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Organochlorine Pesticides in Seals and Porpoises

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Seals and porpoises in Scotland and Canada, far from the sites of application of pesticides, can accumulate high concentrations of residues in their blubber. These chemicals are spreading through the long food chain which ends with seals and porpoises and obviously cannot be confined to their place of discharge.

THE presence of organochlorine residues in marine life in many areas of the world, including the Antarctic, has been reported recently[1-3]. In British waters the accumulation of residue in the eggs of sea birds[3] and the process of concentration of pesticides through certain food chains in the marine environment[4] have been studied. Analyses of various species of marine fish and mammals from Scottish waters during the past 5 years[3,5] have shown that organochlorine residues are now a normal occurrence, the highest concentrations being found in organs or tissues with the highest lipid content. Thus the muscle tissue of salmonids, the livers of cod and the blubber of seals act as storage sites for these residues.

The aquatic mammals, such as whales, seals and porpoises, have a relatively high proportion of the body weight in the form of subcutaneous fat (blubber). Seals feed chiefly on gadoids and salmonids, and porpoises chiefly on small gadoids and clupeoids. The process of concentration of pesticides through their food chain might be expected to result in high pesticide levels in the fat of these mammals, and samples analysed at this laboratory confirm this expectation. In recent years, specimens of the grey seal (*Halichoerus grypus*), common seal (*Phoca vitulina*) and porpoise (*Phocaena phocaena*) have been sampled from the coasts of Scotland. Samples of blubber were available for all the specimens examined, and in a few cases other organs were also available. As well as recognized pesticide residues, other electron-capturing substances have been detected by gas-liquid chromatography and, in order to establish whether such substances are as widely distributed as the pesticides, blubber samples from grey seals and harp seals (*Phoca groenlandica*) on the Canadian Atlantic coast have also been examined and the results of these analyses are reported here. Examination of samples of seal blubber from other areas is continuing in order to assess the variation between different regions of the marine environment.

All tissue samples from Scottish waters were frozen at −18° C until required for analysis. The Canadian samples were preserved in 10 per cent formalin, which showed no evidence of any influence on the analytical procedure, and in this and other cases involving fish it has not affected the efficiency of recovery of pesticide residues. Aliquots of the samples (5 g) were ground to a powder with anhydrous crystalline sodium sulphate (AR grade) and extracted in a Soxhlet apparatus with redistilled AR grade n-hexane (previously tested and found to be free of electron-capturing materials by gas chromatography). The hexane extracts were made up to 100 ml. and 25 ml. aliquots were subjected to clean-up by the hexane–dimethylformamide partition method of de Faubert Maunder *et al.*[1], followed by column clean-up using alumina containing 5 per cent water. The loss of pesticide residues from fat extracts so treated is up to 20 per cent, but, as is customary in pesticide analyses, no correction has been made for such losses in the results presented here.

The cleaned-up hexane extracts were analysed by gas-liquid chromatography on a Varian 'Aerograph 205-2B' instrument employing two glass columns 5 ft. long × ¼ in. outer diameter. One column was packed with 'Chromosorb *W AW/DMCS* 80–100' mesh coated with 10 per cent

DC-200 silicone oil, and the other with the same support coated with 5 per cent *DC*-200 + 7·5 per cent *QF*-1 fluorosilicone. The oven temperature was 200° C, the nitrogen gas flow 60 ml./min and the column resolution for dieldrin equivalent to 1,600 theoretical plates. There was no significant degradation of DDT on the columns. While complete separation of the common pesticide residues is possible on one or other of these columns, interference by polychlorinated biphenyl (PCB) residues[8,9] with *p,p'*-TDE and *p,p'*-DDT peaks necessitated a further analytical stage. Aliquots (5 ml.) of some of the extracts were evaporated to dryness in a stream of cold filtered air, and the residues refluxed with 2 ml. of 2·5 per cent alcoholic potassium hydroxide for 15 min on a warm water-bath. After cooling, 5 ml. of *n*-hexane was added, with mixing, followed by 40 ml. of 2 per cent sodium sulphate solution. The supernatant hexane layer was then analysed by gas-liquid chromatography. By this technique, quantitative conversion of *p,p'*-DDT to *p,p'*-DDE, and of *p,p'*-TDE to *p,p'*-MDE (= DDMU), is obtained, and the conversion products confirm the identity of the original residues. The residual peaks in the *p,p'*-DDT and *p,p'*-TDE positions, where present, can be subtracted from the values of the pesticides originally determined in these positions. Hydrolysis also destroys α and γ-BHC, where present, but polychlorinated biphenyls are unaffected. A cyanosilicone (*XE*-60) column[10] has also been used to separate *p,p'*-DDT and *p,p'*-TDE from PCB interference, confirming the latter.

The principal pesticide residues found in all samples examined were those of the DDT group, with dieldrin in much smaller quantities. Canadian seals contained lower concentrations of dieldrin than Scottish seals, while Scottish porpoises contained remarkably high concentrations of all pesticide residues (see Table 1). Interference on the *DC*-200 silicone column in the *p,p'*-TDE position caused by a non-hydrolysable residue was responsible for about 25 per cent of the total estimated *p,p'*-TDE in the Canadian and Scottish samples examined. In the *p,p'*-DDT position, the non-hydrolysable residue constituted about 10 per cent in the Canadian samples and about 30 per cent in the Scottish samples examined for this interference, the total amount of non-hydrolysable material being smaller in the Canadian samples. The values in Table 1 have not been corrected for this interference, for only a proportion of the samples were hydrolysed. Results from the various species of seals were not noticeably different in a given area, and have been combined in Table 1.

Table 1. CONCENTRATION RANGES OF ORGANOCHLORINE RESIDUES IN FAT OF SEALS AND PORPOISES (p.p.m.)
(Means in parentheses)

Area and type of sample	No. in sample	Dieldrin	*p,p'*-DDE	*p,p'*-TDE	*p,p'*-DDT
E. Scotland, adult seals (1965–66)	18	0·15–2·1 (0·79)	1·0–11·1 (5·8)	0·27–3·0 (1·2)	1·5–23·3 (7·8)
N. and W. Scotland, adult seals (1966–68)	6	0·06–0·39 (0·20)	1·8–7·0 (3·4)	0·17–0·83 (0·41)	1·7–7·7 (3·8)
N. and W. Scotland, seal pups (1966)	9	0·06–0·44 (0·16)	1·0–4·0 (2·5)	0·13–0·59 (0·33)	1·1–3·7 (2·3)
Canada, Magdalen Is., juv. and adult seals (1967)	2	0·02–0·04 (0·03)	0·47–1·6 (1·2)	0·06–0·16 (0·12)	0·76–0·63 (0·26)
Canada, Cabot Straits, juv., imm. and adult seals (1967)	6	0·03–0·10 (0·07)	1·2–17·3 (5·0)	0·35–2·1 (0·78)	2·1–15·6 (5·5)
Scotland, Orkney, adult porpoise (1967)	1	0·50	1·1	0·68	2·2
E. Scotland, adult porpoises (1965, 1967)	3	4·0–18·8 (9·9)	9·6–13·3 (12·8)	3·2–14·3 (8·9)	13·1–25·7 (21·1)

Contamination by pesticides is greater in the seals on the east coast of Scotland (taken in an area roughly from Aberdeen to the Tay estuary) than in specimens from the west and north coasts, including the Orkney Islands. Seal pups from the Orkney and Harris (west coast) breeding grounds contained only slightly lower residues than adults from the same areas. By comparison, the Canadian samples from the Magdalen Islands (Gulf of St. Lawrence) contained very low levels of both dieldrin and the DDT group, while seals from the Cabot Straits (Gulf of St. Lawrence) contained little dieldrin but higher DDT group residues, similar to those of Scottish seals. Of the few porpoises examined, that from Orkney was the least contaminated, but the three taken on the east coast of Scotland had markedly higher contents of all the residues measured. One specimen contained a total residue concentration (dieldrin and DDT group) of 73·3 p.p.m. Analyses of a few tissues other than the blubber have been made, but concentrations in the blubber have always been found to be the highest (Table 2). Concentrations in other tissues seem likely to be consistent with their lower lipid contents.

Table 2. TOTAL DDT CONCENTRATIONS (p.p.m.) IN VARIOUS TISSUES

Sample	Blubber	Liver	Brain	Kidney	Spleen	Muscle
Grey seal	5·6	0·77	0·26	—	—	—
Grey seal	9·2	0·36	0·15	—	—	—
Grey seal	14·6	0·97	0·24	—	0·55	—
Grey seal	8·4	0·48	0·17	—	0·13	—
Grey seal	2·4	0·11	0·07	—	0·06	—
Porpoise	3·8	0·58	0·02	0·04	0·10	0·06

The proportion of blubber extractable by hexane was determined for some samples. Values ranged from 72 to 86 per cent (mean 80 per cent) for the Canadian seals, and from 88 to 98 per cent (mean 93 per cent) for Scottish seals samples on the breeding grounds off the west coast. (Values for other seal samples were not estimated.) Concentrations of pesticides in terms of extractable fat for these samples would thus be up to 40 per cent greater than the values in Table 1, but the general comparison is not significantly affected. The lower extractable fat values for the Canadian samples may possibly be a result of being preserved in formalin.

Concentrations of pesticide residues in the extractable fat of various organs and tissues of one porpoise show an important relationship (Table 3). While the concentrations of the DDT group in the original tissues vary widely, those expressed in terms of extractable fat are in much closer agreement, with the sole exception of the brain. A similar anomaly for tissues of trout was described by Holden[11]. It seems likely that a large proportion of the lipid fraction of the brain may not contain pesticides, and the brain lipid composition is known to differ considerably from that of depot fat.

Table 3. CONCENTRATIONS OF TOTAL DDT (p.p.m.) IN TISSUES OF A PORPOISE

Tissue or organ	Blubber	Muscle	Liver	Spleen	Kidney	Brain
Concentration in tissue	3·8	0·56	0·58	0·12	0·04	0·02
Percentage extractable fat	67·0	6·1	13·2	5·1	1·4	8·8
Concentration in extractable fat	5·6	9·2	4·8	2·4	2·9	0·27

The unidentified peaks on the chromatograms seem to be similar to those produced by polychlorinated biphenyls and, to enable comparison to be made, the ratios (*Rx*) of the retention times of all regularly occurring peaks (relative to dieldrin = 100) have been calculated for the operating column temperature of 200° C, these values being temperature-dependent. The *Rx* values of PCB compounds from commercial PCB formulations were also determined for both types of column (Table 4). At least seven PCB-type peaks were found, including those which interfere with *p,p'*-TDE and *p,p'*-DDT. Heptachlor epoxide and *p,p'*-MDE could not be confirmed on both columns and are not therefore recorded.

The most significant aspect of the results is that, although seals and porpoises inhabit an environment far removed from the site of application or discharge of pesticides, they can contain much greater concentrations of dieldrin and DDT group residues than those recorded for most other species, including man. Robinson and Hunter[12] found a mean concentration of dieldrin of 0·2 p.p.m. (range 0·10–0·73 p.p.m.) and a mean total con-

contration of DDT of 4·0 p.p.m. (range 1·0–11·1 p.p.m.) in samples of human depot fat in England in 1964. No evidence of any significant change was found during 1962–65.

Table 4. RELATIVE RETENTION VALUES (*Re*) OF RESIDUES (DIELDRIN = 100)

DC-200 column			*DC*-200/QF-1 column		
Residue		Sample	Residue		Sample
PCB	= 68·5	68	PCB	= 68·5	70
p,p'-DDE	= 100	100	*p,p'*-DDE	= 84	83·5
Dieldrin			Dieldrin	= 100	100
PCB	= 121	125	*p,p'*-TDE	= 121	121
p,p'-TDE	= 129	128	PCB	= 123	122
PCB	= 148	149	PCB	= 148·5	—
o,p'-DDT	= 170	171	*p,p'*-DDT	= 147	147
PCB	= 174	176	PCB	= 224	221
PCB	= 205	205	PCB	= 282	276
PCB	= 282	287			
PCB	= 359	358			

The seals and porpoises from the east coast of Scotland were usually more seriously contaminated than those from the north and west coasts. This, at least in respect of seals, may be because, as is believed, the populations in the two areas come from different breeding sites. The seals south of Aberdeen breed principally on the Farne Islands, off the north-east coast of England, while the seals of north and west Scotland are primarily from the breeding grounds of the Orkney Islands in the north, and other islands off the west coast of Scotland. The sea off eastern Scotland is also likely to receive more contamination from estuarine discharges than that off north and west Scotland. Similarly, the two separate seal populations sampled off the east coast of Canada may inhabit areas with different levels of contamination. Pups contain pesticide residues in concentrations only slightly lower than in the parent population. Common seals in Holland have been found with concentrations of residue similar to those of seals on the Scottish east coast[13].

While the residue concentrations in subcutaneous fat are high, those of other tissues and organs are found to be much lower. This suggests that there is no risk of physiological effects, unless the metabolism of much of the fat in times of stress leads to a considerable increase in the concentration of the residue in circulating lipids, and thus in residue concentrations in other organs or tissues.

Grey seals on the coast of the Scottish mainland feed primarily on salmon (*Salmo salar*) and cod (*Gadus morrhua*)[14]. The highest concentrations of pesticide in salmonids in the marine environment have so far been found in sea trout (*S. trutta*) on the east coast with up to 0·2 p.p.m. (dieldrin + total DDT) in muscle tissue[15], and salmon from the same area would probably have similar contents. Cod examined from the east coast have contained up to 0·35 p.p.m. (dieldrin + total DDT) in muscle tissue (mean 0·12 p.p.m.) and from the west coast up to 0·60 p.p.m. (mean 0·22 p.p.m.), while cod livers contained up to 4·0 p.p.m. and 12·0 p.p.m., respectively. Grey seals are estimated to eat about 15 lb. of food daily, and thus an adult seal could consume its own weight in food in 20–30 days. Assuming a high proportion of the pesticide intake to be stored in body fat rather than to be excreted or metabolized, an increase of two orders of magnitude in pesticide concentration from food to body fat within a few years is feasible. This degree of accumulation of residue in an environment not deliberately contaminated, and in species ecologically far distant from the target organisms of persistent pesticides, underlines the impossibility of confining such chemicals to the areas of application.

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