INTERNATIONAL COUNCIL FOR THE
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Marine Mammals Committee
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ORGANOCHLORINE RESIDUES IN A HARBOUR PORPOISE (Phocoena phocoena) FOUND DEAD IN THE DUTCH WADDEN SEA IN 1971,

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Introduction.

On December 14th, 1971, a male harbour porpoise (Phocoena phocoena) was found dead on the coast of Ameland in the Dutch Wadden Sea. The animal was in a moderate condition, 136.5 cm long and had a weight of 34.5 kg. The blubber proportion of the animal was 31 % of the total body weight. Blubber, brain, liver and muscle were sampled and stored deep frozen (- 20 °C) until analyzed. In 1975 the concentrations of PCBs and several organochlorine pesticides were determined and the results compared with residues in porpoises from other areas.

Methods.

Extraction - clean-up.

After homogenization in a Waring Blendor, all samples were grounded with anhydrous sodiumsulfate and extracted with n-pentane in Soxhlet extractors for 3 hours.

Column chromatography was used to separate chlorinated hydrocarbons from lipids. Alumina (basic, Aktivitätsstufe I, Merck No. 1076) was deactivated with 10 % H_2O. An aliquot of the pentane extract (containing not more than 250 mg of pentane extractable lipid) was transferred to a column (20 mm i.d.) of anhydrous sodiumsulfate over 15 g deactivated Al_2O_3. The organochlorines were eluted with 200 ml pentane.

The eluate was concentrated in a rotary film evaporator to 2 ml. Separation of pesticides and polychlorinated biphenyls (PCBs) was achieved on a column of silicagel. Two grams of activated silicagel (Merckosorb SI 60. No 10228; 2 hours activated at 150 °C) were slurried into a glass column (6 mm i.d.) with hexane and the solvent drained to the surface of the silicagel. The sample (2 ml) was transferred to the column, washed with 1 ml hexane, eluted first with 10 ml of hexane and then with 10 ml of hexane/diethylether mixture (85 : 15 % v.v.). The first fraction contained HCB, PCBs, nearly all the p,p'-DDE and sometimes some

p,p'-DDT; the second fraction contained the remaining pesticides (1).

Detection - Quantification.

The equipment used was a Packard-Becker gaschromatograph, type 419, provided with a Ni^{63}-Electron Capture Detector. Two glass columns of 1.5 m length and 2 mm i.d. were used packed, with different supports:

column I : 3 % NPGS on Gaschrom Q 100-120 mesh;
column temperature 215 °C; injectiontemperature 220 °C.

column II : 1.95 % QF-1/1.5 % OV-17 on Gaschrom Q 100-120 mesh;
column temperature 195 °C; injectiontemperature 200 °C.

Ar/5 % CH_4 was used as a carrier gas at a flowrate of 30 ml/min. The detector temperature was 305 °C. The peak areas were obtained by electronic integration with a Packard-Becker analog integrator. HCB, p,p'-DDE, dieldrin, α-HCH and γ-HCH were quantified on the NPGS column and p,p'-DDT, p,p'-DDD, o,p'-DDD, β-HCH, heptachloro-epoxide and endrin on the QF-1/OV-17 column by comparing peak areas in sample and standard. The content of PCBs has been calculated on the NPGS column by means of one peak (relative retention time to p,p'-DDE : 1.32). Aroclor 1254 was used as standard.

p,p'-DDE was determined in the first fraction using the oxidation method described by Westöö and Norén. The p,p'-DDE/PCB peak areas were measured before and after oxidation and the p,p'-DDE content was calculated from the difference (2).

The presence of p,p'-DDT and p,p'-DDD was confirmed by dehydrohalogenation with alcoholic Na OH. The confirmation of dieldrin and endrin was performed by reaction with $ZnCl_2$ and HCl in ethyl-alcohol (3).

Results.

The concentrations of organochlorines in the different tissues are reported in Table I on a fat base and in Table II on a product base. In a reaction with alcoholic sodium hydroxide p,p'-DDT and p,p'-DDD were dehydrohalogenated and peaks of p,p'-DDE and p,p'-DDMU appeared in the chromatograms. By treatment with zinc chloride and hydrochloric acid in ethanol the present dieldrin was conferted to the chlorohydrin. The chromatograms on the QF-1/OV-17 column after derivatization showed the peak of the chlorohydrin with a relative retention time to p,p'-DDE of 2.56. In this way the identity of p,p'-DDT, p,p'-DDD and dieldrin in the porpoise was confirmed beyond any doubt.

Discussion.

Among the organochlorine compounds studied, PCBs occurred in highest amounts. The PCB concentration in the blubber (188 ppm on wet weight base) was higher than the maximally detected concentrations in porpoises from the North Sea in 1970-1971 (35-148 ppm on wet weight base) (4). The pesticides found in the highest concentrations are DDT and its metabolites.

Table I - Organochlorine concentrations in different tissues of a harbour porpoise (Phocoena phocoena) found dead in the Dutch Wadden Sea expressed in mg/kg (ppm) on a fat base.

	Brain	Liver	Blubber	Muscle
Percentage fat (%)	10.8	2.6	51.9	0.4
HCB	0.86	10.53	2.42	7.48
α-HCH	3.51	0.26	0.59	1.36
γ-HCH	0.15	0.38	0.80	1.92
heptachloroepoxide	0.24	0.89	2.00	3.85
dieldrin	7.4	27.7	32.0	68.4
p,p'-DDE	3.2	10.5	37.7	45.0
p,p'-DDD	0.7	17.4	16.9	28.3
p,p'-DDT	1.8	n.d.	38.6	54.9
∑p,p'-DDT	5.7	29.9	93.2	128.2
PCB	49	298	363	1290

Table II - Organochlorine concentrations in different tissues of a harbour porpoise (Phocoena phocoena) found dead in the Dutch Wadden Sea, expressed in mg/kg (ppm) on a product (wet weight) base.

	Brain	Liver	Blubber	Muscle
Percentage fat (%)	10.8	2.6	51.9	0.4
HCB	0.093	0.27	1.26	0.029
α-HCH	0.38	0.007	0.31	0.005
γ-HCH	0.016	0.010	0.42	0.008
heptachloroepoxide	0.026	0.023	1.04	0.015
dieldrin	0.80	0.72	16.6	0.27
p,p'-DDE	0.35	0.27	19.6	0.18
p,p'-DDD	0.08	0.45	8.8	0.11
p,p'-DDT	0.19	n.d.	20.0	0.22
∑p,p'-DDT	0.62	0.72	48.4	0.51
PCB	5.3	7.8	188	5.2

The contents of p,p'-DDT, p,p'-DDE and p,p'-DDD are comparable with those reported by Koeman et.al. in blubber of North Sea porpoises (4), whereas harbour porpoises from New Brunswick and Nova Scotia (East Canada, 1969-1971) contained three times more ΣDDT (306 ppm on a fat base) (5).

A significant amount of dieldrin was detected (32 ppm on a fat base), thirty times higher than found before in North Sea porpoises (4). High dieldrin levels have also been cited for porpoises from East Canada. The maximal concentration detected there was 13.1 ppm in extractable fat of the blubber (5).

Considering the contents of pesticides and PCBs on a fat base in the different tissues the highest levels were found in the muscle, followed by blubber, liver and brain (muscle > blubber ≥ liver > brain). Exceptions must be made for HCB especially accumulated in the liver, for p,p'-DDT not at all detected in the liver and α-HCH occurring in high concentration in the brain. The remarkably low concentrations of all organochlorine compounds, except α-HCH, in the brain is a phenomenon also reported by Frank et.al. (6) in harp seals (Pagophilus groenlandicus) from the Gulf of St. Lawrence and the Newfoundland and Labrador coast. He suggested a brain barrier in the accumulation of chlorinated hydrocarbons, another explanation may be the different lipid composition which is responsible for the lower levels in the brain.

On a product base concentrations in brain, muscle and liver were comparable and only the blubber, the depot fat of the animal, contained significant amounts of pesticides and PCBs.

The composition of total p,p'-DDT (Table III) in the muscle and the blubber were almost equal. p,p'-DDT, p,p'-DDE and p,p'-DDD were in the proportion of 2 to 2 to 1. In the brain the percentage of p,p'-DDE was higher (56 %) and the most important metabolite in the liver was p,p'-DDD (62 %), while p,p'-DDT was totally absent.

Table III - Composition of total p,p'-DDT in the different tissues of a harbour porpoise.

	Muscle	Blubber	Brain	Liver
p,p'-DDT (%)	43	41	31	n.d.
p,p'-DDE	35	41	56	38
p,p'-DDD	22	18	13	62

Summary.

Organochlorine residues in blubber, liver, muscle and brain of a harbour porpoise (Phocoena phocoena) found dead in 1971 in the Wadden Sea have been detected. The dieldrin concentration was considerably higher than found before in porpoises from the North Sea. The presence of dieldrin was confirmed undoubtfully by a chemical reaction.

On a fat base the contents of most pesticides and PCBs decreased in the following way: muscle > blubber ≥ liver > brain.

The relatively low concentrations of most organochlorine compounds in the brain may be explained by a brain barrier in the accumulation or by a different composition of the brain lipids.

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