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Levels of Organochlorine Compounds in an Inland Seal Population in Eastern Finland

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Fourteen Saimaa ringed seals (*Phoca hispida saimensis*) were studied for PCB, DDT, chlordane and chlorophenol compounds in blubber, liver, muscle, heart and kidney in Lake Saimaa, eastern Finland, in 1977-1981. Both PCBs and total DDT increased on average from a good 5 mg kg⁻¹ in extractable fat of blubber in a new-born to a good 65 mg kg⁻¹ in sexually mature specimens, the maximum being 93 and 104 mg kg⁻¹ respectively. Concentrations of PCBs and tDDT in blubber and liver were most clearly dependent on the age and weight of the seal. Chlordanes were found in all 14 specimens (mean 0.59 mg kg⁻¹ in blubber), trichlorophenol in one, tetrachlorophenol in two and pentachlorophenol in all three specimens studied. Although some of the Saimaa seals had reached quite high levels of both PCBs and tDDT, the present results do not indicate that the steady decrease of the Saimaa seal population during the last two decades would have been caused by these toxins, at least not in isolation.

It has been suggested that high concentrations of PCB compounds have caused the rapid decrease in the numbers of Baltic seals (Helle *et al.*, 1976) and the North Sea populations of the harbour seal (Reijnders, 1979) during the 1970s. A specific subspecies of the ringed seal, the Saimaa seal (*Phoca hispida saimensis* Nordq.), lives in Lake Saimaa in eastern Finland. The Saimaa seal population has decreased rapidly in recent decades and the population is now estimated to be only 100-odd individuals (Helle *et al.*, 1981). Concentrations of PCB and DDT compounds in Saimaa seals have been studied earlier (Karppanen & Henriksson, 1974), but until now the possible role of environmental toxins in the reduction of the Saimaa seal population has remained undetermined.

Besides the PCB and DDT compounds, other chlorinated

environmental toxins such as chlordane, toxaphene and other neutral organochlorine compounds or chlorinated phenols may be of some importance in the discussion about the present and future existence of the Saimaa ringed seal.

Chlordanes and toxaphenes are insecticides which have been used increasingly during recent decades as a consequence of the restrictions on the use of DDT. Technical chlordane includes some 45 components, mainly polychloromethanoindenes. The major components are α -chlordane, γ -chlordane, *trans*-nonachlor and isomers of chlordane. Residues of chlordanes and toxaphenes have been found in fish in Swedish waters (Jansson *et al.*, 1979) and residues of chlordanes have previously been reported in fish from Lake Saimaa (Pyysalo *et al.*, 1981).

The discovery of chlorinated terpenes and chlordanes in areas like Sweden and Finland, where they have never been used, suggests that they are transported by air or perhaps also formed in industrial processes. Chlorinated phenols are widely used fungicides and they are also produced in the cellulose industry, which has led to their common occurrence in the Finnish aquatic environment (for review, see Paasivirta & Linko, 1980).

The aim of this study is to present the most recent results concerning the concentrations of PCB and DDT compounds, chlordanes, toxaphenes and chlorinated phenols in Saimaa seals and to estimate the possible influence of these environmental toxins on the reduction in the seal population in Lake Saimaa.

Material and Methods

Seal samples

The samples were collected in Lake Saimaa in 1977-1981 (Fig. 1). Of the total of 14 specimens, two were found during the winter about two months after death, so the cause of

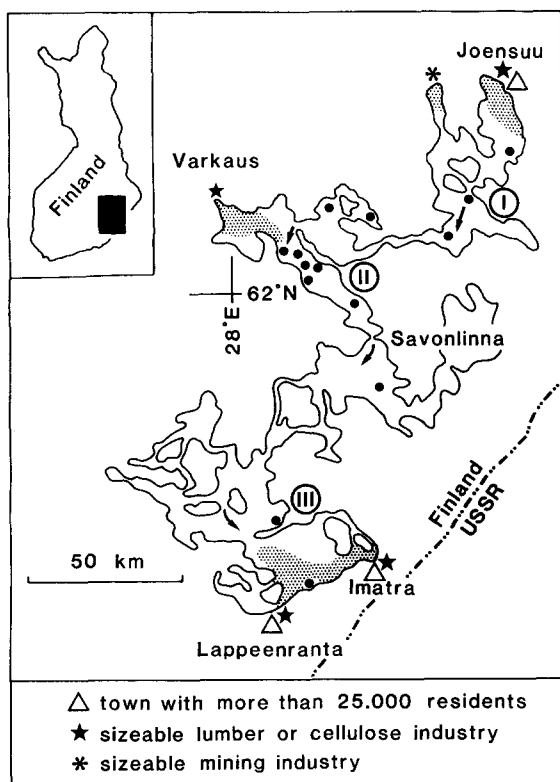


Fig. 1 Study area. Black dots indicate the catching locations of the Saimaa seals. Arrows demonstrate the direction of the current, dotted areas poor or fair usability of the watercourses (according to the National Board of Waters, Finland: Integrated Water Resources Development Plan for the District of Lake Saimaa, 1981).

death remains unknown in both cases. However, PCB and DDT concentrations can be reliably determined in the seals' bodies several months after death (Olsson *et al.*, 1974). The remaining 12 seals were killed in fishing nets, so the samples were obtained soon after death.

Samples from blubber or subcutaneous fat, liver, kidney, heart and shoulder muscle were removed and preserved deep-frozen until analysis was carried out in 1981–1982.

The analytical results have been correlated with the proper age or total weight of the seal. The age determination is based on the layered structure of the tooth cementum (see Helle, 1979).

Analytical procedure

Two grams of the sample was homogenized with 10 g of anhydrous sodium sulphate. Heptachlor (5 mg kg^{-1}) was added as internal standard. The homogenate was extracted at 25°C , once with 35 ml acetonehexane (25 + 10 ml) and twice with 20 ml hexane–diethyl ether (18 + 2 ml) for 30 min. in an ultrasonic bath. The solutions were filtered, combined and shaken in a separatory funnel with 0.9% sodium chloride in 75 ml water. The aqueous solution was extracted with hexane (10 ml), the organic solutions were combined and 10 ml absolute ethyl alcohol was added. The solvents were evaporated at 40°C *in vacuo*, the solid residue was dissolved in 2 ml hexane and 2 ml concentrated sulphuric acid was added. After shaking, the mixture was centrifuged and the organic layer separated. The sulphuric acid cleaning procedure was repeated until the acidic layer was nearly colourless.

The hexane concentrate was fractionated in a Silica gel

column ($10 \times 300 \text{ mm}$, Kieselgel 60, 70–230 mesh, Merck). The silica was first heated at 360°C for 16 h, after which 3% of water was added. The column was successively eluted with 60 ml hexane–diethyl ether (75:25 v/v), and the eluate was evaporated *in vacuo* at 25°C to 0.2 ml.

In the case of seal blubber samples, the concentrate was further cleaned with the aid of thin layer chromatography, using silica gel plates and dichloromethane–hexane (1:3) solvent system. The analysed compounds were collected from the plates ($R_f=0.3\text{--}1.0$) by scratching the silica material into a pasteur pipette with glass wool plug and eluted from the silica with 4 ml cyclohexane–diethyl ether (6:4 v/v), which solution was finally concentrated in a nitrogen flow to 0.2 ml.

The final concentrate was analysed by selective ion monitoring technique (GLC–SIM, mass fragmentography; Hewlett–Packard 5992) and some samples were also analysed with GLC–ECD (Micromat 412 HRGC, Orion Analytica, Finland).

An OV-101 glass capillary or a fused silica column (i.d. 0.32 mm, length 30 m) were used. The GLC oven was temperature programmed from 60 to 240°C , $10^\circ\text{C min}^{-1}$. The monitored ions were m/e 248 (specially for determining *p,p*-DDE), 235 (*p,p*-DDT), 358 (PCB), 326 (PCB), 263 (aldrine, oxychlordan, α - and γ -chlordanes, *trans*-nonachlor), 343 (chlorinated terpenes), 341 (chlorinated terpenes and chlordanes), 377 (α - and γ -chlordanes, chlorinated terpenes), 181 (lindane), 409 (*trans*-nonachlor) and 272 (heptachlor). In ambiguous cases the identifications were confirmed by selected ion monitoring of the six most prominent fragments in the mass spectrum of the compound.

The retention times of the identified compounds were identical with those of the reference compounds kindly supplied by the US Environmental Protection Agency, EPA.

The quantitative determinations were based on the peak areas obtained from the samples, the internal standard and the reference compounds.

The quantitative determination of PCB compounds was based on the total peak areas obtained with selected ion monitoring of the ions 326 and 358. The commercial Clophen A 60-PCB mixture was used as a reference standard.

The sensitivity of the method was about $10 \mu\text{g kg}^{-1}$ for individual compounds. When no interfering compounds were present, especially in the samples of low fat concentration, even higher sensitivity was occasionally obtained.

For the PCB mixture the sensitivity was about 1 mg kg^{-1} and for the toxaphene mixture it was about 5 mg kg^{-1} .

The chlorinated phenols were isolated by grinding the sample with sodium sulphate and extracting with hexane–ether as described for the isolation of the organochlorine compounds. The extracting solvents were acidified by adding 0.1% (v/v) conc. hydrochloric acid to improve the recovery of the phenols. The residue from the evaporation was dissolved in 3 ml diethyl ether, 3 ml 0.2 N sodium hydroxide in water added and the mixture shaken for 5 min. The ether layer was rejected and the aqueous phase was acidified with 0.2 N HCl and extracted with 5 ml diethyl ether. The phenols were methylated using diazomethane (Levitt, 1973) and the chloroanisoles were analysed by the

TABLE 1

Levels (mean \pm SD, range, sample size) of PCBs, total DDT and chlordanes in Saimaa seals in 1977–1981 (mg kg⁻¹; in extractable fat for blubber and in fresh weight for other tissues).

Tissue	PCBs	tDDT	Chlordanes
Blubber	37.1 \pm 26.3 5.1–93 14	36.0 \pm 31.9 5.2–104 14	0.59 \pm 0.48 0.11–1.67 14
Liver	0.70 \pm 0.74 0.16–2.2 10	0.70 \pm 0.66 0.19–2.3 12	0.19 \pm 0.17 0.01–0.43 7
Muscle	3.0 \pm 6.3 0.10–21 11	2.9 \pm 6.3 0.05–21 12	0.02 \pm 0.007 0.01–0.03 4
Heart	0.60 \pm 1.0 0.05–3.6 11	0.17 \pm 0.13 0.04–0.38 11	
Kidney	0.48 \pm 0.55 0.09–1.8 12	0.28 \pm 0.38 0.01–1.4 12	

SIM-technique using the ions 209.9, 211.9 (for trichloroanisoles), 243.9, 245.9 (for tetrachloroanisoles), 277.9 and 279.9 (for pentachloroanisole).

The fat content of the seal blubber, determined by the AOAC (1975) method, was, on average, 96%.

Results

Concentrations of PCBs, total DDT and chlordanes are presented in Table 1. The levels are presented in mg kg⁻¹ (ppm) as calculated in extractable fat in the case of blubber and in fresh weight in the case of other tissues.

The range of PCB and DDT concentrations vary widely, the highest ones reaching 93 and 104 ppm respectively in blubber. Mean PCB concentrations are clearly higher than those of DDT only in the hearts and kidneys. Significant correlation in blubber emerges between PCBs and tDDT ($r=0.577$, $p<0.05$), but not between PCBs and chlordanes or tDDT and chlordanes.

The total DDT consists of *p,p*-DDE, *p,p*-DDD and *p,p*-DDT, the proportion of *p,p*-DDE being dominant in most of the samples and in all the tissues studied (Table 2). The proportion of *p,p*-DDD in blubber varies from 0 to 16% and that of *p,p*-DDT from 2 to 41% of the total DDT.

All the samples contain chlordanes compounds, the maximum being 1.67 mg kg⁻¹ in the blubber. The total chlordanes concentration consists of *trans*-nonachlor (an average of

TABLE 2

Percentage proportion (mean, range; sample size in brackets) of *p,p*-DDE, *p,p*-DDD and *p,p*-DDT of the total DDT in different tissues of the Saimaa seal in 1977–1981.

Tissue	<i>p,p</i> -DDE	<i>p,p</i> -DDD	<i>p,p</i> -DDT
Blubber (14)	83.3 46–96	3.9 0–16	13.1 2–41
Liver (12)	58.5 39–74	22.7 8–38	19.3 0–40
Muscle (12)	65.5 3–96	13.0 4–47	21.5 0–88
Heart (11)	80.5 50–100	16.7 0–50	2.7 0–11
Kidney (11)	78.5 50–100	15.1 0–50	5.9 0–30

TABLE 3

Levels (mean \pm SD, range; sample size in brackets) of PCBs, total DDT and chlordanes in Saimaa seal blubber (mg kg⁻¹ in extractable fat) in 1977–1981, by age of the seal.

Compound	Age of seal			
	Full-term foetus/new-born (1)	2–4 months (4)	5–15 months (6)	Sexually mature (4–12 years) (3)
PCBs	5.1	17.3 \pm 11.3 9–34	41.5 \pm 21.6 28–82	65.3 \pm 24.4 47–93
tDDT	7.1	15.1 \pm 8.4 5.2–23	39.3 \pm 27.1 15–82	67.0 \pm 43.6 19–104
<i>p,p</i> -DDE	6.5	13.5 \pm 7.2 5.0–20	31.8 \pm 24.9 6.9–75	55.3 \pm 39.1 15–93
<i>p,p</i> -DDD	0.32	0.16 \pm 0.16 0–0.35	1.7 \pm 2.2 0.17–5.9	3.8 \pm 4.3 0.46–8.6
<i>p,p</i> -DDT	0.26	1.2 \pm 0.93 0.15–2.2	5.9 \pm 4.2 1.3–13	7.8 \pm 8.8 2.3–18
Chlordanes	0.11	0.41 \pm 0.48 0.10–1.12	0.71 \pm 0.55 0.12–1.67	0.74 \pm 0.39 0.45–1.18

43.8%), oxychlordanes (37.5%), γ -chlordanes (10.3%) and α -chlordanes (8.4%). The proportion of oxychlordanes in the total chlordanes in the present seal samples is higher than in fish muscle and liver (see Moilanen *et al.*, 1982), which indicates that oxychlordanes is formed via metabolism.

Three specimens were analysed for chlorophenols (blubber and liver). Trichlorophenol was found in none of the samples, tetrachlorophenol in two of the blubber

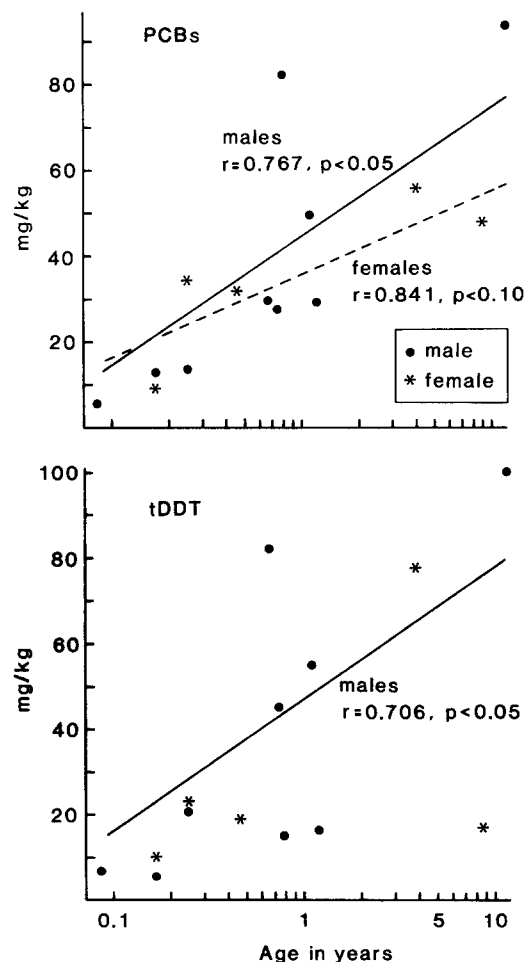


Fig. 2 Levels of PCBs and total DDT in blubber (in extractable fat) versus the age of the Saimaa seals in 1977–1981. Formulae for regressions: PCBs for males $y = 28.8 \log x + 46.1$ and for females $y = 19.6 \log x + 36.2$; tDDT for males $y = 30.2 \log x + 47.9$.

TABLE 4

Dependence of PCBs, total DDT and chlordanes levels on the age and weight of the Saimaa seal in different tissues in 1977–81. Symbols of probability: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; °, $p < 0.10$; ns, $p > 0.10$.

Tissue	PCBs		tDDT		Chlordanes	
	Age	Weight	Age	Weight	Age	Weight
Blubber	0.668**	0.540*	0.540*	0.550*	0.274 ^{ns}	0.182 ^{ns}
Liver	0.722*	0.732*	0.543°	0.633*		
Muscle	0.087 ^{ns}	0.115 ^{ns}	0.084 ^{ns}	0.100 ^{ns}		
Heart	-0.029 ^{ns}	0.023 ^{ns}	0.610*	0.722*		
Kidney	0.634*	0.518°	0.313 ^{ns}	0.304 ^{ns}		

samples (0.40 and 0.80 mg kg⁻¹) and pentachlorophenol in all the blubber samples (mean 0.61, range 0.23–1.0 mg kg⁻¹) and in two of the liver samples (0.06 and 0.11 mg kg⁻¹).

Lindane and aldrin could not be found in the samples with the sensitivity available. For the occurrence of toxaphene, see Discussion section.

Tables 3 and 4 give the concentrations of PCB and DDT compounds versus the age and weight of the seal. The concentrations in the new-born specimen demonstrate the contamination from mother to foetus through the placenta, and those in the age class of 2–4 months illustrate additionally the contamination through suckling. Unlike the DDT and PCB concentrations, chlordanes levels do not increase any longer in the oldest age group.

In addition to the age of the seal, concentrations of environmental toxins in marine mammals are also dependent on sex (e.g. Addison & Brodie, 1977). The present material is too small and it contains too few mature specimens (see Fig. 2) for this point to be studied thoroughly, since the intersexual difference begins to be clear only at maturity (see Discussion).

The concentrations in various tissues are given in Tables 1 and 2. The distribution of environmental toxins in various tissues deviates to some extent in different specimens. Thus, in the case of PCBs only the concentrations in blubber and liver and in muscle and kidney are correlated with each other (Table 5). In the case of tDDT the strongest correlation is found between muscle and kidney.

Sampling locations, largest towns and the location of industry as well as their pollution influence on Lake Saimaa are depicted in Fig. 1. The present material does not offer any firm basis for considering regional differences in the levels of environmental toxins. However, on the basis of the present few comparable seal samples (young specimens) the levels of PCBs are highest in the heavily industrialized southern part of the lake (III in Fig. 1), and the levels of both tDDT and PCBs seem to be lowest in the specimens originating from the central part of Lake Saimaa (II in Fig. 1).

TABLE 5

Correlations of toxin levels between different tissues in Saimaa seals in 1977–81. Correlations for total DDT are presented in the lower left-hand triangle and those for PCBs in the upper right-hand triangle. For symbols of probability, see legend for Table 4.

	Blubber	Liver	Muscle	Heart	Kidney
Blubber					
Liver	0.100 ^{ns}				
Muscle	0.134 ^{ns}	0.112 ^{ns}			
Heart	0.110 ^{ns}	0.860***	0.596°		
Kidney	0.111 ^{ns}	0.013 ^{ns}	0.924***	0.858**	

tDDT

PCBs

Discussion

In comparing the present results with those found earlier in Lake Saimaa (Karppanen & Henriksson, 1974) it has to be remembered that the analytical procedure is somewhat different. However, the results are completely comparable in this respect with those obtained from the Baltic seals (Helle *et al.*, 1983).

The concentrations of the organochloro compounds in Saimaa seals are higher than those in many oceanic races (see Wageman & Muir, 1981), but considerably lower than those in the Baltic seals (see Helle *et al.*, 1983), where the concentrations of PCB compounds commonly exceed 200 mg kg⁻¹ in blubber. The latter difference is in accordance with the general levels found in fish (see Paasivirta & Linko, 1980).

The present concentrations of PCBs and tDDT are clearly lower than those found 10 years earlier (see Karppanen & Henriksson, 1974), but the unspecified ages of the previous seal material must be taken into consideration, and so the difference may be at least partly due to possible differences in the age structure of the samples. It is known, however, that levels of DDT compounds have been widely decreasing in Finnish wildlife during the 1970s, and that the reduction in PCB levels has been found up to now only in Baltic Sea fish, but not in fish from Finnish inland lakes (see Paasivirta *et al.*, 1981; Moilanen *et al.*, 1982).

Chlordanes have been discovered in Baltic seals at a level of 5–10 mg kg⁻¹ in blubber (Jansson *et al.*, 1979; Helle *et al.*, 1983). The average levels of chlordanes in Saimaa seals are considerable lower, which results are also in accordance with the results obtained from fish (Pyysalo *et al.*, 1981).

Toxaphenes are also found in the Baltic seal blubber at an average level of 10 mg kg⁻¹ (Jansson *et al.*, 1979). Due to the instrumental facilities and the complexity of toxaphene mixtures, the levels of toxaphenes could not be reliably determined in the present material. However, GLC/MS analysis gave signals which most likely were due to chlorinated terpenes. The level of toxaphenes in Lake Saimaa seal is thus suspected to be about the same as that of chlordanes.

The concentrations of chlorinated phenols in Finnish fish has been studied by Paasivirta *et al.* (1981). The main component in pike muscle was pentachlorophenol, ranging from 0.012 to 0.07 mg kg⁻¹. So the maximum present concentration of pentachlorophenol of 1.0 mg kg⁻¹ of blubber tissue is one of the highest chlorophenol concentrations ever found in Finnish wildlife.

The PCB:DDT ratio was 0.3 in the seal blubber in Lake Saimaa around 1970 (Karppanen & Henriksson, 1974) but is now, about ten years later, 1.5. This change reflects the faster reduction of DDT concentration when compared to PCBs, which has been brought both by the more effective

restrictions on the DDT and by the faster completion in animals.

The concentrations of accumulative environmental toxins increase in male marine mammals with age, whereas in females the increase slows down at maturity (Addison *et al.*, 1973; Addison & Smith, 1974). This is due to the fact that females get rid of part of their body burden of environmental toxins in giving birth and in subsequent lactation (e.g. Addison & Brodie, 1977). This concerns marine mammal populations with normal reproductive functions, whereas such clear differences do not occur e.g. in the Baltic ringed seal population, which suffer from serious reproductive disturbances (Helle *et al.*, 1976).

The present material indicates that the accumulation pattern in Saimaa seal males is of the normal marine mammal type (Fig. 2), but in the case of females the specimens are too young for discussion of the pattern. The transfer of toxin burden from mother to pup also occurs in the Saimaa seal population, however, which is demonstrated by the rather high concentrations in the new-born and the rapid increase of levels during the suckling period (see Table 3).

In the light of the general levels of the toxins in question, it seems that hydrocarbons are most probably not responsible for reproductive disturbances, at least not in isolation. This may be deduced, for example, from the fact that the lowest concentrations of normal pregnant females in the Baltic ringed seal population exceed even the highest PCB levels found in females of the Saimaa seal (see Helle *et al.*, 1983).

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Enumeration of the Bacterial Contamination of Bivalves in Monitoring the Marine Bacterial Pollution

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Marine organisms such as shellfish are capable of filtering large amounts of sea water and of retaining pathogenic bacteria. This property constitutes a way for evaluating the level of bacterial contamination in sea water. Despite some limitations, the use of shellfish as concentrating material appears to improve the bacterial enumeration results both in accuracy and consistency.

Despite the improvement of enumeration techniques, estimation of the bacteriological quality of surface marine waters remains controversial. In particular, the evaluation of the quality of bathing waters remains a litigious subject. Among the major reasons are the infrequency of sampling versus the heterogeneity, as well as continuous variations of marine environmental conditions, particularly in the bays