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Organochlorines in harbour porpoise (Phocoena phocoena) from Scandinavian waters

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ABSTRACT

This paper presents information on the organochlorine (OC) levels in harbour porpoises (Phocoena phocoena) from Norwegian coastal and adjacent waters. Blubber samples of 34 male harbour porpoises were analysed and the presence of PCBs, DDTs, chlordanes, cyclodienes, HCHs and HCB were examined. Concentrations of ΣPCB (47 congeners) and ΣDDT were raging from 3.7 - 65.3 and 3.2 - 45.0 μg g-1 lipid weight, respectively. The range of median concentrations of dieldrin, endrin and trans-nonachlor was 1-3 μg g-1 lipid weight, while median concentrations of ΣHCH, heptachlor epoxid and HCB were < 1.5 μg g-1 lipid weight. A significant OC accumulation with age was apparent, with the exception of HCB and HCHs.

A geographical difference in the level of OCs was apparent for all compounds except for dieldrin and heptachlor epoxide. Significantly higher levels of chlordane metabolites (trans-nonachlor and oxychlordane) and endrin were found in the group of animals from the northern location Tufjord, while ΣDDT, ΣHCH and ΣPCB were highest in the group of animals from the southern location Gilleleje. These findings may indicate little or no regular migration of harbour porpoise between these three locations. No correlations was found between OC concentrations and blubber thickness.

Although mean values of ΣDDT and ΣPCB were in the lower end of previously reported levels in harbour porpoise from adjacent waters in the eastern part of the North Atlantic Ocean, these levels are relatively high.

The organochlorine concentrations in harbour porpoises in the present study were 2-3 times higher than corresponding OC levels detected in harbour seals (Phoca vitulina) from the same areas.

INTRODUCTION

The lipophilic nature and persistence of the organochlorines contribute to their high bioaccumulating potential and their biomagnification in nature (Tanabe *et al.*, 1983; Muir *et al.*, 1988, Reijnders 1988), resultant that top predator species of marine food webs are especially exposed to high organochlorine contamination (Reijnders, 1980, 1986; Skaare *et al.*, 1990; Tatsukawa, 1992; Muir *et al.* 1992; Norheim *et al.*, 1992; Wang-Andersen *et al.*, 1993). The harbour porpoise is a top predator inhabiting coastal waters of Europe, and is therefor subject to an accumulation of these persistent environmental pollutants.

Ottar *et al.* (1981) suggested that the main source of OC contamination in Norway is atmospheric fallout. Generally, in winter, air currents arrive from the southwest along the Norwegian coast. During summer in near coastal areas of the Barents Sea, north-easterly to easterly air currents are more common (Anon, 1989), and may contribute to seasonal transport of airborn pollution from Eurasia. High levels of PCB are found in the Baltic fauna, and particularly in Baltic seal populations (Helle *et al.*, 1976A; Reutergårdh, 1988; Blomkvist *et al.*, 1992). However, no information on the transport of organochlorines in the Baltic current is available, and one can only speculate on the degree of input of these chemicals into Kattegat, Skagerak and the coastal waters of Norway.

The main objective of the present study was to examine the levels PCB and certain organochlorine pesticides in harbour porpoise from Scandinavian waters. Corresponding tissue samples from northeastern Norwegian Sea (Tufjord), northern North Sea (Vestlandet) and Kattegat (Gilleleje) were compared. To reveal eventually seasonal and bioaccumulating effects, an effort was made to assess the influence of physical parameters like blubber thickness and age on OC levels

MATERIALS AND METHODS

During Norwegian and Danish harbour porpoise projects 1987-1991, subcutaneous blubber samples of 34 male harbour porpoises were collected. Animals from three geographical locations, ranging from 56°N, 12°E to 71°N, 26°E, were chosen in order to study the organochlorine (OC) contamination in this species inhabiting the northeastern part of the North Atlantic Ocean, the northern North Sea and Kattegat, at

the locations of Tufjord (71°N, n=7, Norway), Vestlandet (58-62°N, n=15, Norway) and Gilleleje (56°N, n=12, Danmark), respectively (Fig. 1). Porpoises from the southern location Gilleleje were caught in March and April, approximately two month earlier than animals from Vestlandet and Tufjord. Subcutaneous blubber samples were taken in front of the dorsal fin and stored at -18°C or colder until analyses. The analyses were carried out at the Institute for Forestry and Nature Research, Arnhem, Holland.

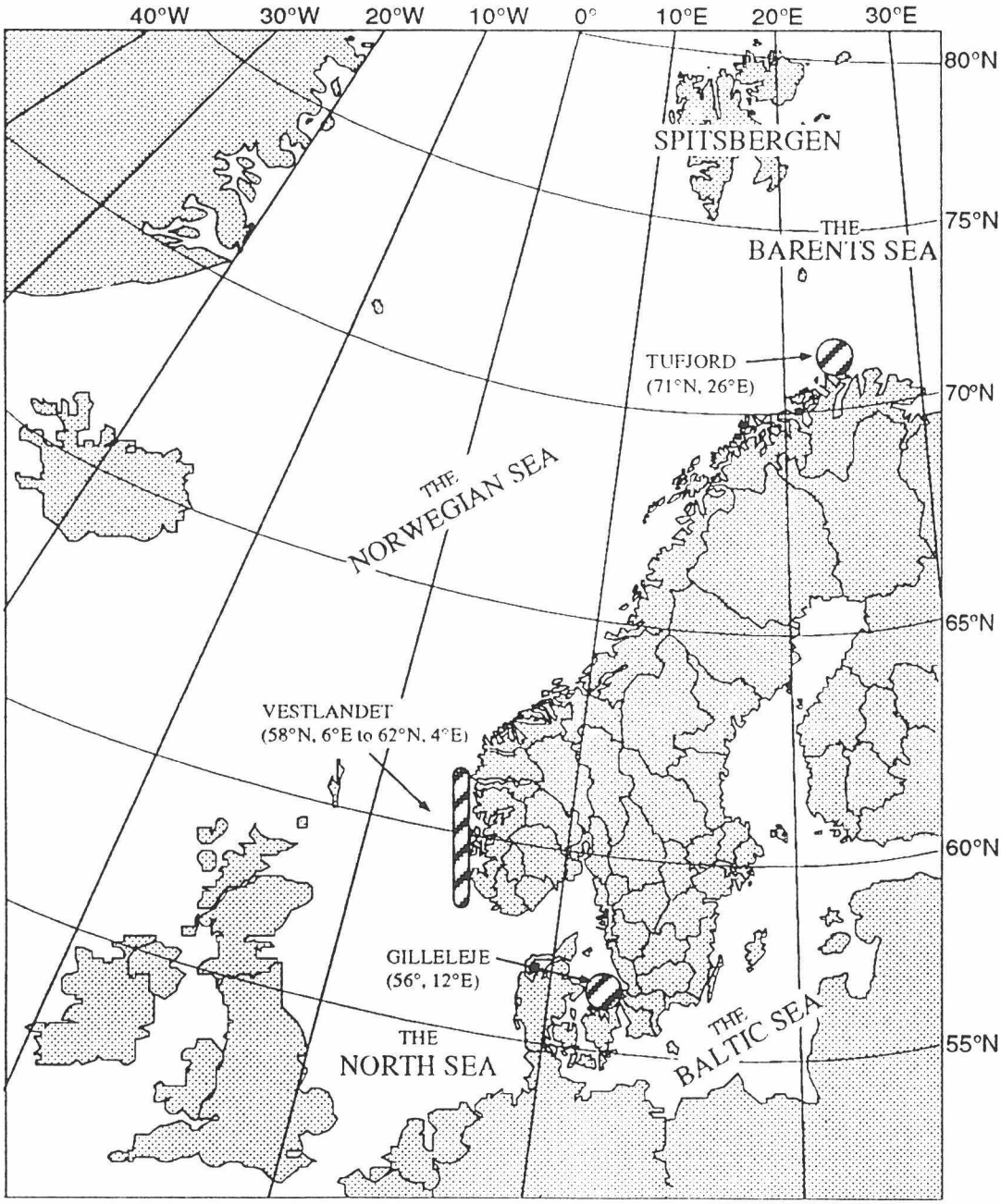

Figure 1. Harbour porpoises from Danish (gilleleje) and Norwegian (Vestlandet and Tufjord) waters collected for organochlorine analyses.

Chemical analyses

Clean-up and separation steps were combined in a single aluminium oxide/silica gel column (Holden & Marsden, 1969), and separation of PCBs (first hexane fraction) and organochlorine pesticide residues (second dichlormethane/ hexane fraction) were obtained using a silica gel column eluted with solvents of variable polarity (Kadoum, 1968).

The first hexane eluate (PCBs) was injected into a gas chromatograph (GC-ECD: Carlo Erba, HRGC 5140) equipped with ^{63}Ni electron capture detectors and an on column injection system. Temperature program; heating rates were 30, 10 and 5°C/min from the injection temperature at 60°C to 140°C, from 140°C to 200°C and from 200°C to the end temperature at 280°C, respectively. Intervals of 5 and 17 min were set at 200 and 280°C, respectively. The second eluate (OC pesticides) was injected into a gas chromatograph (GC-ECD: Packard model 438) equipped with ^{63}Ni electron capture detectors and a splitter injection system (splitting ratio 1:30). The temperature program; heating rates were 5 and 20°C/min from the injection temperature at 180°C to 200°C and from 200°C to the end temperature at 240°C, respectively. Intervals of 5 and 6 min were set at 180 and 240°C, respectively. Both columns were J&W DB-5 30 m x 0.25 mm fused silica capillary columns, with a 0.25 micron filmlayer. Helium was used as carrier gas, while purified nitrogen was used as makeup gas in the detectors (rate of 30 ml/min). Chromatograms were written on line (Kipp & Zonen/BD 9) and data were recorded on a spectra physics SP 4100 computing integrator and on a digital professional 360 PC. In addition to the OC pesticides (ΣPEST; DDTs (o,p'- and p,p'-DDT, p,p'-DDE, o,p'- and p,p'-DDD), HCHs (γ-HCH, α-HCH, ß-HCH), endrin, dieldrin, oxychlordane, trans-nonachlor, heptachlor epoxide and HCB), the standards included 22 PCB congeners (52, 61, 66, 101, 136, 82, 151, 153, 105, 141, 138, 129, 183, 202, 180, 170, 194, 206, 209) and 6 technical mixtures from which all the PCB congener standards (104 PCBs) were calculated. The industrial mixtures where analysed by means of Microwave Plasma Detector which give quantitative results for the amounts of Carbon, Hydrogen and Chlorine. The ΣPCB include 47 congeners or groups of congeners; PCB no. 26, 52, 47/48, 63/58, 95, 91, 60/65, 101/90, 99/113, 87, 11, 148, 110, 151, 107/108, 147/123, 118, 114, 146, 153, 132/105, 141, 179, 130, 137/176, 138/163/164, 158, 178, 182/187, 183, 128, 167, 185, 174, 177, 202, 200/157, 192/172, 180, 193, 170, 201, 196/203, 208/195, 194, 206 and 209 (IUPAC numbering system, Ballschmiter & Zell, 1980).

Analyses were carried out in duplicate, and if the difference between duplicates was more than 10%, the sample was reanalysed. Through the hole analytical procedure, for each 10 samples a PCB mixture was analysed to determine the recovery. The total mean of recovery was 106 ± 5%.

Age structure

All age groups were represented, but with plain dissimilar age distribution at the three locations (Table 1). An influence on OC concentrations during maturation, and an important OC-transfer during lactation have been reported (Gaskin *et al.*, 1983; Duinker

& Hillebrand, 1979). For these reasons age-groups were assembled into three age-classes, pups, immatures and adults (age-group 0, age-groups 1 and 2, and age-group >2, respectively). The majority of specimens in age-group 2 from Vestlandet and Tufjord were immatures (Bjørge *et al.*, 1991). Animals older than 8 years were pooled in an age-group 8^{+}, due to difficulties in reading dental growth layer groups in old specimens.

Statistical analyses

Simple linear regression analyses and multiple analyses of variance (MANOVA) were applied to test any relationship between the OC levels in harbour porpoise and their homerange (Zar, 1984). The relationships between OC levels and blubber thickness, as well as age related aspects, were examined by oneway-analyses of variance (ANOVA), where the Tukey test in a multiple range analyses with the level of compound by age-classes was used to separate groups (Zar, 1984).

RESULTS

Table 1 and 2 presents median levels and ranges of the OC residues, where ΣPCB and ΣDDT (ΣDDT = DDT substitutes + 1·1 (DDE + DDD substitutes)) were the major contaminants with maximum concentrations of 65 and 45 $\mu g\ g^{-1}$ lipid weight, respectively.

Nutritional status aspects

Mean blubber thickness of dorsal, lateral and ventral measures in front of the dorsal fin, varied from 1.3 to 3.3 cm. The group of animals caught at Gilleleje had a significantly thicker ($p<0{\cdot}05$) blubber layer (2.8 cm) compared to animals from Vestlandet and Tufjord (1.9 cm). When including all animals, however, no significant relationship was found between the levels of OCs and blubber thickness. The mean value of extractable fat in blubber was 92%.

Age-related aspects

When all animals were considered, no significant inter-relationship between levels of ΣHCH or HCB and age was found (Fig. 2). Opposite to these findings, a significant age-dependent accumulation was found for ΣPEST, ΣDDT and ΣPCB ($p<0{\cdot}01$) (Fig. 3). A significant increase with age was also found in the levels of the cyclodienes dieldrin and endrin, and heptachlor epoxide, trans-nonachlor and oxychlordane ($p<0{\cdot}01$, for all).

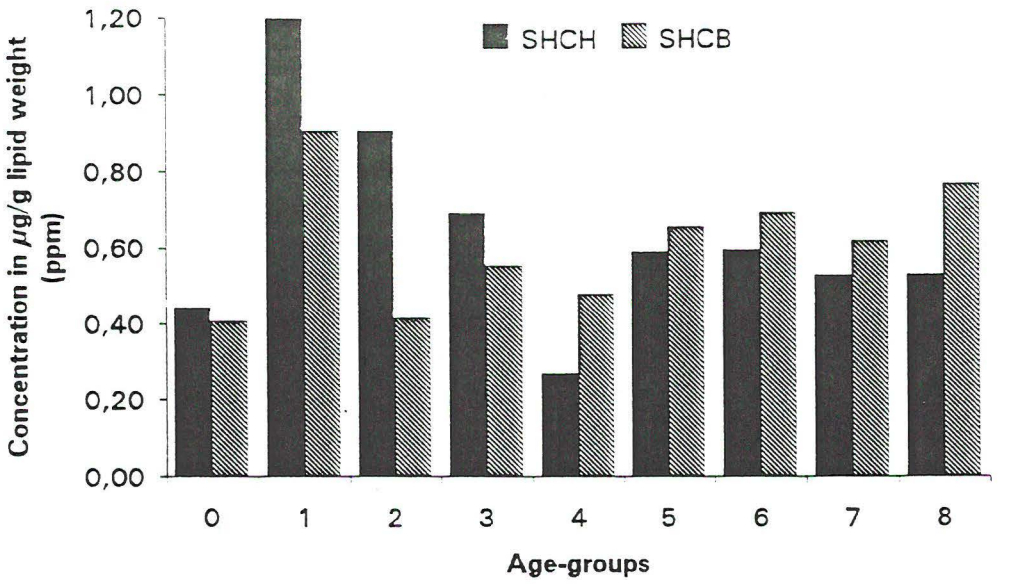

Figure 2. Mean concentration of Sum HCH and Sum HCB in harbour porpoises belonging to different age-groups; Scandinavian waters.

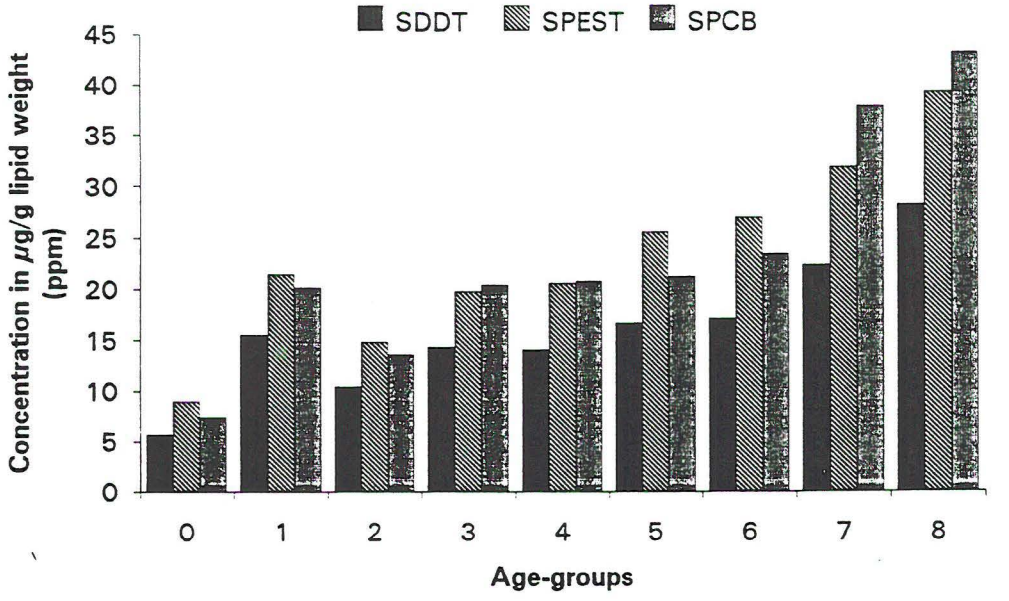

Figure 3. Mean concentration of Sum PCB, Sum DDT and Sum PEST in harbour porpoises belonging to different age-groups; Scandinavian waters.

Geographical aspects

Effects of dissimilar age distributions at the three locations were reduced by using age as a covariate in the MANOVA analyses.

Significant geographical differences ($p < 0.05$) in levels were found for the cyclodienes, endrin, trans-nonachlor and oxychlordane, with the highest and lowest levels of these OCs in the groups of porpoises caught at the northern location, Tufjord, and at Gilleleje, respectively (Table 2). This is also demonstrated in figure 4, with a higher ratio of ΣPEST/ΣPCB at the northern location. Levels of dieldrin and heptachlor epoxide revealed no geographical differences. Although not statistically significant, slightly elevated levels of HCB were found in animals from Tufjord, compared with the two other locations.
Significantly higher ($p < 0.05$) levels of ΣPCB and ΣHCH were found in the group of porpoises caught at the southern location, Gilleleje, compared to levels detected at Vestlandet and Tufjord. Although not significant, a decreasing trend was also apparent from the south to the north with regard to ΣDDT.
Table 1 presents the ratios of ΣDDT/ΣPCB and DDE/ΣDDT. The percentage of DDE to ΣDDT at Gilleleje, Vestlandet and Tufjord were 49, 41 and 43, respectively.

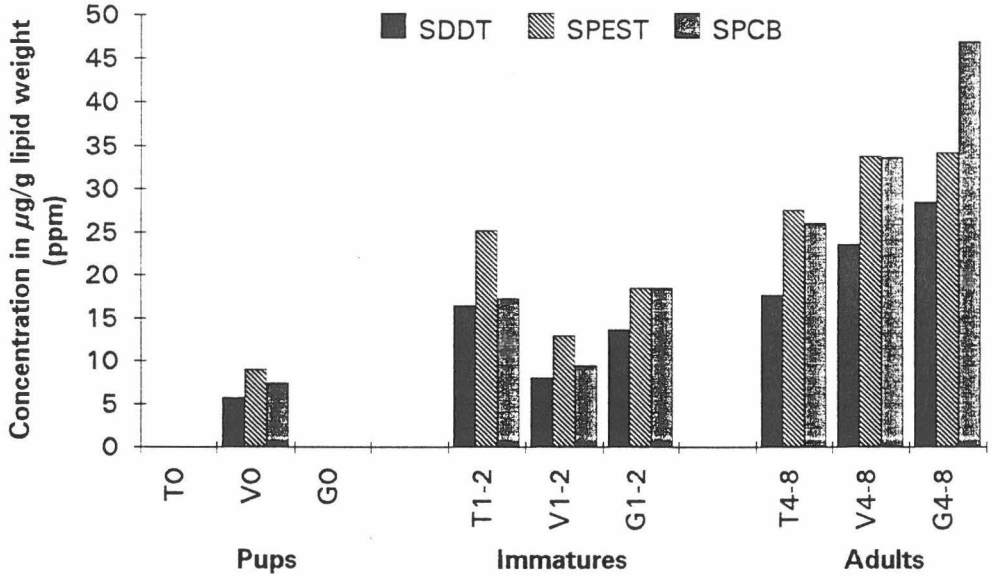

Figure 4. Mean concentrations of Sum DDT, Sum PEST and Sum PCB in harbour porpoises from different locations. The T, V and G are abbr. for Tufjord, Vestlandet and Gilleleje, respectively. Age-group 3 is omitted due to high varians

Table 1. Concentration (mean/median (range), μg g^{-1} lipid) of ΣDDT and ΣPCB, and ratios of ΣDDT/DDE and ΣDDT/ΣPCB in harbour porpoises belonging to different age-groups; Scandinavian waters.

		ΣDDT		ΣPCB*		ΣDDT/DDE		ΣDDT/ΣPCB	
Location	Age/n	Mean/Median	(Range)	Mean/Median	(Range	Mean/Median	(Range)	Mean/Median	(Range
Gilleleje	1/5	16.85/10.93	(7.49 - 45.09)	22.04/14.79	(10.20 - 50.80)	2.28/2.00	(1.80 - 2.42)	0.78/0.73	(0.59 - 0.89)
	2/4	9.73/10.06	(7.27 - 11.52)	13.99/13.65	(12.66 - 16.03)	2.09/1.94	(1.74 - 2.25)	0.71/0.69	(0.57 - 0.81)
	3/1	12.78		19.09		1.98		0.67	
	4/1	17.77		28.40		2.31		0.63	
	8+/1	39.17		65.26		2.18		0.60	
	Total 12					2.19/2.05	(1.74 - 2.43)	0.76/0.66	(0.57 - 0.89)
Vestlandet	0/3	5.70/3.70	(3.22 - 10.18)	7.41/5.66	(3.71 - 12.86)	2.04/2.33	(2.06 - 2.60)	0.73/0.79	(0.65 - 0.87)
	1/1	8.92		10.59		2.42		0.84	
	2/1	7.05		8.20		2.20		0.86	
	3/4	14.74/9.75	(6.16 - 25.35)	20.69/16.27	(8.32 - 41.88)	2.38/2.55	(2.48 - 2.76)	0.68/0.78	(0.61 - 0.88)
	4/2	17.50	(12.58 - 22.02)	18.84	(14.59 - 23.07)	2.60	(2.58 - 2.63)	0.91	(0.86 - 0.95)
	5/1	14.40		17.73		2.18		0.81	
	7/1	22.35		37.79		2.29		0.59	
	8+/2	32.30	(28.52 - 38.07)	47.68	(41.16 - 54.20)	2.52	(2.14 - 2.91)	0.73	(0.53 - 0.92)
	Total 15					2.31/2.48	(2.06 - 2.91)	0.74/0.81	(0.53 - 0.95)
Tufjord	2/1	16.47		17.25		2.60		0.95	
	5/1	13.59		22.73		2.17		0.60	
	6/2	17.07	(12.04 - 22.14)	23.89	(19.39 - 27.39)	2.31	(2.15 - 2.47)	0.72	(0.62 - 0.81)
	8+/3	21.25/14.63	(12.94 - 36.18)	31.17/27.23	(22.25 - 44.02)	2.28	(2.18 - 2.53)	0.58	(0.54 - 0.82)
	Total 7					2.32/2.28	(2.15 - 2.60)	0.65/0.62	(0.54 - 0.95)

* significant higher levels at the southern location ($p<0.05$)

Table 2. Concentration (mean/median (range), μg g^{-1} lipid) of cyclodienes, ΣHCH and HCB in blubber of harbour porpoises belonging to different age-groups; Scandinavian waters.

Location	Age/n	Heptachlor Epoxide	Oxychlordane*	Trans-nonachlor*	Dieldrin	Endrin*	ΣHCH**	HCB
		Mean/Median (Range)	Mean/Median (Range)	Mean/Median (Range)	Mean/Median (Range)	Mean/Median (Range)	Mean/Median (Range)	Mean/Median (range)
Gilleleje	1/5	0.48/0.26 (0.21-1.39)	0.24/0.15 (0.07-0.65)	0.70/0.52 (0.32-1.67)	3.40/1.86 (1.41-10.38)	1.00/0.59 (0.38-2.96)	1.36/1.09 (0.90-2.5)	0.82/0.41 (0.29-2.59)
	2/4	0.24/0.24 (0.13-0.33)	0.13/0.13 (0.10-0.17)	0.42/0.40 (0.34-0.55)	1.60/1.62 (1.20- 1.94)	0.42/0.45 (0.28-0.49)	1.14/1.13 (0.91-1.4)	0.36/0.35 (0.33-0.39)
	3/1	0.31	0.15	0.42	2.07	0.53	1.23	0.48
	4/1	0.48	0.23	0.82	3.24	0.99	1.23	0.77
	8+/1	0.53	0.36	1.30	3.78	1.42	0.80	0.51
Vestlandet	0/3	0.21/0.16 (0.07-0.41)	0.18/0.10 (0.09-0.35)	0.65/0.33 (0.30-1.32)	1.51/1.24 (0.52-2.77)	0.67/0.36 (0.34-1.29)	0.44/0.48 (0.32-0.53)	0.41/0.27 (0.19-0.76)
	1/1	0.23	0.25	1.39	1.67	1.91	0.29	0.81
	2/1	0.24	0.20	1.25	1.75	1.30	0.34	0.41
	3/4	0.32/0.31 (0.27-0.39)	0.32/0.31 (0.26-0.41)	1.70/1.36 (1.34-2.34)	2.32/2.19 (1.52-3.35)	1.52/1.52 (1.13-1.89)	0.42/0.43 (0.29-0.54)	0.52/0.49 (0.31-0.80)
	4/2	0.46 (0.28-0.65)	0.35 (0.27-0.44)	2.05 (1.61-2.50)	3.24 (2.13-4.36)	2.29 (1.71-2.88)	0.62 (0.27-0.70)	0.60 (0.48-0.73)
	5/1	0.44	0.45	1.85	3.50	1.42	0.66	0.55
	7/1	0.56	0.59	2.81	4.17	2.18	0.53	0.62
	8+/2	0.77 (0.26-1.28)	0.63 (0.50-0.76)	3.80 (3.09-4.51)	4.21 (1.87-6.54)	3.28 (1.96-4.61)	0.56 (0.52-0.62)	0.54 (0.32-0.77)
Tufjord	2/1	0.54	0.47	2.49	3.20	2.52	0.54	0.65
	5/1	0.49	0.47	2.43	3.42	2.61	0.52	0.70
	6/2	0.43 (0.39-0.48)	0.55 (0.39-0.73)	2.95 (1.50-4.41)	3.28 (2.57-3.80)	3.16 (2.01-4.31)	0.60 (0.57-0.63)	0.69 (0.58-0.81)
	8+/3	0.52/0.54 (0.39-0.63)	0.55/0.53 (0.50-0.62)	3.33/2.64 (2.33-5.01)	3.71/3.41 (3.11-4.62)	3.78/2.36 (2.30-6.68)	0.41/0.39 (0.25-0.58)	1.07/1.24 (0.72-1.25)

* significant higher levels at the northern location ($p<0.05$)
** significant higher levels at the southern location ($p<0.05$)

DISCUSSION

The present study reveals considerable levels of a number of organochlorines in the blubber of male harbour porpoises inhabiting Kattegat, northern North Sea and the north-eastern Norwegian Sea.

Organochlorines and nutritional status

Seasonal changes, the sex and the age are all parameters that might influence on the blubber thickness of an animal (Møhl-Hansen, 1954). Differences in blubber thickness between porpoises caught in Kattegat (Gilleleje) and along the Norwegian coast (Vestlandet and Tufjord) may be partly explained by differences in the time of year of catching. Availability of food and ambient water temperature at different locations are also factors that may affect the blubber thickness. Van der Zande and de Ruiter (1983), and Drescher *et al.* (1977) found a negative correlation between blubber thickness and the PCB concentration in blubber of harbour seals. No such correlation was found in the present study. This may either demonstrate a metabolic difference between these species, and/or indicate that the exposure of PCBs is even higher at the southern location Gilleleje, but that the pollutants are diluted in a thicker blubber layer.

Age-related aspects

Different lipid solubilities may to some extent explain the differences between OCs with regard to accumulation, distribution and elimination (Tanabe *et al.*, 1982; Aguilar, 1985). The absence of accumulation of HCH isomers and HCB with age may indicate that these compounds are easier metabolized and/or more water soluble so that a distribution equilibrium has been reached. Gaskin *et al.* (1983) revealed sex-related differences associated with OC-accumulation in harbour porpoise, and found no correlation between OC levels (blubber)and age in mature females. This indicates an important OC transfer from mother to offspring during the 6-8 month lactation period (Duinker & Hillebrand, 1979; Gaskin *et al.*, 1984). Due to possible OC-effects on maturation (Gaskin *et al.*, 1983), it was essential to separate younger animals in the present study. Age-group profiles of ΣHCH and HCB levels reveal a relatively high transfer from mother to pup, with a peak of ΣHCH in pups at the end of the lactation period (Fig. 2). Levels of other OCs (PCBs, DDTs and the cyclodienes) were significantly higher in older age-groups, thus revealing an age-dependent accumulation. Differences in food preferences with age may also play a role. These results are comparable with corresponding findings of Tanabe *et al.* (1981) on striped dolphin (*Stenella coeruleoalba*), and indicate a relatively low metabolic capacity of small cetaceans with regard to these compounds. However this can not be solely attributed to a relatively low metabolic capacity, since it has been demonstrated by Reijnders (1994) that harbour porpoises exhibit phenobarbital-type (PB-type) of P450-enzyme activity.
Specific bioaccumulation of PCBs, DDTs, HCHs and HCB between pups and older harbour porpoises indicate different kinetics for these pollutants.

Geographical aspects

Aarefjord (1993) found overall a higher degree of benthic prey species in porpoises from the southern location (Gilleleje) as compared to the northern location (Tufjord). However, irrespective a difference in prey species composition, there is likely a difference in pollution burden at the three locations. Resuspension and run-off from old deposits of PCBs and DDTs are likely higher in locations located closer to industrialized areas. Furthermore, sparse information on the use of these chemicals is available from eastern countries bordering the Baltic Sea. Present use cannot be disregarded. These factors may explain the higher concentrations of the DDTs and the PCBs in the group of animals caught at Gilleleje. Elevated concentrations of the ΣHCH at the southern location (Gilleleje) indicate a current utilization of this pesticide in countries surrounding these water masses. The ratio ΣPEST/ΣDDT was higest at the nortern location (Fig.4).In the Scandinavian countries, the pesticide chlordane was only used to a limited extent up to the 1960-ies (Norheim *et al.*, 1992). However, little is known about uses of chlordane and other pesticides in eastern countries, and unknown applications may explain the higher concentrations of ΣPEST in porpoises from Tufjord.

Aguilar and Borrell (1990) reported a ΣDDT/ΣPCB ratio characteristic of the eastern North Atlantic harbour porpoise in the period 1979-1988 to 0·54. This is lower than the mean ΣDDT/ΣPCB ratios calculated from Gilleleje, Vestlandet and Tufjord (0·64, 0·78 and 0·70, respectively). The mean ΣDDT/ΣPCB ratio in harbour porpoise caught in Danish waters in 1980/81 (Clausen & Andersen, 1988) was half the value calculated in animals caught at Gilleleje. This indicates a relative decrease of PCBs compared to DDTs in Danish waters in the period from 1980/81 to 1987/88. This corresponds with a recent study of Granby and Kinze (1991), indicating decreasing OCs levels in Danish waters. The relatively high ratio of DDE/ΣDDT shown in this study, indicate an old-established DDT composition in these waters.
Migration of harbour porpoise in Norwegian waters is poorly known, but the results from the present study may indicate that there is no regular migration of this species between these locations.

The present levels of ΣDDT and ΣPCB at Gilleleje, Vestlandet and in Tufjord, were at the lower end of previously reported OC levels in harbour porpoise from adjacent waters in the eastern part of the North Atlantic Ocean and along the coasts of North America (Gaskin et al., 1983; Clausen & Andersen, 1988; Morris et al., 1989; Granby & Kinze, 1991). The levels of ΣDDT and ΣPCB were 2-3 times higher than corresponding values in harbour seal (Skaare *et al.*, 1990) from the same areas. This could be due to interspecies differences in the metabolism of these compounds. However, differences in food preferences may also play a role.

Allthough the evidences are not unambiguous, there are a number of reports on a decline in the stocks of harbour porpoises of the North Sea region (Anon, 1977; Kinze, 1986; Smeenk, 1987; Evens *et al.*, 1987; Berggren & Petterson, 1990). Convincing evidence on detrimental impact of chemical pollutants on cetaceans is still not available, it seems possible that these animals, and especially small cetaceans, are vulnerable to the chronic toxicity of persistent organochlorines. Thus, it cannot be excluded that present OC levels in harbour porpoise from Scandinavian waters may have contributed

to the reported decline of this species in adjacant waters of the southern location, Gilleleje.

Acknowledgement

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