. and Zucker, H. 1990. The majority of vitamin A is transported as retinyl esters in the blood of most carnivores. *Comp. Biochem. Physiol. B* 95A(4):573-8.

Schweigert, F.J., Thomann, E. and Zucker, H. 1991a. Vitamin A in the urine of carnivores. *Int. J. Vitam. Nutr. Res.* 61:110-3.

Schweigert, F.J., Uehlein-Harrel, S., Hegel, G.V. and Wiesner, H. 1991b. Vitamin A (retinol and retinyl esters), alpha-tocopherol and lipid levels in plasma of captive wild mammals and birds. *J. Vet. Med. Sci.* 38:35-42.

Schweigert, F.J., Bucholz, I. and Bonitz, K. 1998. Effect of age on the levels of retinol and retinyl esters in blood plasma, liver and kidney of dogs. *Int. J. Vitam. Nutr. Res.* 68:237-41.

Scrofano, M.M., Jhngen-Hodge, J., Nowell, T.R., Jr., Gong, X., Smith, D.E., Perrone, G., Asmmundsson, G., Dallal, G., Gindleesky, B., Mura, C.V. and Taylor, A. 1998. The effects of ageing and calorie restriction on plasma nutrient levels in male and female Emory mice. *Mech. Ageing Develop.* 105:31-44.

Seaman, G.A., Lowry, L.F. and Frost, K.J. 1982. Foods of belukha whales *Delphinapterus leucas* in Western Alaska. *Cetology* 44:1-19.

Shrestha, S.P., Ullrey, D.E., Bernard, J.B., Wemmer, C. and Kraemer, D.C. 1998. Plasma vitamin E and other analyte levels in Nepalese camp elephants (*Elephas maximus*). *J. Zoo Wildl. Med.* 29(3):269-78.

Simms, W. and Ross, P.S. 2000. Developmental changes in circulatory vitamin A (retinol) and its transport proteins in free-ranging harbour seal (*Phoca vitulina*) pups. *Can. J. Zool.* 78:1862-8.

Simms, W., Jeffries, S.J., Ikonomou, M.G. and Ross, P.S. 2000. Contaminant-related disruption of vitamin A dynamics in free-ranging harbor seal (*Phoca vitulina*) pups from British Columbia, Canada and Washington State, USA. *Environ. Toxicol. Chem.* 19:2844-9.

Simpson, V.R., Bain, M.S., Brown, R., Brown, B.F. and Lacey, R.F. 2000. A long-term study of vitamin A and polychlorinated hydrocarbon levels in otters (*Lutra lutra*) in south west England. *Environ. Pollut.* 110:267-75.

Skaare, J.U., Bernhoft, A., Wiig, O., Norum, K.R., Haug, E., Eide, D.M. and Derocher, A.E. 2001. Relationships between plasma levels or organochlorines, retinol and thyroid hormones from polar bears (*Ursus maritimus*) at Svalbard. *J. Toxicol. Environ. Health* 62(4):227-41.

Smith, R.J. and Read, A.J. 1992. Consumption of euphausiids by harbour porpoise (*Phocoena phocoena*) calves in the Bay of Fundy. *Can. J. Zool.* 70(8):1629-32.

Sommer, A. and West, K.P., Jr. 1996. *Vitamin A Deficiency: Health, Survivial and Vision*. Oxford University Press Inc, New York, NY. 242pp.

Soprano, D.R. and Blaner, W.S. 1994. Plasma retinol binding protein. pp. 257-81. *In:* M.B. Sporn, A.B. Roberts and D.S. Goodman (eds.) *The Retinoids: Biology, Chemistry and Medicine*. Raven Press, New York. 679pp.

Southcott, R.V., Chesterfield, N.J. and Warneke, R.M. 1974. The vitamin A content of the liver of the Australian fur seal, *Arctocephalus pusillus doriferus*. *Aust. Wildl. Res.* 1:145-9.

Stephenson, C.B. and Gildengorin, G. 2000. Serum retinol, the acute phase reponse, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. *Am. J. Clin. Nutr.* 72(5):1170-8.

Stewart, R.E.A. and Murie, D.J. 1986. Food habits of lactating harp seals (*Phoca groenlandica*) in the Gulf of St. Lawrence in March. *J. Mammal.* 67(1):186-8.

Succari, M., Garric, B., Ponteziere, C., Miocque, M. and Cals, M.J. 1991. Influence of sex and age on vitamin A and E status. *Age and Ageing* 20:413-6.

Tawara, T. and Fukazawa, R. 1950a. Studies in kitol. II. Influence of kitol fraction on the determination of the International Unit of Vitamin A. *Sci. Rep. Whales Res. Inst., Tokyo* 3:89-91.

Tawara, T. and Fukazawa, R. 1950b. Studies on kitol. I. Preparation of kitol from whale liver oil. *Sci. Rep. Whales Res. Inst., Tokyo* 3:85-8.

Thompson, J.N. 1976. Fat-soluble vitamins. *Comp. Anim. Nutr.* 1:99-135.

Underwood, B.A. 1984. Vitamin A in animal and human nutrition. pp. 282-392. *In:* M.B. Sporn, A.B. Roberts and D.S. Goodman (eds.) Vol. 2. *The Retinoids*. Academic Press, Orlando, FL.

Van Birgelen, A.P.J.M., Van Der Kolk, J., Fase, K.M., Bol, I., Poiger, H., Brouwer, A. and Van Den Berg, M. 1994a. Toxic potency of 3,3',4,4',5-pentachlorobiphenyl relative to and in combination with 2,3,7,8-tetrachlordibenzo-p-dioxin in a subchronic feeding study in the rat. *Toxicol. Appl. Pharmacol.* 127:209-21.

Van Birgelen, A.P.J.M., Van Der Kolk, J., Fase, K.M., Bol, I., Poiger, H., Van Den Berg, M. and Brouwer, A. 1994b. Toxic potency of 2,3,3',4,4',5-hexachlorobiphenyl relative to and in combination with 2,3,7,8-tetrachlorodibenzo-p-dioxin in a subchronic feeding study in the rat. *Toxicol. Appl. Pharmacol.* 126:202-13.

Wetlesen, C.U. 1938. Whale liver. *Norsk Hvalfangsttid.* 27:262-4.

Wolf, G. 1984. Multiple functions of vitamin A. *Physiol. Rev.* 64:873-973.

Zile, M.H. 1992. Vitamin A homeostasis endangered by environmental pollutants. *Proceed. Soc. Exper. Biol. Med.* 201:141-53.