ACTA CHEMICA SCANDINAVICA 24 (1970) 1639–1644

Determination of Organochlorine Pesticides and Polychlorinated Biphenyls in Animal Foods

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The determination by gas chromatography and thin-layer chromatography of organochlorine pesticides (OCP) in animal foods in the presence of polychlorinated biphenyls (PCB) is described. If PCB standards are available, the content of the PCB components can also be determined.

In gas and thin-layer chromatograms of extracts of organochlorine pesticides (OCP) from animal foods, peaks and spots caused by various polychlorinated hydrocarbons, mainly polychlorinated biphenyls (PCB), are often observed (Figs. 1 and 2). They were first identified in wildlife samples by Jensen [1] and have also been studied by, *e.g.*, Holmes *et al.*[2] and Reynolds.[3] As is to be expected by their composition, these substances are not influenced by the clean-up procedures usually used when OCP are determined in foods.

As is well known,[1–3] some PCB components and OCP compounds, *e.g.* DDT, DDD, and DDE, on gas-liquid chromatography have adjacent retention times. The PCB peaks thus disturb the gas chromatographic analysis of one or several of these pesticides (Figs. 3 and 4) in foods, especially in fish where the PCB level occasionally is high. In such cases the analysis of the OCP and PCB can be performed in the following way.

Analysis of lindane, α-BHC, β-BHC, aldrin, and heptachlor is not disturbed by PCB in several of the gas chromatographic systems applied to analysis of OCP. Accordingly these substances can be determined directly in an extract cleaned up in a regular fashion. We have used the extraction, clean-up and gas chromatographic methods described by Norén and Westöö.[4] Using an aluminium oxide thin-layer chromatogram, eluted 15 cm with heptane, dieldrin, heptachlor epoxide, *p,p'*-DDD, and *p,p'*-DDT (area I, Fig. 2) can be separated from *p,p'*-DDE, *o,p'*-DDT, *o,p'*-DDE, and PCB (area II, Fig. 2). The compounds in area I are then easily determined in a gas chromatogram of a hexane extract of this area. The determination of the compounds extracted from area II offers more problems. However, *p,p'*-DDE is usually the only OCP compound in this extract, and all the PCB components except Nos. 4

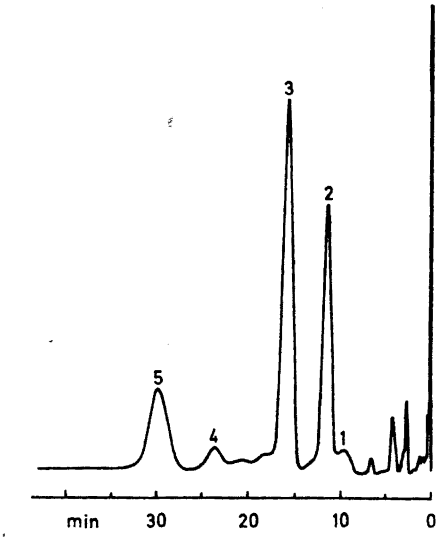

Fig. 1. Gas chromatogram of an extract of human milk (heptachlor epoxide added as internal standard). 1. PCB peak 2. 2. Heptachlor epoxide. 3. *p,p'*-DDE, disturbed by PCB peaks 4 and 5. 4. PCB peak 8. 5. *p,p'*-DDT, disturbed by PCB peak 10. *Cf.* Fig. 4.

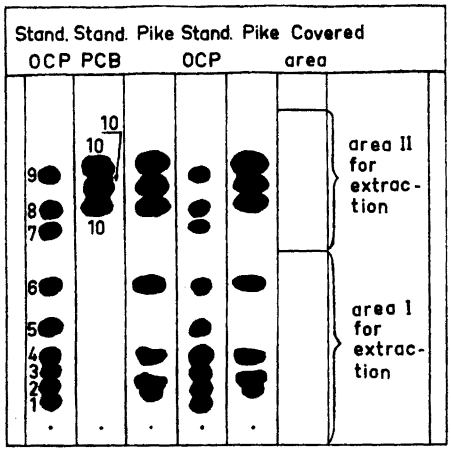

Fig. 2. Thin-layer chromatograms of standards and extracts of pike. 1. Dieldrin. 2. Heptachlor epoxide. 3. *p,p'*-DDD. 4. Lindane. 5. α-BHC. 6. *p,p'*-DDT. 7. *o,p'*-DDT, *o,p'*-DDE. 8. *p,p'*-DDE, heptachlor. 9. Aldrin. 10. PCB.

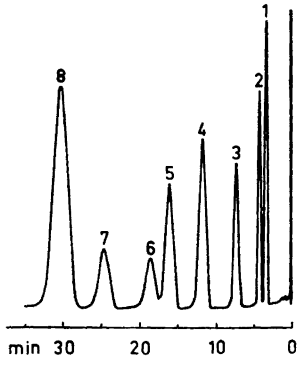

Fig. 3. Gas chromatogram of some chlorinated pesticides. 1. α-BHC. 2. Lindane. 3. Aldrin. 4. Heptachlor epoxide. 5. *p,p'*-DDE. 6. Dieldrin. 7. *p,p'*-DDD. 8. *p,p'*-DDT.

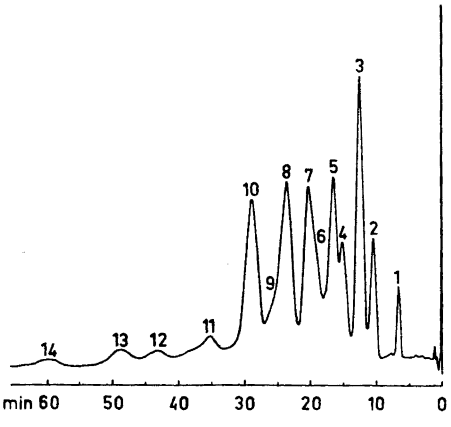

Fig. 4. Gas chromatogram of a PCB standard.

and 5, Fig. 4, can be determined by gas chromatography, if PCB standards are available. *o,p'*-DDT, only rarely present in animal foods in appreciable amounts, can be dehydrohalogenated to *o,p'*-DDE by boiling with alkali solution. *o,p'*-DDE (rarely present) and *p,p'*-DDE are decomposed by oxidation with chromic acid at room temperature without affecting PCB.

In a gas chromatogram performed after the oxidation, all the PCB components, except sometimes No. 2, Fig. 4, (*cf.* below can be determined. The *p,p'*-DDE, *o,p'*-DDE, and *o,p'*-DDT positions of the OCP – PCB peaks (Fig. 5 b and c) are measured before and after the oxidation, and the *p,p'*-DDE, *o,p'*-DDE and *o,p'*-DDT are calculated as differences.

When 0.1 – 1 μg *p,p'*-DDE/ml is present before the oxidation (if >1 μg/ml, the solution is diluted), a peak of *p,p'*-dichlorobenzophenone (verified by thin-layer chromatography) is seen in the gas chromatogram after the oxidation, at about the same retention time as PCB peak No. 2.

Aldrin and heptachlor are rarely observed in animal foods, but if they should be present, they will be found in the *p,p'*-DDE – PCB area of the thin-layer chromatogram. They do not disturb the analysis, since after oxidation heptachlor will give a peak with the same retention time as before, which does not interfere with the PCB peaks, and aldrin will vanish from the chromatogram.

Table 1. Recoveries of PCB and OCP added to purified fish extracts before application to thin-layer plate, or added to fish flesh before first extraction.

Compound, added		Recovery on addition to extract before application to thin-layer plate			Recovery on addition to fish flesh		
	mg/kg fish flesh	Number of samples	Average recovery %	Recovery range, %	Number of samples	Average recovery %	Recovery range, %
PCB, peak 2	0.026	3	96	82 – 105	3	95	82 – 109
3	0.033	3	96	92 – 102	3	92	89 – 95
5	0.023	3	106	97 – 115	3	100	99 – 101
7	0.024	3	100	89 – 110	3	94	94 – 95
8	0.025	3	100	94 – 111	3	89	84 – 95
10	0.020	3	101	88 – 116	3	83	76 – 91
α-BHC	0.050				5	87	82 – 92
Lindane	0.050				5	89	80 – 108
Heptachlor epoxide	0.100				5	92	89 – 96
p,p'-DDE	0.100				5	94	89 – 108
Dieldrin	0.050				5	93	86 – 102
p,p'-DDD	0.100				5	94	92 – 100
p,p'-DDT	0.500				5	94	89 – 98

Table 1 shows the results obtained when known amounts of PCB and OCP were added to fish samples, which were then analysed according to the procedure described. The average recoveries were 83 – 100 %. In Table 2 the

Table 2. *p,p'*-DDT and *p,p'*-DDD levels of a few samples of animal foodstuffs, calculated both from the gas chromatograms of the extracts from area I (Fig. 2), and as differences from the gas chromatograms of the original, purified extracts [4] and the oxidized extracts of area II (Fig. 2).

Sample	*p,p'*-DDT, mg/kg		*p,p'*-DDD, mg/kg	
	Calculated from chromatogram of extract from area I	Calculated as difference	Calculated from chromatogram of extract from area I	Calculated as difference
Egg yolk	0.021	0.020	—	0.002
Human milk	0.033	0.029	—	0.001
»	0.010	0.010	—	0.003
»	0.026	0.024	0.001	0.001
Pork	0.020	0.022	0.002	0.002
Eel	0.42	0.42	0.052	0.080
»	0.14	0.12	0.11	0.07
»	0.42	0.42	0.098	0.094
»	0.14	0.15	0.037	0.038

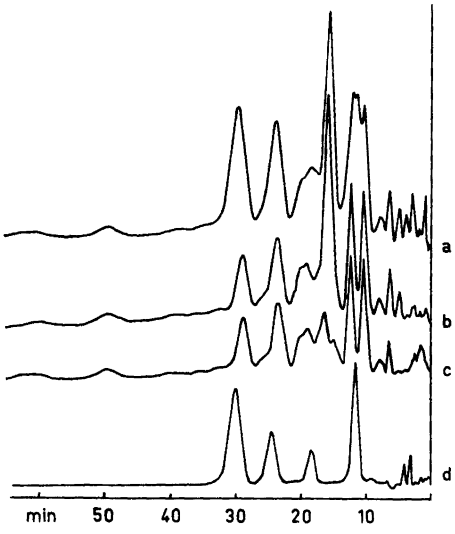

Fig. 5. Gas chromatograms performed for the analysis of PCB and OCP in a sample of Baltic herring (heptachlor epoxide added as internal standard). For standards, *cf.* Figs. 3 and 4.
a) Original extract.
b) Extract of area II, Fig. 2, before oxidation.
c) Extract of area II, Fig. 2, after oxidation.
d) Extract of area I, Fig. 2.

p,p'-DDT and *p,p'*-DDD levels of a few samples of animal foods are reported. The results for *p,p'*-DDT calculated from the gas chromatograms of the extracts from area I (Fig. 2) agree with those calculated as differences between the disturbed *p,p'*-DDT peaks obtained before the thin-layer separation, and the PCB interferences at *p,p'*-DDT positions in the chromatograms of the oxidized extracts of area II (a relative retention time of *p,p'*-DDT to PCB peak 10 of 1.03 and of *p,p'*-DDD to PCB peak 8 of 1.02 was used at the calcula-

tions; *cf.* Figs. 3 – 5). This shows that also the *p,p'*-DDE, *o,p'*-DDT, and *o,p'*-DDE figures, which are calculated only as differences in the present procedure, should be reliable, when these compounds are present in appreciable amounts.

EXPERIMENTAL

Reagents

Methanol, analytical reagent grade. *Hexane*, redistilled. *Water*, distilled. *Sodium hydroxide solution*, 2 N in water. *Chromic acid solution*. Dissolve 16 g of potassium dichromate in 185 ml of water and 1000 g of concentrated sulphuric acid. *Sodium sulphate*, anhydrous.

Procedure

Extraction of organochlorine pesticides and polychlorinated biphenyls from animal foods and routine clean-up of the extract were performed according to Norén and Westöö.[4]

Gas chromatography and thin-layer chromatography. For the main procedures, see Norén and Westöö.[4] Elute the samples on the thin-layer plates 15 cm with heptane.

Special procedure for samples containing PCB. Inject the cleaned-up extract into the gas chromatograph. Calculate the content of lindane, α- and β-BHC from this chromatogram, and also heptachlor and aldrin, if they should be present.

For each sample concentrate two portions of the extract, corresponding to 5 and 4 g of foodstuff, to about 0.1 ml, and apply the solutions to an aluminium oxide thin-layer plate together with standards. After elution with heptane (15 cm), cover the areas for the 5 g samples on the plate with aluminium foil. Spray the areas of the standards and the uncovered samples according to the procedure for OCP,[4] and expose them to UV light for identification of the components. When the spots of OCP are visible, collect the aluminium oxide area I of the covered samples (Fig. 2), containing, *e.g.*, dieldrin, heptachlor epoxide, *p,p'*-DDD and *p,p'*-DDT, and area II (Fig. 2), containing, *e.g.*, *p,p'*-DDE, *o,p'*-DDE, *o,p'*-DDT, and PCB, in separate tubes. Add 1 ml of methanol and 5.00 ml of hexane to each tube, and shake for 1½ min. Add 2 ml of water and shake for another minute. Centrifuge. Discard the water layers. Remove remaining methanol from the hexane solution by shaking with 10 ml of water. Dry the extracts with anhydrous sodium sulphate. As prolonged contact of, *e.g.*, α-BHC and lindane, with the aluminium oxide results in losses, the whole thin-layer procedure, inclusive the extraction, should be performed as fast as possible.

Put aside about 1 ml of each extract for gas chromatographic analysis before continuing with dehydrohalogenation or oxidation.

When more than 1 μg of *p,p'*-DDE/ml is likely to be present in the extract (A) from area II, dilute to less than 1 μg/ml (extract B) to facilitate oxidation.

If *o,p'*-DDT seems to be present, reflux 3.00 ml of the extract from area II (A or B) for 1 h with an equal volume of 2 N sodium hydroxide solution. Rinse the condensor with 2 ml of hexane and transfer the cool solution with 2 ml of hexane to a separatory funnel. Reject the water layer and wash the hexane solution twice with 5 ml of water. Transfer the hexane layer quantitatively to a graduated tube and concentrate to 3.0 ml (extract C).

Shake about 3 ml of the extract (A, B, or C) with 15 ml of chromic acid solution at room temperature for 6 min. Wash the hexane layer by shaking twice with 5 ml of water. Dry it with anhydrous sodium sulphate. Avoid evaporation of the hexane layer during the whole procedure. Inject the oxidized extract into the gas chromatograph and compare with the chromatograms of the cleaned-up food extract, the extracts of the areas I and II of the thin-layer chromatogram (Fig. 2), OCP standard, and, if available, PCB standard.

We are obliged to Dr. Sören Jensen for the gift of a PCB standard solution.

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Received November 15, 1969.