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ORGANOCHLORINES AND METALS IN HARBOUR SEALS (DUTCH WADDEN SEA)

by

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MAXIMUM CONCENTRATIONS OF PCB AND MEMBERS OF THE DDT FAMILY IN LIVER, BRAINS, KIDNEY, SPLEEN AND HEART AND CU, PB, ZN AND CD IN BRAINS OF HARBOUR SEALS FOUND DEAD IN THE DUTCH WADDEN SEA ARE HIGHER THAN THOSE REPORTED FOR SPECIMENS FROM THE GERMAN WADDEN SEA, WHERE THE POPULATION IS STABLE IN CONTRAST TO THE STRONG REDUCTION OBSERVED FOR THE POPULATION IN THE FORMER PART. RESULTS ARE ALSO COMPARED WITH DATA FROM THE EASTCOAST OF ENGLAND.

INTRODUCTION

High concentrations of certain organochlorines and metals have been associated with reproductive failure and increased juvenile mortality in marine mammals (Le Boeuf and Bonnell, 1971; De Long et al., 1973; Helle et al., 1976a, b). In seals from the North Sea, high concentrations have been detected in specimens from the Dutch coast (Koeman and van Genderen, 1966; Koeman et al., 1972; Koeman et al., 1975), the east coast of Britain (Holden, 1972, 1975, 1978; Heppleston, 1973; Heppleston and French, 1973) and the German Wadden Sea (Drescher, Harms and Huschenbeth, 1977). The Dutch population of the harbour seal has been strongly declining during the last decades (Reijnders, 1976). It has been established (Reijnders, 1978) that pup production in this population is lower and initial juvenile mortality higher than in the more stable population in the eastern Wadden Sea, i.e. the German part. The present paper reports concentrations of organochlorines and some metals detected in tissue of stranded seals from the Dutch Wadden Sea, including data on blubber, kidney, heart, liver, spleen and brains.

MATERIALS AND METHODS

All data refer to specimens found dead. Data were also obtained by analysing metals in a fetus and placenta, produced by a harbour seal after having been transferred from the beach, where she was found in a poor condition, to one of the aquaria of Texel Museum for a recovery period. In cases where the condition of the carcasses allowed, samples were taken from various tissues. These were kept deepfrozen until analysis, within six months.

For organochlorine analysis, between 0.5 and 10 g tissue were ground with anhydrous sodium sulfate and extracted with acetone-hexane

(2 : 1) in a Soxhlet for 10 hours. The extracts were filtered through a Whatman 540 filter and concentrated in a Kuderna-Danish evaporator equipped with a three-ball Snyder column. Between 50 and 100 mg lipid material was cleaned-up and fractionated according to the method designed by Holden and Marsden (1969). However, basic alumina was used, and the alumina and silica microcolumns, glassware and chemicals were specially treated for improving blank chromatograms (Duinker and Hillebrand, 1978). Components in the two silica fractions were identified by electron capture gas chromatography on two 1.8 m packed columns: 1.5% SP 2250-1.95% SP 2401 on Supelcoport 100-200 at 215°C and 3% DEGS-1% H_3PO_4 on Chromosorb-W-AWDMCS at 200°C, with ^{63}Ni-ECD and Ar/CH_4 (90:10) as carrier gas.

p,p'-DDE, eluting together with a PCB peak, was quantified as the difference between the chromatograms of the PCB fraction before and after oxidation of p,p'-DDE with chromic acid. PCB was quantified by comparing total area of peaks in the sample and matching peaks of Phenoclor DP6 (Fig. 1). All peaks of the DP6 standard have a matching peak in the chromatogram of the sample extract, practically at the appropriate retention time. Various peaks in the standard are composed of several components, not necessarily in the same ratio as in the corresponding peak in the chromatogram of the sample. This results in shifts of retention times. Some of the lower chlorinated components, in particular, penta- and hexachloro-PCB are very weak in the samples. Thus, a relatively large uncertainty should be taken into account when comparing PCB concentrations reported by different authors selecting different peaks for quantitation, especially when the method used is not specified in any detail. The striking similarity that we have observed in the chromatograms of the first fraction from the various tissues of all specimens result in PCB concentrations that can be compared reliably.

Additionally, the components listed in Table I have been identified by GC/MS techniques in the multiple ion monitoring mode using Finnigan mass spectrometers 3200 and 4000 with INCOS data system. Tetra-, penta-, hexa-, hepta- and octachloro-PCB components have been distinguished. Several pronounced GLC peaks in the second fraction could not yet be identified. The possibility of heptachloroepoxid being one of these, as suggested by retention on the SP column, could

be eliminated on the basis of retention on the polar DEGS column, although its presence in tissue of a harbour porpoise from Dutch coastal waters has been reported (Kerkhoff and Boer, 1977). Peaks with the retention times of endrin and β-HCH are present in the chromatograms obtained by the use of both columns. Their presence could not be proved by GC/MS techniques.

For trace metal analysis, between 0.5 and 1.0 g tissue were heated in a teflon digestion bomb at 110°C in an oven for two hours with a mixture of 5 ml H_2SO_4 and 2 ml HNO_3. After cooling, solutions were diluted to 50 ml with quartz distilled water in a polypropylene flask. Cu, Cd, Pb and Cr were determined by flameless AAS; Zn, Fe and Mn by flame AAS with Deuterium background correction. Standard addition techniques were applied for all elements. No reliable data for Hg were obtained.

RESULTS

For all tissues, the chromatogram of the first fraction dominates over that of the second fraction. Similar to the observations made for the PCB fractions, the chromatograms of the second fractions of all tissues of all specimens are equal in the number and positions of peaks. Relatively large variations occur in the relative distribution of each compound over the various tissues within the seal body (Table I). In each tissue of each specimen analyzed, the highest concentrations (wet weight) are of PCB followed, although an order of magnitude (or more) smaller, by total p,p'-DDT, mirex, dieldrin, α-HCH and γ-HCH. In most cases, the highest concentration of each compound occurs in blubber.

The present range of concentrations of PCB, p,p'-DDT, p,p'-DDD, p,p'-DDE and dieldrin in blubber is very similar to that reported earlier for harbour seals from the Dutch Wadden Sea (Koeman and van Genderen, 1966; Koeman et al., 1972), the German Wadden Sea (Drescher et al., 1977), East Anglia (Heppleston, 1973) and grey seals from East England (Holden, 1975). Considerable higher concentrations of PCB in blubber were reported by Koeman et al. (1972) for adult harbour seals in the Dutch area, up to 2530 mg / kg. Both minimum and in particular maximum values of the range of concentrations of PCB, p,p'-DDE and total p,p'-DDT in liver, kidney and brains in the present results are

higher than the corresponding ones in the eastern Wadden Sea (Table 2). No concentration data in spleen and heart were reported for the latter area. Present p,p'-DDT concentrations in liver, spleen, kidney and heart are similar to those dected in specimens from East Anglia; present values in brains are considerably higher. Moreover, present maximum concentrations of PCB in liver, spleen, kidney and heart are higher than those in East Anglia-between two and fifteen times. No significant differences are present for dieldrin. It was possible to quantify mirex, but only in a limited number of samples. The concentrations are similar to those of dieldrin. Mirex was identified in seal blubber by ten Noever de Brauw et al. (1973): no concentrations have been reported however.

Drescher, Harms and Huschenbeth (1977) reported a higher average fraction of p,p'-DDE in total p,p'-DDT in blubber of sick (49%) and dead (51%) than in healthy (42%) animals and increasing values for p,p'-DDD within the series dead (14%), sick (16%) and healthy (24%) animals. Present values (Table III) for the Western Wadden Sea are higher for DDE (43-100%). The occurrence of both lower and higher values for DDD (3-18% in blubber and 22-33% in liver) must be partly attributed to the lower accuracy in the DDD data than in those for DDE.

Residue concentrations on an extractable fat basis vary among the tissues; these may thus depend on other mechanisms in addition to partition between aqueous and lipid phases. Alternatively, the lipids that determine solubility may differ between the various tissues. Thus, maximum concentration of PCB was found in heart lipids and of α-HCH and γ-HCH in brain lipids. Low values of ΣDDT and PCB on a fat basis in brain tissue with respect to blubber and other tissue were reported for sealions (Le Boeuf and Bonnell, 1971), grey seals (Holden, 1975) and harp seals (Frank et al., 1973). The latter authors reported ratios between 1:5 and 1:9. It was suggested that this may be due to a blood-brain barrier. The present ratios for brains and blubber are considerably larger. Values for PCB, ΣDDT and mirex are typically in the order 1:1, while α-HCH and γ-HCH values are well above this ratio. These data suggest the absence of a blood-brain barrier for the latter compounds or it may be less active at the concentration levels present in the harbour seals from the Dutch Wadden Sea. Obviously, more data are necessary for a reliable conclusion.

The presence of high concentrations already in young animals has also been observed in other areas, including the Eastern Wadden Sea and the East coast of England. The large similarity between the chromatograms of tissues from juveniles and adults suggests that mobilization of organochlorines from female tissue and transplacental transfer during parturition (as found for harbour porpoises, Duinker and Hillebrand, 1979) and transfer to pups during lactation (as established for grey seals, Addison and Brodie, 1977) may be important sources of organochlorines in juveniles of the harbour seal, in addition to food ingested.

The range of concentrations of Zn, Cu, Pb and Cd in liver and kidney is very similar to those in the eastern part (Table IV). Present maximum values in brains are higher for all metals, especially for Cd. Concentrations in the placenta and fetus were lower than or similar to those in tissues of juveniles and adults, except for Cu and Zn in liver. The relatively small differences in the concentration levels of metals in seals from different areas suggest that environmental variations in the concentration levels of these elements may only be partly reflected in tissue levels in seals, with a possible exception for Cd.

It has not yet been established which are the main factors that may be responsible for the strong reduction in the population of the harbour seal and the extreme reduction in the practically and completely vanished populations of the harbour porpoise (*Phocoena phocoena*) and the bottle-nosed dolphin (*Tursiops truncatus*) from Dutch coastal waters in the last decades (Reijnders, 1976; Verweij, 1975). It seems important that all specimens of these populations, once found dead, are analysed in detail. The present data may assist in future analysis of the problems associated with pollutants and seals, the essential aspects of which have been reviewed elegantly in a recent paper (Holden, 1978).

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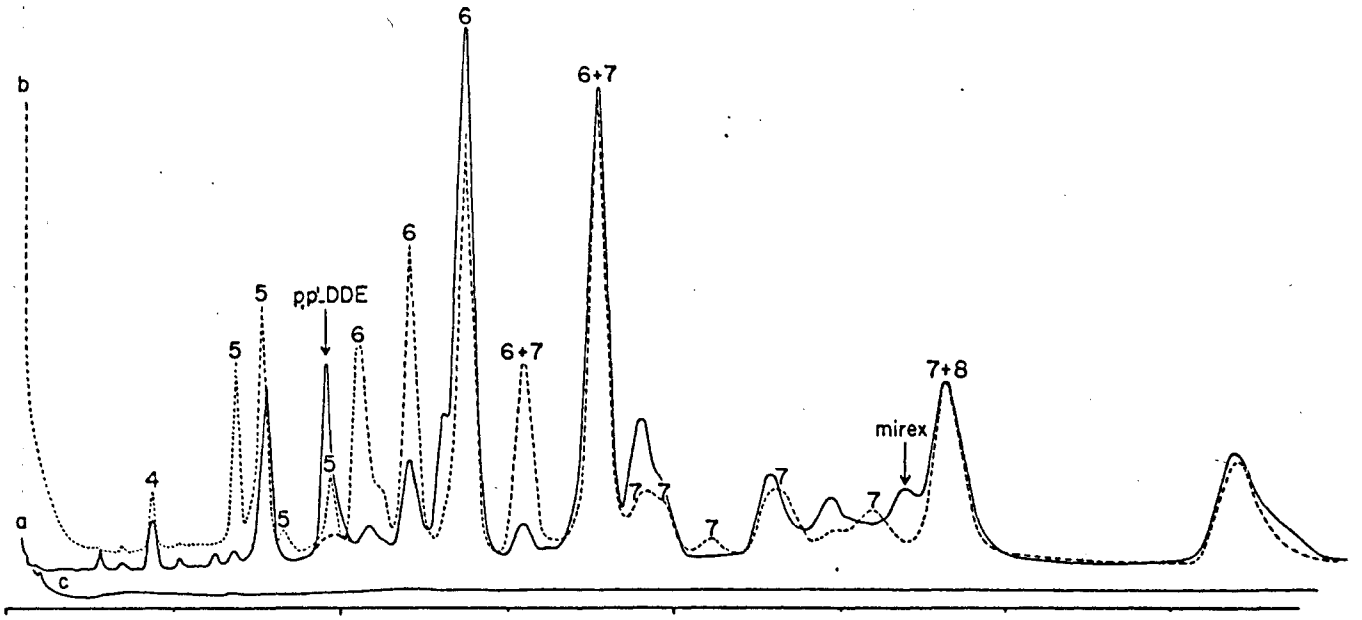
Fig. 1.

Chromatograms of seal blubber extract (a), Phenoclor DP 6 standard (b) and blank chromatogram for the complete procedure without sample however (c), involving cleanup over alumina and fractionation over silica, resulting in a hexane fraction (first fraction, top chromatogram), and a 10% diethylether in n-hexane fraction (second fraction, bottom). Oxidation of the first fraction with chromic acid results in a chromatogram that only differs due to the disappearance of p,p'-DDE (- - - in top chromatogram a). The numbers indicate the number of Cl atoms in the eluting PCB components. A=α-hexachlorocyclohexane, B=γ-hexachlorocyclohexane, C=β-hexachlorocyclohexane (not proved by MS), D=dieldrin, E=endrin (not proved by MS), F=p,p'-DDD, G=p,p'-DDT.



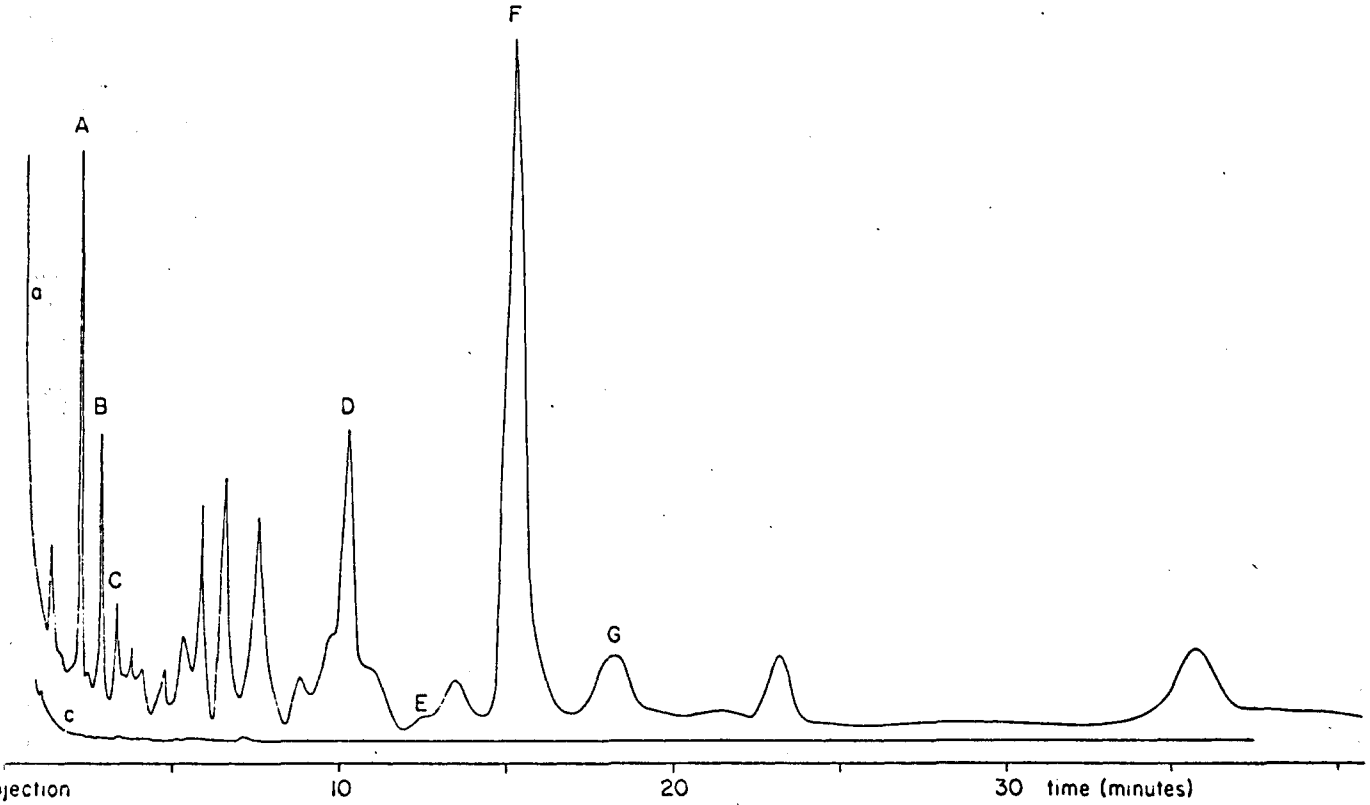


Table 1 .

Concentrations of organochlorines in seal tissue in mg/kg on an hexane extractable lipid basis (L) and on a wet weight basis (W).

Number, sex, age	Tissue	% HEL	PCB		α-HCH		γ-HCH		Dieldrin		o,p'-DDD		p,p'-DDD		p,p'-DDT		p,p'-DDE		Mirex	
			L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1. ♀, 23yrs	Liver	7.9	455	36	0.31	0.02	0.01	0.001	0.50	0.04	<0.02	<0.002	5.91	0.46	<0.1	<0.008	11.2	0.88	<0.1	<0.001
	Brains	14.2	66	9	1.10	0.16	<0.01	<0.001	<0.02	<0.003	<0.02	<0.003	1.12	0.20	<0.1	<0.010	3.4	0.48	<0.1	<0.01
2. ♀, 1yr	Blubber	86.6	58	50	0.27	0.23	0.03	0.03	0.30	0.26	<0.02	<0.02	0.53	0.46	2.9	2.5	3.9	3.4	<0.1	<0.1
	Liver	2.2	105	2.4	0.36	0.01	0.03	0.001	0.46	0.01	<0.02	<0.001	2.9	0.07	2.0	0.05	4.8	0.11	<0.1	<0.002
3. ♀, 1yr	Blubber	92	24	22	0.03	0.03	0.10	0.09	<0.02	<0.02	1.28	1.18	<0.05	<0.05	<0.1	<0.1	0.56	0.51	<0.1	<0.1
	Kidney	6.4	25	1.6	<0.01	<0.001	<0.01	<0.001	<0.02	<0.001	<0.02	<0.001	<0.05	<0.003	<0.1	<0.006	0.72	0.05	<0.1	<0.006
	Spleen	3.4	33	1.1	<0.01	<0.001	0.04	0.001	0.06	0.002	<0.02	<0.001	<0.05	<0.001	<0.1	<0.003	0.84	0.03	<0.1	<0.003
	Heart	5.1	40	2.1	0.08	0.004	0.12	0.006	0.23	0.012	<0.02	<0.001	0.99	0.051	1.6	0.08	2.35	0.12	<0.1	<0.005
	Liver	7.3	21	1.5	<0.01	<0.001	0.13	0.01	0.34	0.02	<0.02	<0.001	0.64	0.048	0.38	0.03	0.99	0.07	<0.1	<0.007
	Brains	12.8	11	1.4	0.88	0.11	1.04	0.13	0.03	0.004	<0.02	<0.003	<0.1	<0.01	<0.1	<0.01	0.46	0.06	<0.1	<0.01
4. ♀, 1yr	Blubber	77.3	290	223	0.27	0.20	0.06	0.05	<0.02	<0.02	<0.02	<0.02	0.44	0.34	0.79	0.61	13.4	10.4	1.1	0.8
	Kidney	8.2	380	31	0.13	0.01	0.35	0.03	0.18	0.01	<0.02	<0.002	1.16	0.10	<0.1	<0.008	8.1	0.66	0.9	0.006
	Spleen	3.5	128	5	0.22	0.01	0.31	0.01	0.32	0.01	<0.02	<0.001	2.06	0.07	<0.1	<0.004	3.0	0.11	0.9	0.003
	Heart	5.6	708	40	0.20	0.01	0.54	0.03	1.33	0.07	0.12	0.007	2.16	0.12	2.47	0.14	6.0	0.34	2.1	0.11
	Liver	4.6	368	17	0.29	0.01	0.15	0.007	0.21	0.009	0.09	0.004	3.6	0.16	1.36	0.06	9.9	0.46	1.0	0.05
	Brains	25.2	141	46	0.33	0.08	0.50	0.13	0.09	0.02	0.09	0.02	0.86	0.22	3.56	0.90	7.8	1.97	0.9	0.25
5. ♂, 1yr	Blubber	96.3	42	41	0.98	0.95	0.14	0.14	0.48	0.46	<0.02	<0.02	0.15	0.15	0.95	0.92	1.7	1.63	1.1	1.1
6. ♂, 1yr	Blubber	92.2	211	194	0.37	0.34	0.18	0.16	0.73	0.67	0.03	0.03	0.60	0.55	4.9	4.5	10.2	9.4	1.4	1.3
7. ♀, 2yrs	Blubber	90.0	640	576	0.38	0.34	0.26	0.23	0.15	0.14	<0.02	<0.02	5.0	4.5	0.71	0.64	22.5	20.3	1.2	1.1
8. ♂, 24yrs	Blubber	64.8	350	220	0.32	0.22	0.60	0.39	2.1	1.4	0.11	0.07	1.49	0.96	10.6	6.9	9.3	6.03	0.9	0.6
	Liver	4.2	666	28	0.14	0.06	0.38	0.02	0.68	0.03	0.02	0.001	2.01	0.08	1.2	0.05	5.5	0.23	0.6	0.02

Table 2. Range of concentrations of organochlorines in tissue of seals from the North Sea. Data refer to harbour seals from the Western (ref. a, b, c) and Eastern (d) Wadden Sea and grey (four) and harbour (five) seals from the East coast of England. Data in mg/kg wet weight.

Tissue	Number of data	PCB	p,p'-DDT	p,p'-DDD	p,p'-DDE	$\sum$ p,p'-DDT	γ-HCH	Dieldrin	Reference
Blubber	7	22-576	<0.1-6.9	<0.05-4.5	0.5-20.3	0.51-25.4	0.03-0.39	<0.02-1.4	a
	3		3.5-9.8	0.7-3.6	5.4-14.0			0.3-2.3	b
	3	47-600	<0.71	<0.16	0.33-12			<0.014-0.05	c
	46	27.3-564	0.54-10.7	0.3-2.84	0.98-17.14	2.2-23.3	0.04-0.98	0.06-0.9	d
	9	90.40±110.23				12.55±6.23		0.21±0.29	e
Liver	5	1.5-36	<0.008-0.06	0.05-0.46	0.01-0.88	0.15-1.3	0.001-0.02	0.01-0.04	a
	2					0.1-0.4		0.07-0.07	b
	5	0.38-2.02	0.02-0.07	0.02-0.13	0.02 -0.05	0.06-0.25	0.005-0.006	0.01-0.024	d
	9	6.12±6.66				1.42±1.38		0.05±0.06	e
Kidney	2	1.6-31	<0.008	<0.003-0.10	0.05-0.66	0.05-0.76	<0.001-0.03	<0.001 -0.01	a
	4	0.22-0.87	0.03-0.13	0.01-0.08	0.01-0.04	0.05-0.25		tr	d
	9	2.11±2.16				0.38±0.24		0.02±0.03	e
Brains	3	1.4-46	<0.01-0.90	<0.01-0.22	0.06-2.0	0.06-3.10	<0.001-0.13	<0.003-0.02	a
	8	0.25-2.96	0.023-0.64	0.006-0.028	0.005-0.065	0.038-0.161	tr	tr	d
	9	1.13±0.96				0.19±0.12		0.01±0.02	e
Spleen	2	1.1-5	<0.004	<0.001-0.07	0.03 -0.11	0.029-0.18	0.001-0.01	0.002-0.01	a
	9	0.78±0.85				0.14±0.12		0.01±0.00	e
Heart	2	2.1-40	0.08-0.14	0.05-0.12	0.12-0.34	0.25-0.60	0.006-0.03	0.01-0.07	a
	9	1.56±1.40				0.32±0.25		0.01±0.06	e

References: a. Duinker, Nolting & Hillebrand (1975)

;b: Koeman and van Genderen, 1966; c: Koeman et al., 1972;

d: Drescher et al., 1977; e: Heppleston, 1973.

Table 3.

Range of p,p'-DDD and p,p'-DDE in the various seal tissues, given as the fraction (in %) in total p,p'-DDT. Number of data in parentheses.

		p,p'-DDD	p,p'-DDE
Blubber	(7)	3-18	43-100
Kidney	(2)	6-13	87-100
Spleen	(2)	3-39	60-100
Heart	(2)	20	47-57
Liver	(5)	22-33	45-67
Brains	(3)	7-29	64-100

Table 4. Range of concentrations of metals determined in various tissues of seals from the Dutch Wadden Sea and comparison with the range of levels reported for adjacent areas of the North Sea.
Concentrations in mg/kg wet weight.

Tissue	Number of data	Zn	Fe	Cu	Mn	Pb	Cd	Cr	area	Reference
Blubber	3	3-14	27-75	0.9-3.0	<0.04-2.7	<0.05-1.0	<0.01-0.02	0.49	west wadden sea	a
	5	4-13	-	-	-	-	-	-	east england	b
Liver	8	16-64	28-3240	2-20	2-6	<0.05-2.3	0.03-0.21	-		a
	1 (fetus)	89	510	49	0.7	<0.05	<0.24	-	west wadeen sea	a
	5	25-34	-	-	-	-	-	0.05-0.30		c
	57	27-56	-	2.6-17	-	0.1-0.57	0.01-0.20	-	east wadden sea	d
	5	43-61	-	9-23	-	10-12	0.9-1.4	-	east england	b
	20	-	-	-	-	2.31 ± 1.27 (is)	0.2-0.8	-		e
Kidney	2	15-25	31-66	4.8-5.1	1.9-3.4	0.16-0.23	0.15-0.17	0.15-0.59	west wadden sea	a
	16	16.3-32.5	-	2.3-4.0	-	0.14-0.55	0.06-0.38	-	east wadden sea	d
	5	28-51	-	-	-	-	-	-	east england	b
	9	-	-	-	-	-	0.1 - 0.6	-		e
Brains	7	8-27	62-119	2.5-9.5	<0.04-8	<0.05-2	<0.01-0.14	1.0-2.8	west wadden sea	a
	1 (fetus)	8	13	<1	0.3	<0.05	<0.01	-		a
	16	10.8-15.0	-	2.4-4.0	-	0.04-0.20	0.002-0.014	-	east wadden sea	d
	5	19-36	-	-	-	-	-	-	east england	b
Spleen	2	26-31	120-150	3.3-4.0	2.7-4.4	0.16-0.40	0.04-0.09	0.8-1.34	west wadden sea	a
	5	28-35	-	-	-	-	-	-	east england	b
	20	-	-	-	-	1.18 ± 0.35	-	-		
Heart	2	31	106-149	5.8-8.2	2.6-4.4	0.29-0.61	0.06-0.47	0.73-1.15	west wadden sea	a
	5	28-32	-	-	-	-	-	-	east england	b
Placenta	1	11	180	2	0.3	<0.05	<0.01		west wadden sea	a

References:

a: present work, b: Holden, 1975, c: Koeman et al., 1972; d: Drescher et al., 1977.
e: Roberts et al., 1976