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REMOVAL OF DDT AND ITS METABOLITES FROM FISH OILS BY MOLECULAR DISTILLATION

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INTRODUCTION

As a result of world wide production and application, DDT (2,2 bis-(p-chlorophenyl)-1,1,1-trichloroethane) and its degradation products are found everywhere in the global environment. Numerous studies also report on the accumulation of often relatively high concentrations of DDT and its metabolites in the fat depots of marine organisms and fish and fish products (HOLDEN & MARSDEN, 1967; WESTØØ & NOREN, 1970: STENERSEN & KVALVAAG, 1972; ADDISON et al. 1973; EICHNER, 1973; and FRANK et al., 1973). The potential hazards of these compounds for both humans and animals have caused a growing concern in many countries, and legislative restrictions as to acceptable contamination in food and foodstuffs have emerged.

This paper reports on the levels of organochlorine pesticides in some fish oils. Considering the low vapour pressure of DDT, 1.5×10^{-3} mm at 20°C (METCALF, 1973), removal of the pesticide by molecular distillation (stripping) should be possible. The present study also reports on experiments in which attempts have been made to remove DDT and its metabolities from a cod liver oil by molecular distillation without extensive losses of vitamin A.

SAMPLES

The cod liver oils derived from different processing plants along the Northern coast of Norway. They had been refined by deacidification and cold-filtration. Further were analysed a liver oil from dogfish (*Squalus acanthis*), and body oils from capelin (*Mallotus villiosus*), herring (*Clupea harengus*), mackerel (*Scomber scombrus*) and mink whale (*Balaenoptera acustorostrata*).

The «stripped» oils were produced in a CMS-5 molecular distillation laboratory still (BIEHLER et al. 1949). The still was filled with about 400 g of deacidified, filtered cod liver oil. The oil was fed to the evaporating, rotating dish at a rate of about 15 gm/min. Degassing was performed by passing the oil once over the evaporator at a temperature of 95°C, and a pressure of 15—20 microns. The temperature was subsequently raised to the stripping temperature, while circulating the oil, and the stripping was performed by passing the oil once more over the evaporator at the chosen temperature. Distillate and rest-oil were collected in the normal manner. Part of the distillate condensed and crystalized on the wall of the dome on areas outside where the distillate

is normally collected. These crystals were recovered by washing down the dome with acetone after the withdrawel of the rest-oil. The acetone was removed by evaporation before the samples were analyzed. The material trapped in the cooling trap was also washed out with acetone, and analyzed after removal of the acetone by evaporation. The distillation data are summarized in Table 1.

Table 1. Distillation data.

Run	Stripping temperature °C	Stripping pressure 10^{-3}mm Hg	Fractions as percentage of oil stripped			
			Distillate	Dome	Trap	Rest
A	223—228	17	3.50	0.33	0.05	96.12
B	199—206	16	1.43	0.38	0.03	98.16
C	174—178	18	0.68	0.44	0.20	98.78
D	150—152	13	0.29	0.35	0.04	99.32
E	123—129	13	0.22	0.27	0.04	99.47
F[2])	95	14	0	0.25	0.036	99.71

[1]) The temperature refers to the rest oil when leaving the rotating disc.
[2]) This sample was only degassed.

METHOD

0.2 to 1.0 g of different oils or fractions from the stripping of the cod liver oil were dissolved in 5 ml petrol ether (MERCK, DARMSTAD 1775, p.a.). The solution was cleaned up by chromatography on a celite-sulfuric acid column (400—500 mm, Ø 22 mm), which was fitted with a Teflon stopcock. 3 parts by weight celite (Celite 545, John-Manville Product) were carefully mixed with 2 parts by volume of conc. sulfuric acid (p.a.). A petrol ether extract of the celite should be free from electron capturing substances. To each column were added 20 g of this mixture. The chlorinated hydrocarbons were eluted with petrol ether (JENSEN et al. 1957). The eluate, normally 200 ml, was evaporated nearly to dryness in a vacuum evaporator (Heidolph) and transferred by washing 3 times with 1 ml petrol ether to vials of appropriate size. Now the petrol ether was totally evaporated by blowing nitrogen through the vial while it was kept in hot water. The residue was finally dissolved in a volume of hexane giving a suitable concentration for gas chromatography. The hexane should be of reagent grade and double distilled in an all glass apparatus.

1 μl of the extracts was injected into a Perkin Elmer 900 gas chromatograph equipped with a Ni^{63} discharge electron capture detector

and a 200 × 0.4 cm i.d. glass column packed with 11 % of an equal mixture of OV—17 and QF—1 on Gas Chrom Q (80/100 mesh), (Applied Science Laboratories). The glass column was treated with DMCS (dimethyldichlorosilane) before the packing. The gas chromatograph was operated at column, injection and detector temperatures of 190, 225 and 215°C, respectively. A nitrogen flow rate of 60 ml/min. was applied. The chromatogram was recorded on a Perkin Elmer Modell 56 Hitachi Recorder. The areas were calculated on a Infotronic CRS 208 integrator and read out on an Addo-X printer. As standard were used DDT and its metabolited (Analytical Standard Ltd.) and Chlopene A—50 (industrial grade) dissolved in nanograde hexane.

Differentiation and determination of the particular chlorinated hydrocarbons in the samples were confirmed by reference to standard curves with known concentrations. The gas chromatographic analyses showed that the PCB in samples corresponded to Chlophene A—50 (Fig. 1) and that the DDT residues consisted of pp'-DDE, pp'-DDD, op'-DDT and pp'-DDT. The Chlophene A—50 as well as other PCB-standards which were tried, interfered with the peaks of DDT and its metabolities on the chromatogrammes, with the exception of pp'-DDD, and peak nine of Chlophene A—50. The further differentiation and determination of the particular organo chlorine pesticides were carried out according to the method of SMITH et al. (1973) with minor modifications. Instead of the height of the peaks, the ratios of the areas were applied in the present study. Where PCB interfered in the ratios for DDT and its metabolities, a correction was possible as peaks 5, 7, 9, 13 and 14 in a standard run of Chlophene A—50 could be specifically and with no interference identified in the chromatographic pattern from the oils. The indirect method of calculation was checked by a quantitative determination of DDT and its metabolities applying the more elaborate method with separation on activated 3% silica acid-celite column described ARMOUR & BURKE (1970). The recoveries were checked periodically by addition of the pesticides to the oil, and were as follows: pp'-DDE 87.1%, pp'-DDD 92.0%, op'DDT 98,2% and pp'-DDT 91.2%.

RESULTS AND DISCUSSIONS

In Table 2 are reported the results from analysis of some fish oils. The 11 samples of cod liver oil showed total DDT from 0.94 to 2.22, average (1.50 ± 0.31) mg/kg. The dogfish liver oil contained 2.44 mg DDT/kg, and the body oils from capelin, herring and mackerel showed 0.40, 0.31 and 0.45 mg DDT/kg, respectively. Finally the whale oil

had a content of 1.33 mg DDT/kg. On the basis of duplicate analyses on similar samples in our laboratory, the accuracy of these results is within ± 10% for concentrations above 0.5 mg DDT/kg. At lower concentration the precision is reduced.

With one exception the oils derived from fishes showed pp'-DDT as the major component, confirming the apparently slow degradation of DDT observed in cod by WESTØØ (1970). In the whale liver oil, however, pp'-DDE and pp'-DDD were the major components.

In Table 3 are reported the results from experiments to remove DDT from cod liver oil by molecular distillation, and the corresponding loss of vitamin A. The starting oil had a content of 710 I.U. vitamin A/g and 1.90 mg DDT/kg. The results are further presented graphically in Fig. 2. When the removal of DDT is considered in relation to the losses of vitamin A, a run at a temperature of 170°C seems to give the optimum condition. At this temperature 65% DDT was removed with a loss of about 25% vitamin A. At higher temperatures the loss of vitamin A increased rapidly, whereas the distillation (removal) of DDT leveled off. The practical applicability of the findings is difficult to evaluate, as the economy of the process depends on several factors.

SUMMARY

The distribution of DDT and its metabolities in cod liver oil and some other marine oils are reported. Cod liver oil showed an average content of (1.50 ± 0.31) mg DDT/kg. Dogfish liver oil contained 2.44 mg DDT/kg, and body oil from capelin, herring and mackerel, showed 0.40, 0.31 and 0.45 mg/kg, respectively. A whale oil showed a content of 1.33 mg DDT/kg.

Stripping of a cod liver oil by molecular distillation is reported. When the removal of DDT is seen in relation to the losses of vitamin A, a run at a temperature of 170°C seemed to give optimum conditions. At this temperature 65% DDT was removed with a loss of about 25% vitamin A.

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Table 2. Residues of DDT and its metabolites in different fish oils.

No Sample	Fish oils	Species	pp'-DDT mg/kg (%)	op'-DDT mg/kg (%)	pp'-DDE mg/kg (%)	pp'-DDD mg/kg (%)	Total DDT mg/kg
1	Cod liver oil	*Gadus morrhua*	1.26 (57)	0.12 (5)	0.45 (20)	0.39 (18)	2.22
2	—»—	—»—	0.55 (41)	0.06 (5)	0.34 (25)	0.39 (29)	1.34
3	—»—	—»—	0.62 (38)	0.10 (6)	0.58 (36)	0.32 (20)	1.62
4	—»—	—»—	0.90 (56)	0.14 (9)	0.30 (19)	0.26 (16)	1.60
5	—»—	—»—	0.31 (27)	0.02 (2)	0.34 (29)	0.49 (42)	1.16
6	—»—	—»—	0.95 (58)	0.16 (10)	0.31 (19)	0.21 (13)	1.63
7	—»—	—»—	0.54 (57)	0.05 (5)	0.14 (15)	0.21 (23)	0.94
8	—»—	—»—	0.95 (58)	0.12 (7)	0.23 (20)	0.24 (15)	1.64
9	—»—	—»—	0.76 (58)	0.06 (5)	0.31 (24)	0.17 (13)	1.30
10	—»—	—»—	0.78 (49)	0.12 (8)	0.45 (28)	0.24 (15)	1.59
11	—»—	—»—	0.46 (31)	0.03 (2)	0.47 (31)	0.55 (36)	1.51
12	Dogfish oil	*Squalus acanthias*	1.55 (64)	0.20 (8)	0.43 (18)	0.26 (11)	2.44
13	Capelin oil	*Mallotus villosus*	0.17 (43)	trace	0.11 (28)	0.12 (31)	0.40
14	Herring oil	*Clupea harengus*	0.16 (52)	trace	0.095 (30)	0.055 (18)	0.31
15	Mackerel oil	*Scomber scombrus*	0.30 (67)	0.01 (2)	0.06 (13)	0.08 (18)	0.45
16	Whale oil	*Balaenoptera acustorostrata*	0.31 (23)	0.02	0.55 (41)	0.45 (34)	1.33

Table 3. Removal of DDT and its metabolities and losses of vitamin A by stripping of a cod liver oil.[1])

Run	Vitamin A in rest-oil	Vitamin A losses (%)	DDT in Distillate (%)	DDT in Dome (%)	DDT in Cooling trap (%)	DDT in Rest-oil mg/g	DDT removed by M.D. (%)
A	200	71.8	47.3	8.2	16.0	0.57	71.5
B	365	48.6	31.8	17.9	20.6	0.58	70.3
C	555	21.8	23.7	17.0	23.7	0.68	64.4
D	590	16.9	22.0	10.1	22.3	0.89	54.4
E	560	21.1	20.2	1.2	13.3	1.25	34.7
F[2])	685	3.5		1.0	9.9	1.71	10.9

[1]) Cod liver oil, 710 I.U. vitamin A and 1.90 mg DDT/kg.

[2]) This sample was only degassed.

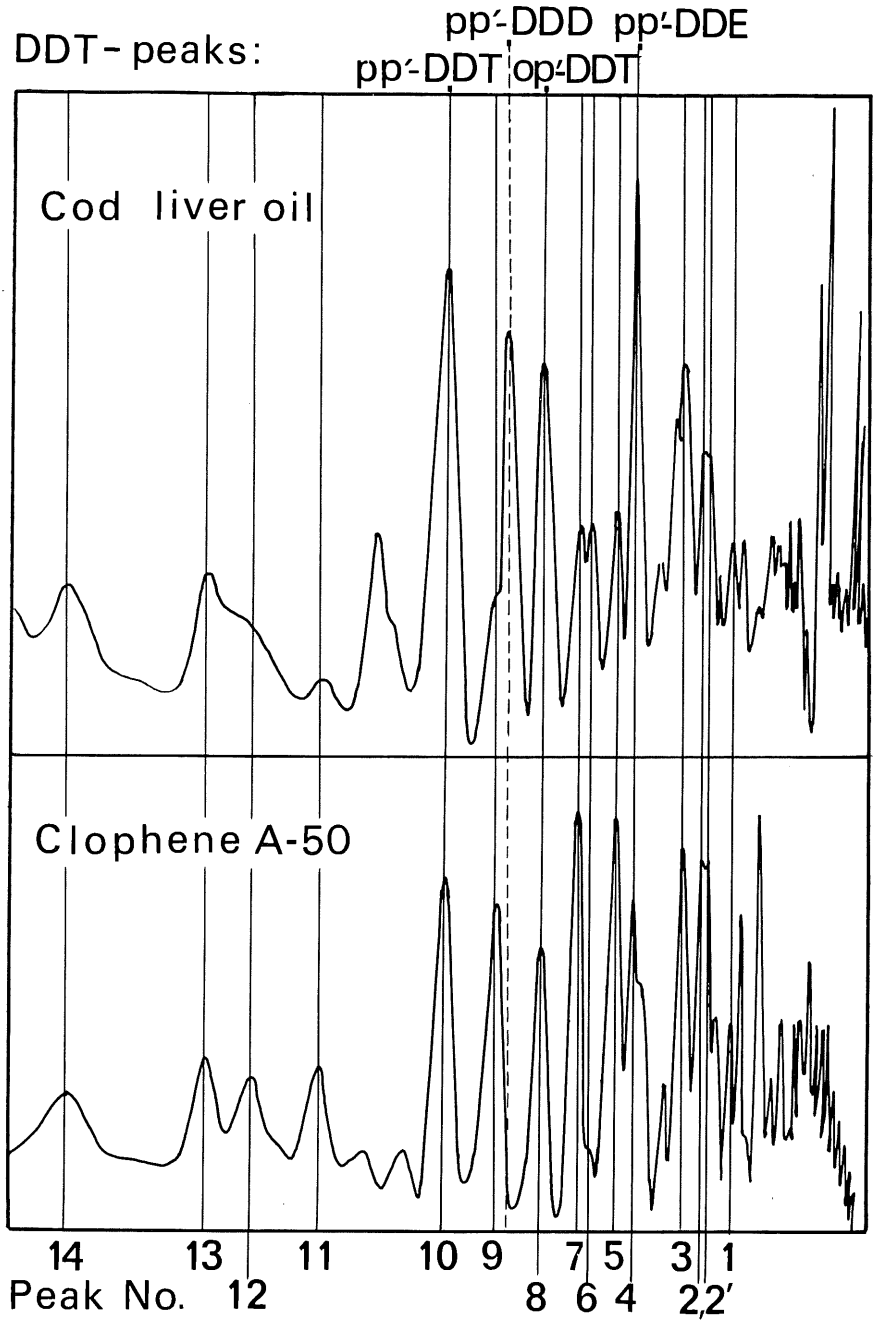

Fig. 1. Chromatograms of a cod liver oil and Chlophene A-50. The chromatographic patterns show where the peaks of Chlophene A-50 interfere with the peaks of DDT in the cod liver oil. Note the non-interference for pp'-DDD.

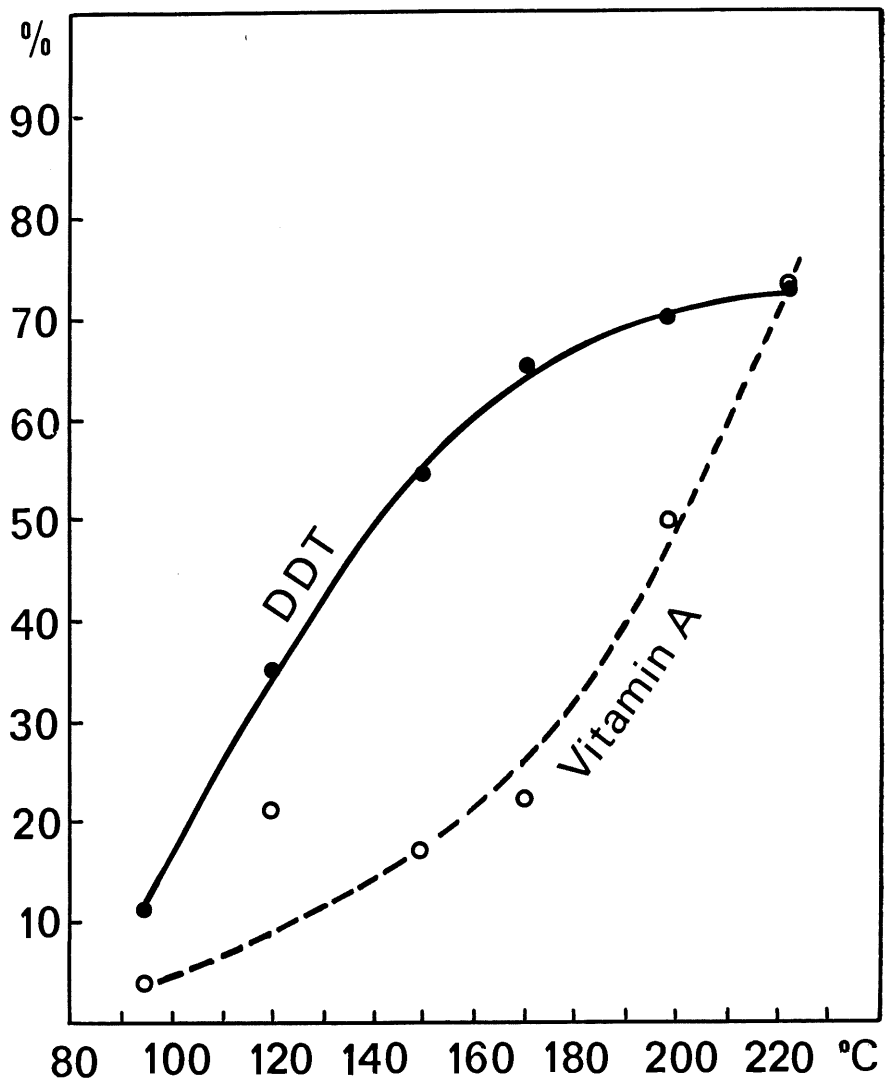

Fig. 2. Relation between removal of DDT and the corresponding losses of vitamin A from a cod liver oil containing 710 I.U. vitamin A/g and 1.90 mg DDT/kg.

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