Some Aspects of the Recovery of Chlorinated Residues (DDT-type Compounds and PCB) from Fish Tissue by Using Different Extraction Methods

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Because of their fat solubility both organochlorine pesticide- and PCB are stored in the fat tissue of organisms. Quantitative fat extraction is therefore essential for the complete extraction of residues. In the report of HOLDEN (1970a) it was stated that the analytical methods, especially extraction and cleanup, varied widely among those 17 laboratories which took part in the study of the report. Extraction involved cold or hot solvents of various types, for short or long periods in vertical columns by using an adsorbent material, in Soxhlet extractors or sometimes in open dishes [HOLDEN (1970b)].

The solvents most frequently used in extraction are acetone, acetonitrile, ether, hexane, petroleum-ether, methanol and chloroform [ARMOUR and BURKE (1971), COLE et al. (1971), HENDERSON et al. (1969), KOEMAN et al. (1969), LYMAN et al. (1968), REINERT (1970), ROTE et al. (1971) and TATTON et al. (1967)]. JENSEN (1970) paid special attention to the errors arising in the extraction method. According to his study the common method of using hexane and Na_2SO_4 -dried material may give pesticides quantitatively by not the fat content, the result of which is that the pesticide values calculated on fat basis are too big. Also the fat % in fish may vary from 2 to 10 % in herring from the same school and this causes a variation in pesticide contents also. In the publication of ABBOT (1971) it was recommended that the expression of analytical results on a fat basis was not advisable. It was also noted that great variations took place in the extraction efficiences of different methods concerning fish meat and poultry tissue. The method of PORTER et al. (1970) was recommended for the residue extraction of fish and meat.

In this study 4 solvent systems were studied and used as cold extraction in glass columns and as hot extraction in Soxhlet apparatus.

MATERIALS AND METHODS

a) Fish

The fish material consisted of pike, perch and bream from the northern part of the Baltic Sea. These fishes represent lean, medium fatty and fatty fish, respectively.

Two specimens of pike weighing 1 kg each, 4 perch weighing 0.5 kg and two bream weighing 1 kg were made into fillets which

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were homogenized in a Waring blender and the homogenate was further mixed for half an hour in a household mixer. Each fish homogenate was devided into 5 ± 0.05 g portions and dried with anhydrous Na_2SO_4 (Merck, p.a., Darmstadt, Germany). The mixing took place in a mortar and the homogenate was transferred to an aluminium foil sheet and dried for 48 hours at room temperature.

b) Equipment and solvents used

a. Glass columns with a sinter and a stopcock at the lower end (length 50 cm and inside diameter 2 cm)
b. Volumetrix glasses of 100 ml
c. Centrifuge tubes with a glass stopper
d. Soxhlet apparatus
e. Waterbath with a thermostat
f. Pure nitrogen gas

Solvents:

a. Diethylether (Merck p.a.)
b. n-pentane (Uvasol, Merck p.a.)
c. n-hexane (M&B, Dagenham, England, redistilled over KOH)
d. Acetone (Merck p.a.)
e. Petroleum ether b.p. 40-60°C (M&B, redistilled over KOH)
f. Chloroform (Merck p.a., redistilled)
g. Methanol (Merck p.a.)

c) Gas chromatographic equipment

Varian Model Aerograph 600-D with ^3H EC detector, glass columns 1.5 m -inside diameter 1.5 mm, injector, column and detector temperature 225°, 190° and 200°C, respectively. The column fillings used were (a) SF-96 on Chromosorb W (HMDS) 100-120 mesh and (b) a mixture filling containing 65 parts of 8 % QF-1 and 35 parts of 4 % SF-96 on Chromosorb W 100-120 mesh.

d) PCB and pesticide standards used

PCB standard: Clophen® A 60 , Bayer Leverkusen, Germany
p,p'DDE and p,p'DDT: Unilab Research Corporation, Berkeley, California, USA.

Extraction procedure

Method 1. Diethylether applied cold
Method 2. Diethylether and n-pentane 1:1 (v/v) applied cold
Method 3. 25 % n-hexane in acetone and 10 % diethylether in petroleumether 1:1 (v/v) applied cold
Method 4. Methanol and chloroform 1:1 (v/v) applied cold
Method 5. The solvent of method 1 applied warm in Soxhlet
Method 6. The solvents of method 2 - " -
Method 7. The solvents of method 3 (2.5 : 5.5 : 1 : 9 v/v) applied warm in Soxhlet
Method 8. The solvents of method 4 applied warm in Soxhlet

Method 1 has been used by BJERK and HOLT (1971), methods 2 and 3 have been obtained by personal communication and method 4 used in Soxhlet extraction is recommended as the basic extraction method to which other method should be compared ,[ABBOT (1971), PORTER et al. (1970)].

COLD EXTRACTION

15 samples of each fish were extracted as follows:

The dried homogenate was transferred quantitatively into the column through a funnel and 50 ml solvent was poured into the column. The columns were loosely packed because tight packing decreased the recovery. The columns were covered with aluminium foil. After two hours (the time chosen on the basis of preliminary experiments) the solvent was drawn off into a volumetric glass which contained acid washed anti-bumping granules. The column was washed (2x25 ml) with the same solvent to make the final volume 100 ml. For solvent III an exception was made in the respect that the dried fish homogenate was first kept for two hours in a hexane-acetone solution and then washed twice with 25 ml ether-petroleumether solution.

The solvent was evaporated to near dryness in a waterbath except for the chloroform-methanol which was evaporated in a Büchi-Rotavapor to avoid the possible losses of pesticides at high temperatures.

When 10 ml was left the residue was transferred into a weighed tube and the rest of the solvent was evaporated by nitrogen flow. The fat was dried to constant weight, in a desiccator overnight and weighed.

When methanol-chloroform was used for extraction, proteinaceous material was extracted also, and the amount varied from 80 to 100 mg/5g of fish tissue. The protein made the quantitative determination of fat difficult because the solution had to be filtrated at least once before the final evaporation.

SOXHLET EXTRACTION

In preliminary experiments the fish were extracted for different periods of time from 2 to 16 hours. Although in most cases the yield did not increase after 4 hours period the final time of 6 hours was chosen for the final determinations.

The dried fish homogenate was placed into a hexane washed thimble and extracted for 6 hours (the reflux 7 ml/min) after which the evaporation was carried out in the same manner as when the cold method was used. The final evaporation and fat weighing was made in identical fashion to the cold extraction.

RESULTS OF THE FAT EXTRACTION

For final results series of 15 tissue samples were extracted by the cold method and 10 samples by Soxhlet and the results of all the extraction methods are shown in table 1.

The results were analyzed by one way analysis of variance and the t-test. The results of the analysis of variance showed that the difference between methods was significant whatever risk level was chosen (from 0.1 to 5 %). The difference between individual methods was tested by the t-test. A 5 % risk level was chosen to reject the hypothesis that the difference between methods was not significant. When the efficiency of each extraction method of different species of fish was inspected, the results showed that when pike material was extracted the highest yield of fat was obtained by method 8 to which no other method was comparable. As for perch, the highest yield was obtained by method 7 to which 8 was comparable. For

TABLE 1

The amount of fat (mg) in 5 g of wet tissue in three species of fish obtained by 8 extraction methods

	cold				Soxhlet			
	1	2	3	4	5	6	7	8
pike	14.4	13.4	23	39	29	15.4	41	62
s_d	1.1	0.7	2	6	3	0.3	4	6
perch	65	59	67	66	85	83	92	92
s_d	6	7	7	9	9	3	8	13
bream	150	134	160	210	176	163	190	230
s_d	20	14	30	40	14	9	20	40

bream, method 8 was the best one to which method 4 was comparable. The extraction results were then inspected independently according to the specied of fish and the results from the t-test showed that the highest yield expressed as mg fat in 5 g of any tissue was obtained by method 8 which consisted of extracting by methanol and chloroform 1:1 in a Soxhlet apparatus. However, the difference was not significant if methods 5 and 7 were used. One of the cold extraction methods, method 4, was comparable to Soxhlet extraction.

THE RELATION OF THE RESIDUE YIELD TO THE FAT YIELD

To study if the maximum yield of fat results in the maximum yield of PCB and pesticides, the determination of PCB and chlorinated pesticides was carried out. The sulfuric acid method [AHLING and JENSEN (1970), BJERK and HOLT (1971) and JENSEN et al. (1972)] was chosen for cleanup. Because the absolute amount of fat in some cases was so small that the volumetric error in making a 1 % solution of the fat had grown too large, the extracts were combined to make homogeneous solutions. The cold extracts of each method were combined to make 5 solutions and the hot extracts 3 solutions.

After cleanup the samples were chromatographed and duplicate chromatograms were taken. Even if the amount of fat was sufficient for individual analysis, the combining was made to keep the results comparable. The only residues detected in the material were PCB, p,p'DDE and p,p'DDT. The PCB was determined as Clophen A 60. The calculation of PCB was carried out by using the method of HOLDEN (1970a) and the heights of 9 peaks of the total 13 were included in the calculations. For determining p,p'DDE the method of WESTÖÖ and NORÉN (1970) was used.

The final results are expressed as ng residues in 15 g of the wet tissue of the fish and are presented in table 2.

The results of the extraction experiment were tested by the analysis of variance and the t-test. The analysis of variance showed again that there were differences between the methods whatever risk level from 0.1 to 5 % was chosen. The differences between individual methods were tested by t-test. The risk level of 5 % was

TABLE 2

The amount of PCB, pp'DDE and p,p'DDT expressed ng/15 g wet fish tissue obtained by different extraction methods

Method	cold				Soxhlet			
	1	2	3	4	5	6	7	8
pike	1400	1770	2700	2700	3700	3000	4000	3700
PCB s_d	100	90	200	300	700	500	800	600
	350	424	460	720	450	420	720	1100
DDE s_d	30	12	20	60	100	94	140	180
	380	480	590	690	530	580	810	710
DDT s_d	42	52	36	47	110	110	160	110
perch	1540	1560	1890	880	3500	3300	3500	2200
PCB s_d	44	24	36	37	560	550	500	300
	450	620	600	490	770	770	840	710
DDE s_d	12	30	20	20	120	120	140	120
	1330	1600	1830	1500	2400	2200	2500	1300
DDT s_d	60	20	40	70	400	400	400	200
bream	1030	1120	1220	1340	2300	2300	2100	2100
PCB s_d	30	20	20	20	300	300	300	300
	405	518	560	597	810	760	950	840
DDE s_d	6	14	11	6	130	120	150	110
DDT	-	-	-	-	-	-	-	-

chosen to reject the hypothesis that the difference between methods was not significant. First the effect of the extraction method on every species of fish and every type of residue (PCB, DDE, DDT) was examined by one way analysis of variance. Then the effect of methods on every residue was examined regardless of the species of the fish. Finally the extraction effect was examined regardless of the species of the fish or residue and the results are presented in table 3.

The results of the t-test are presented so that the significant difference between the methods is denoted by a (+) and when the difference is not significant at the 5 % risk level it is denoted by a (-).

According to the results expressed as ng of residues (PCB+DDE+DDT) method 7 in which the mixture of hexane, acetone, diethylether and petroleumether 2.5 : 5.5 : 1 : 9 (v/v) was used for a 6 hours Soxhlet extraction gave the highest yield. The results of the t-test showed that all the other hot extraction methods were comparable with method 7.

As to the cold extraction methods, not one gave a yield comparable to the yield obtained by Soxhlet extraction. On the basis of the means of total residues in the materials examined, the best yield using cold methods was obtained by method 3, the yield of which was 64 % of the maximum.

TABLE 3

Significance between extraction methods regardless the species of the fish or the residue

Method	1	2	3	4	5	6	7
2	-						
3	+	+					
4	+	-	-				
5	+	+	+	+			
6	+	+	+	+	-		
7	+	+	+	+	-	-	
8	+	+	+	+	-	-	-

DISCUSSION

The factors affecting the final results, which are expressed as quantities of residues, are mainly the extraction process, clean-up and the determination by gas liquid chromatography. According to JENSEN et al. (1972) the extraction may not be effective and a conventional recovery experiment is not satisfactory because it is based on a post mortem addition of a standard. Only if radioactive marked test substances have been added to a animal alive, can a relevant recovery experiment be carried out.

Because Cl-labelled PCB was not available the efficiency of the methods used was compared with the method accepted as an official method [JENSEN et al. (1972), PORTER et al. (1970)], viz. extraction with methanol-chloroform 1:1 (v/v). The method which gave the highest yield of PCB and total DDT (DDT+DDE) had been modified from JENSEN's (1970) method in which the use of acetone is recommended for opening tissue cells.

Because of the great standard deviation, the method, when tested statistically was not better than the official method [JENSEN et al. (1972) and PORTER et al. (1970)]. The difficulties encountered in extraction when a chloroform-methanol mixture was used, for example, the precipitation of protein into the fat, would seem to favour the method used in this work. Because the method was not, however, statistically better than any other Soxhlet method, simpler solvent systems could be used which would reduce the work of redistilling. Also the evaporation of pentane and diethylether could be carried out at low temperatures.

The results showed obviously that cold extraction is not comparable with hot extraction at least when the residue concentration of the material is low (the average value of PCB in the material studied was 0.22 ppm and DDT+DDE 0.15 ppm, of the wet weight of the fish tissue).

The results also showed that great variation takes place in the fat extraction process even if homogenous material is extracted. It is therefore essential that the extraction method should tested for different materials and when the results are given the limits of errors which are statistically tested should be given.

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