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Determination of Some Chlorinated Pesticides in Vegetable Oils, Margarine, Butter, Milk, Eggs, Meat, and Fish by Gas Chromatography and Thin-Layer Chromatography

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A procedure is described for clean-up of extracts of fats and animal foods for analysis of chlorinated pesticide residues by gas chromatography and thin-layer chromatography. The method has been applied to butter, margarine, vegetable oils, milk, eggs, meat, and fish.

A study of the content of chlorinated pesticides in vegetable oils, margarine, butter, milk, eggs, meat, and fish was started in this laboratory at the end of 1965. About 1000 samples of vegetable oils, margarine, butter, meat,[1] milk,[2] eggs, and fish have so far been analysed with the method described below.

In 1965 many procedures for analysis of chlorinated pesticide residues in fats by gas chromatography were based on partition between two solvent systems, *e.g.* acetonitrile,[3–6] dimethyl sulphoxide,[7] or dimethylformamide [8–10] and hexane or light petroleum, and additional chromatographic clean-up. Later Tolbert [11] and Giuffrida *et al.*[12] described more efficient clean-up methods on a similar basis.

For a thin-layer chromatography control [13] of small amounts of pesticides (0.01 mg/kg of food, necessitating application of an extract corresponding to 5—10 g of fat to the chromatographic plate) the extracts obtained by the methods available in 1965 usually contained too much residual fat. The gas chromatographic column and detector also were contaminated rather rapidly. Therefore a modification of the dimethylformamide-hexane partition of the pesticides and the fat was worked out. Water was added to the dimethylformamide to diminish the extraction of fat by the amide. Water contents from 6 to 10 % (v/v) were tried.

Recovery experiments were carried out with addition of standard solutions of α-BHC, lindane, heptachlor, heptachlor epoxide, aldrin, dieldrin, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, and *p,p'*-DDT to the fat solution before

Table 1. Recoveries of chlorinated pesticides added to vegetable oil, margarine, or butter using dimethylformamide containing 8 % of water in the clean-up procedure.

Pesticide	Pesticide added, mg/kg	Number of samples	Average recovery %	Recovery range %
α-BHC	0.050	7	86	80—92
Lindane	0.050	11	90	86— 97
Heptachlor	0.050	4	90	85—93
Heptachlor epoxide	0.100	18	94	91— 99
Aldrin	0.050	13	66	56— 74
Dieldrin	0.050	12	95	86—102
p,p'-DDT	0.50	15	96	90—101
o,p'-DDT	0.100	4	93	88 —99
p,p'-DDE	0.100	11	87	84— 91
p,p'-DDD	0.100	8	94	86—102

extraction. When 6 or 7 % of water was used, the dimethylformamide extracts were not clean enough. 8 % of water in dimethylformamide was preferred, as it gave a sufficiently clean extract for thin-layer chromatography of 10 g of fat and good recoveries for all the pesticides investigated except aldrin (Table 1). With the addition of 10 % of water low recoveries for heptachlor and aldrin were obtained. Recovery determinations were performed also with milk, meat, eggs, and fish, but only with dimethylformamide containing 8 % of water (Table 2). Besides, hexane solutions of methoxychlor, Perthane, α-chlordane, and toxaphene were investigated. They all passed the procedure with 83—92 % recovery.

The method is not convenient for aldrin. If aldrin should be found, the content must be corrected with a recovery factor calculated from the results in Tables 1 or 2. So far aldrin has not been detected in any sample.

Table 2. Recoveries of chlorinated pesticides added to meat, eggs, fish, or milk using dimethylformamide containing 8 % of water in the clean-up procedure.

Pesticide	Pesticide added mg/kg of meat, fish, or egg	mg/kg of milk	Number of samples	Average recovery %	Recovery range %
α-BHC	0.050	0.0025	5	87	82— 92
Lindane	0.050	0.0025	12	86	80—100
Heptachlor	0.050	0.0025	2	86	80— 92
Heptachlor epoxide	0.100	0.0125	17	93	89— 96
Aldrin	0.050	0.0025	12	67	59— 74
Dieldrin	0.050	0.0025	12	93	86—102
p,p'-DDT	0.50	0.0250	17	94	89—100
o,p'-DDT	0.100	0.0050	4	84	80— 87
p,p'-DDE	0.100	0.0250	15	89	86— 99
p,p'-DDD	0.100	0.0050	12	95	92—100

When samples of foods were analysed for pesticides, a known amount of heptachlor or heptachlor epoxide was added to the food and used as internal standard[14] in the procedure. In the routine work the pesticide contents in the samples were corrected according to the recovery of the internal standard in each sample. The samples of margarine and vegetable oils were analysed using the gas chromatographic column I. For samples containing *p,p'*-DDE, which was not separated from dieldrin by column I, (milk, butter, meat, eggs, and fish) column II was used. All the samples were subjected to thin-layer chromatography. For safe identification of the pesticides both columns were often used for gas chromatography of a sample together with thin-layer chromatography. When necessary the spots of pesticides on the thin-layer chromatograms were extracted and injected on the gas chromatograph as an extra control.

Polychlorinated biphenyls[15] are sometimes present in fish in considerable amounts. They interfere with the peaks of the chlorinated pesticides and thus make a more elaborate procedure necessary. 80—90 % of added polychlorinated biphenyls are recovered.

For retention times of the pesticides on the columns used and for R_F values, see Westöö *et al.*[16]

EXPERIMENTAL

Apparatus.

Volumetric flasks, 10-ml, 200-ml. *Separating funnels*, 250-ml, 500-ml, 1-l. *Chromatographic column*, 2 cm diameter. *Evaporator*, rotating, for evaporation under reduced pressure.

Gas chromatograph. Varian Aerograph Hy—Fi 600 and Varian Aerograph Hy—Fi III 1 200 equipped with electron capture detectors were used. Column I: Pyrex glass 1/8″ × 5′, packed with 5 % DC 11 on Chromosorb W 60/80 mesh. Gas flow rate: 75 ml of nitrogen/min. Temperature of column: 195°. Temperature of injector: 210°. Temperature of detector: 195°. Column II: Pyrex glass 1/8″ × 5′, packed with a mixture of equal parts of 5 % DC 11 on Chromosorb W 60/80 mesh and 15 % QF-1 on Chromosorb W 60/80 mesh. The column was conditioned for 72 h at 240°. Gas flow rate: 30 ml of nitrogen/min. Temperature of column: 171°. Temperature of injector: 200°. Temperature of detector: 200°. *Recorder*, Texas Instrument, model PWS—IMVC-05-A 25—BT.

Thin-layer chromatography apparatus, Desaga applicator and Camag "sandwich chamber".

Ultraviolet light source. Two Westinghouse WL-782-30L, 2 537 A.

Reagents

Dimethylformamide, Baker & Adamson or J. T. Baker, analytical reagent grade. Mix 92 ml of dimethylformamide and 8 ml of water. *Ethanol. Ethyl ether*, anhydrous, analytical reagent grade. *Hexane*, redistilled. *Light petroleum*, redistilled. *Heptachlor solution*, 0.50 μg of heptachlor per ml of hexane. *Heptachlor epoxide solution.* 1.00 μg of heptachlor epoxide per ml of hexane (0.50 μg/ml for milk samples). *Standard solutions for gas chromatography.* 0.01—0.05 μg of α-BHC, lindane, heptachlor, aldrin and dieldrin, 0.10—0.25 μg of heptachlor epoxide, 0.02—0.10 μg of *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT and 0.1—0.5 μg of *p,p'*-DDT per ml of hexane. *Standard solutions for thin-layer chromatography.* 0.01 or 0.1 μg of α-BHC, lindane, heptachlor, aldrin, dieldrin, heptachlor epoxide, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT and *p,p'*-DDT per μl of hexane. *Sodium sulphate solution*, 2 % in water. *Anhydrous sodium sulphate*, heated overnight at 450°. Store in tightly stoppered flask. *Potassium oxalate*, analytical reagent grade. *Aluminium oxide*, Brock-

mann, activated. Heat aluminium oxide in an oven at 800° for 4 h. After cooling add 5 % (v/w) of water and mix carefully by rotation in a sealed flask until homogeneous (about 2 h). When the oxide is stored in a tightly stoppered flask, the activity remains for 7 days. *Aluminium oxide* G, with 15 % $CaSO_4$, for thin-layer chromatography. *Developing solvent for thin-layer chromatography.* Hexane, redistilled + anhydrous ethyl ether, analytical reagent grade (40+0.8). *Spraying agent for thin-layer chromatography.* Dissolve 0.10 g of silver nitrate in 1 ml of water and add 10 ml of 2-phenoxyethanol. Dilute the solution to 200 ml with acetone and add one drop of 30 % hydrogen peroxide.[13]

Preparation of thin-layer plates. Apply a 0.25 mm thick layer of aluminium oxide to glass plates and pre-wash the adsorbent layer according to Kovacs.[13] Activate the plates at 105° for 2 h before use. After application of standards (5 µl) and samples, develop the plates 11 cm, spray with the silver nitrate spraying agent and expose to short wave UV light for ½−2 h according to the concentration of the samples.

Procedure I (for butter, margarine, and vegetable oils)

Melt 100 g of butter or margarine and filter through a folded paper. Transfer 40.0 g of vegetable oil or filtered fat to a 200-ml volumetric flask and make up to the mark with hexane.

Transfer 50.0 ml of the sample solution to a 250-ml separating funnel and add 1.00 ml of internal standard solution. Shake with 50 ml of dimethylformamide solution for 2 min. After separation transfer the dimethylformamide layer to a 1-l separating funnel containing 400 ml of 2 % sodium sulphate solution. Repeat the extraction of the sample solution with dimethylformamide solution 3 times and transfer the extracts to the 1-l funnel. Add 50 ml of hexane to the combined extracts and shake vigorously for 2 min. Discard the aqueous layer and wash the hexane layer with 200 ml of sodium sulphate solution.

Transfer the hexane layer to an evaporator, rinse the separating funnel with small portions of hexane, and evaporate the combined extract and washings at reduced pressure at 35° to 2 ml.

Pack a chromatographic column (Ø 2 cm) with 10 g of activated aluminium oxide, and put a 3.5 cm layer of anhydrous sodium sulphate on the top. Pre-wet the absorbent with 25 ml of hexane.

Transfer the evaporated extract quantitatively to the column and elute with 100 ml of hexane. Evaporate the eluate at reduced pressure at 35° to 5 ml, transfer quantitatively to a 10-ml volumetric flask and make up to the mark with hexane. Examine the solution by gas chromatography and after further concentration by thin-layer chromatography. Using this clean-up an amount of extract corresponding to 10 g of fat can be investigated by thin-layer chromatography. Thus the presence of less than 0.01 ppm of the chlorinated pesticides can be checked.

Procedure II (for milk)

Add 1.00 ml of heptachlor epoxide solution (0.50 µg of heptachlor epoxide/ml of hexane), 1 g of potassium oxalate and 100 ml of ethanol to 100 g of milk in each of two 500 ml centrifuge bottles and shake for 10 min in a shaking-machine. Treat the contents in each centrifuge bottle as follows. Add 50 ml of ethyl ether and shake vigorously. Add 50 ml of light petroleum and shake for 5 min. Centrifuge for 45 min (or until the phases separate). Transfer the light petroleum−ethyl ether layers from both bottles with a pipette to the same 1 l (for meat, eggs, and fish 500 ml) separating funnel.

Shake the residue in the centrifuge flask vigorously with 50 ml of light petroleum-ethyl ether (1+1) for 5 min. Centrifuge. Repeat the extraction. Collect the light petroleum-ethyl ether phases in the 1 l (500-ml for meat, egg, and fish) separating funnel and shake with 100 ml (50 ml for meat, egg, and fish) of water. Discard the aqueous layer. Evaporate the organic solvents at reduced pressure at 35° to *ca.* 1 ml. Transfer the residue with 50 ml of hexane to a 250 ml separating funnel. Continue according to procedure I starting with the shaking with 50 ml of dimethylformamide solution.

Procedure III (for meat, eggs, and fish)

Mince 100 g of meat in a mincing machine and mix thoroughly. Homogenize 10.00 g of minced meat, egg, or fish in a homogenizer with 50 ml of ethanol. Transfer the mixture to a 500 ml centrifuge flask with 90 ml of water. Rinse the homogenizer with 50 ml of ethanol and transfer to the flask. Add 1.00 ml of heptachlor epoxide solution (1.00 μg of heptachlor epoxide/ml of hexane) and shake for 10 min in a shaking-machine. Add 50 ml of ethyl ether, using part of it for rinsing the homogenizer. Shake vigorously. Continue according to procedure II, starting with the addition of 50 ml of light petroleum.

Blank. Proceed according to the above descriptions, only exchanging the sample for water.

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REFERENCES

1. Andersson, M., Norén, K. and Westöö, G. *To be published.*
2. Norén, K. and Westöö, G. *To be published.*
3. Jones, L. R. and Riddick, J. A. *Anal. Chem.* **24** (1952) 569.
4. Mills, P. A. *J. Assoc. Offic. Agr. Chemists* **42** (1959) 734.
5. McKinley, W. P. and Mahon, J. H. *J. Assoc. Offic. Agr. Chemists* **42** (1959) 725.
6. Klein, A. K., Watts, J. O. and Damico, J. N. *J. Assoc. Offic. Agr. Chemists* **46** (1963) 165.
7. Eidelman, M. *J. Assoc. Offic. Agr. Chemists* **45** (1962) 672.
8. Burchfield, H. P. and Storrs, E. E. *Contrib. Boyce Thomson Inst.* **17** (1953) 333.
9. Abdallah, M. D. and Landheer, C. A. *J. Chromatog.* **9** (1962) 245.
10. de Faubert Maunder, M. J., Egan, H., Godly, E. W., Hammond, E. W., Roburn, J. and Thomson, J. *Analyst* **89** (1964) 168.
11. Tolbert, C. E. *J. Assoc. Offic. Agr. Chemists* **49** (1966) 386.
12. Giuffrida, L., Bostwick, D. C. and Ives, N. F. *J. Assoc. Offic. Agr. Chemists* **49** (1966) 634.
13. Kovacs, M. F. *J. Assoc. Offic. Agr. Chemists* **46** (1963) 884.
14. Storrs, E. E. and Burchfield, H. P. *Contrib. Boyce Thomson Inst.* **21** (1962) 423.
15. Jensen, S. *New Sci.* **32** (1966) 612.
16. Westöö, G., Norén, K. and Andersson, M. *To be published.*

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