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# Rapid Determination of Chlorinated Pesticides, Polychlorinated Biphenyls and a Number of Phosphated Insecticides in Fatty Foods

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## *Introduction*

It is well known that fatty foods such as milk, meat, fish, eggs and vegetable oils often contain detectable amounts of organochlorine pesticides. In recent years, several authors have also reported the presence of polychlorinated biphenyls, especially in fish (1—5).

Furthermore, some of the less polar and fat soluble organophosphorus insecticides are increasingly found in foods with a high fat content (6). The analytical methods which are at present in use for the determination of these residues are mostly modifications of the multiresidue method as developed by the specialists of the U.S. Food and Drug administration (7). Although this procedure has been found to be reliable, as illustrated by a large number of publications (8), it has some limitations which narrow its field of application:

1. The method is not so easy to carry out by relatively untrained staff. Reliable results are only obtained after considerable training and supervision.
2. It also takes much time, especially for the analysis of fresh milk because the fat has to be isolated prior to extraction and clean-up of the pesticides.
3. It is not suitable for the determination of hexachlorobenzene (HCB) residues. This fungicide is not quantitatively carried through the acetonitrile partition step and the recommended gas chromatographic column is not able to give a complete separation of HCB and BHC and these residues often occur together (9).

An alternative method which finds far less application is the procedure of *Langlois, Stemp* and *Liska* (10).

It has gained some popularity in France (11) and in Australia (12), but no validation studies have been published.

The present authors undertook the study of this method in 1969 and introduced several modifications which considerably extended its field of applica-

tion. Since then, the procedure as now described in this paper is successfully used by a number of residue laboratories checking Nestlé products and the raw materials used for their manufacture all over the world.

## *Method*

1. *Field of application*

The method can be applied to meat, fish, all fats and oils, eggs, egg yolk powder, milk and dairy products, cocoa powder and chocolate.

2. *Principle of the method*

From meat, fish and cheese the fat is first extracted by a suitable method. A quantity of oil or fat not exceeding 1 g is placed on the top of a chromatographic column composed of partly deactivated Florisil. For the analysis of milk, dairy products, eggs, egg yolk powder, cocoa powder and chocolate an adsorption clean-up is performed by mixing a certain quantity of the sample (in presence of water) with enough Florisil to form a free-flowing powder. During this procedure the Florisil is deactivated and all the sample's constituents such as fat, protein etc. are coated as a thin film on the adsorbent particles. In this way, the sample becomes easily accessible to extracting solvents. The powder thus prepared is brought on top of an ordinary chromatographic column of partly deactivated Florisil immersed in light petroleum. Subsequently, the pesticides are eluted with methylene chloride in light petroleum (20 + 80) and the eluate is evaporated just to dryness, after which the residues are taken up in a small volume of light petroleum.

Tentative identification and quantitation of possible present residues is achieved by gaschromatography, both with an electron capture and a phosphorus detector. Confirmatory analysis is carried out by thin-layer chromatography in two suitable systems.

3. *Reagents*

3.1 *Light petroleum*

This solvent should be purified, preferably by distillation in an all-glass apparatus equipped with a 1 meter long fractionating column filled with Raschig rings. The distillate should pass the following general purity test for solvents:

Place 200 ml of the light petroleum in a Rotavapor apparatus and evaporate until about 5 ml remain. Inject 5 microliters of the concentrate into the gas chromatograph under the conditions described under 5.8.1. The concentrate must not cause recorder deflection from baseline for 1—45 minutes after injection.

3.2 *Methylene chloride, pure*

Any purum quality of Merck or Fluka may be used. Purify by distillation in an all-glass apparatus, collecting the fraction which distills at 39 ° C.

3.3 *Eluting mixture*

Dilute 200 ml of distilled methylene chloride to 1 litre with distilled light petroleum.

3.4 *Florisil, synthetic magnesium silicate, 60—100 mesh*

Quite often this commercial product contains impurities which interfere with the determination of chlorinated pesticides. These impurities, mainly polychlorinated biphenyls (PCB) can be removed by heating the Florisil overnight at 550 ° C. Store in a well-closed glass container.

Before use, standardize the adsorbent in the following way:

Heat at least 5 hours or preferably overnight at 130 ° C. Pour the Florisil in a 1 liter flask and add 3 percent by weight of distilled water. Mix by shaking the flask for at least 20 minutes and let equilibrate during 10—12 hours.

The partly deactivated adsorbent thus obtained can only be used up to three days after its preparation, after which time it should be standardized again.

Test the standardized adsorbent before use by checking the recovery: Add known amounts of HCB, lindane, DDT, dieldrin, bromophos and ethion to samples of fat in which pesticides have not been previously detected. Proceed with the clean-up and gas chromatographic determination as described under 5.4—5.8.2 and calculate the recovery for each pesticide.

The recovery for HCB, lindane and DDT should be at least 95 %. For dieldrin, bromophos and ethion a recovery of about 80 % may be considered acceptable, provided that the concentrated sample extract contains not more than traces of fat.

If the recoveries for the three more polar pesticides are too low, repeat the recovery experiment either using Florisil containing 4 % of water or increase the volume of the eluting mixture by 50 ml.

3.5 *Toluene p. a.*

Test for absence of electron capturing compounds by simply injecting 5 microliters of the solvent as such into the gas chromatograph under the conditions described under 5.8.1.

3.6 *Acetone p. a.*

3.7 *Ethanol 96 % p. a.*

3.8 *Methanol p. a.*

3.9 *Silver nitrate p. a.*

3.10 *Chromogenic reagent for the TLC of chlorinated pesticides*

Dissolve 0,5 g of silver nitrate in about 1 ml of distilled water. Add 99 ml of 96 % ethanol and mix.

3. 11 *Lyophilised human blood serum*

The quality supplied by the Nutritional Biochemical Corp. Cleveland Ohio 44128, U.S.A. was found to be suitable.

3.12 *Beta-naphthyl acetate,* naphthol free

3.13 *Fast Blue salt* (Di-o-anisidine-tetrazolium chloride)

The quality of Merck's Echtblau salz was found to be suitable.

3.14 *Anhydrous sodium sulfate p. a. quality*

Remove interfering impurities by heating overnight at 550 ° C.

3.15 *Analytical standards of pesticides*

High purity standard compounds were obtained from:

Dr. S. u. I. Ehrenstorfer
89 *Augsburg*
Fritz-Hintermayerstr. 3
Bundesrepublik (Germany)

3.16 *Standard solutions for chromatographic purposes*

Prepare solutions containing 1 mg of each pesticide pro ml in pure toluene. From these stock solutions prepare the followig dilutions:

a) *Reference solutions for thin-layer chromatography (TLC)*

For chlorinated compounds: dilute accurately the stock solution 20 times with toluene. This dilution contains 0,05 mg/ml or 0,05 mcg pro microliter.

For phosphated pesticides: dilute accurately the stock solution 100 times with toluene. This dilution contains 0,01 mg/ml or 0,01 mcg pro microliter.

b) *Reference solutions for gas chromatography (GLC)*

Prepare intermediate reference solutions containing 1 mcg/ml or 1 nanogram pro microliter. The concentration of the final reference solutions depends on the magnitude of the GLC-response of the individual pesticides. For most chlorinated pesticides, solutions containing 0,002, 0,005, 0,01 and 0,02 nanogram pro microliter are needed. A notable exception is toxaphene which gives only measurable peaks at the nanogram

level. Phosphated pesticides give far less response both to the electron capture and the phosphorus detector. For these compounds, prepare dilutions containing 0,05, 0,1 and 0,2 nanogram pro microliter.
In order to save costly solvent and to keep the number of standards within reasonable limits, it is recommended to prepare mixed standard solutions. For example, one reference solution containing the four isomers of BHC, another containing DDT plus its analogues, etc.

3.17 Viscous paraffin. Merck no. 7160

4. *Apparatus*

4.1 Gas chromatograph, equipped with dual column arrangement, electron capture detector and phosphorus detector.

4.2 Gas chromatographic columns: 5 feet long pyrex glass columns with an inside diameter of approximately $^1/_8$ inch filled with the following stationary phases:

— 1,5 % OV-17 + 1,95 % QF-1 coated on Chromosorb W, H.P. 100—120 mesh DMCS treated.
— 2 % DEGS polyester + 0,5 % phosphoric acid also coated on Chromosorb W 100—120 mesh.

Condition both columns at 235 ° C with a nitrogen flow of 50 ml during at least 36 hours. During this time the column should be disconnected from the detector.
When using a freshly conditioned column, check performance by injecting a small quantity of p,p'DDT. From a good column this compound will be eluted as a perfect gaussian peak in about 15—20 minutes. Calculate the number of theoretical plates, N, using the equation:

$$N = 16\,(x/y)^2$$

where N = total theoretical plates, x = distance in mm from injection point to peak center, and y = width of peak base in mm.
Generally speaking, a good column should yield not less than 3000 theoretical plates. Columns with a significantly lower efficiency will exhibit poor separation characteristics such as broad overlapping peaks and an inability to separate critical pairs. Examples of good separations are given in figures 1 and 2.
Sensitivity, i. e. the magnitude of response for the individual pesticides, depends to a large extent on the quality of the column. Peak height response may be improved by injecting microgram quantities of the individual pesticides. Often, however, more significant improvement occurs after injection of a number of sample extracts, especially for the responses of p,p'DDT and endrin.

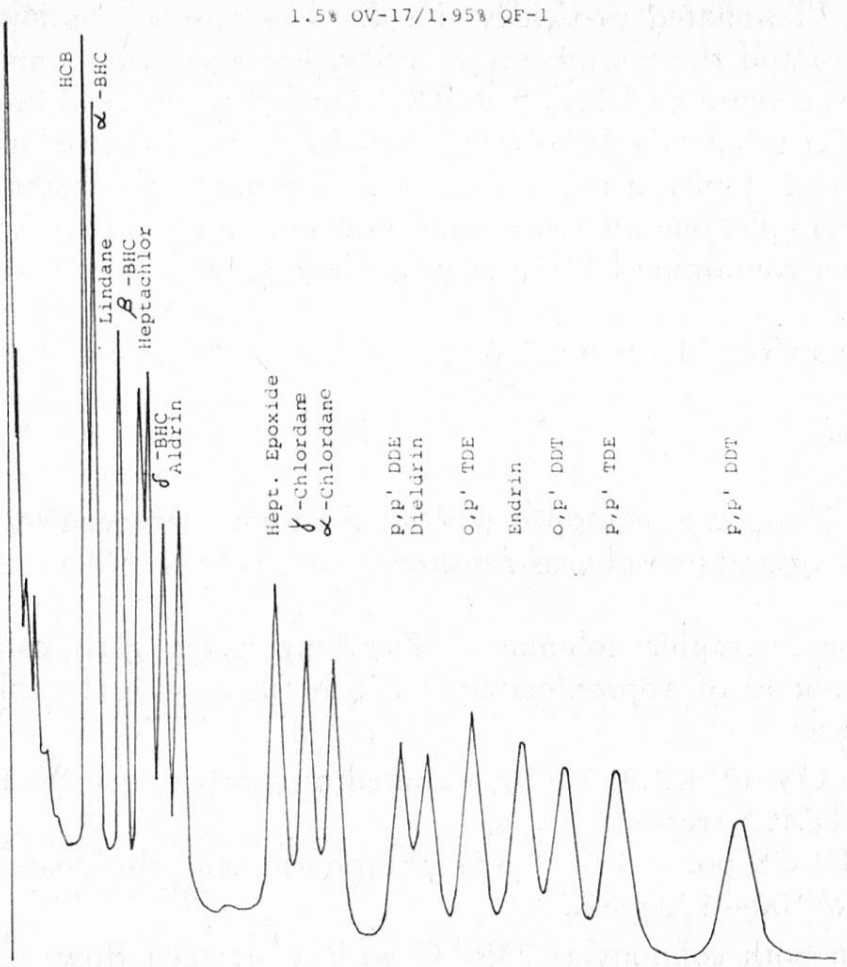


Fig. 1. Separation of 17 chlorinated pesticide compounds on a 1,5 % OV-17/1,95 % QF-1 column.

4.3 Ready made plates for thin-layer chromatography.

— For chlorinated pesticides: aluminium oxide, type E.F254, alufoil sheets from Merck. Catalogue number 5550/0025.

— For phosphated pesticides: $SiO_2$ 60 Merck, precoated plates an glass, layer thickness 0,25 mm, catalogue number 5721.

4.4 Ultraviolet lamp for photochemical revelation of chlorinated pesticides on TLC:

Recommended is the «Quarzbrenner für photochemische Zwecke» of Philips, type HPK 125 W/L with a transformer VG 1/HP 125 W.

4.5 Rotavapor apparatus — rotative evaporator connected to a waterjet pump.

4.6 Chromatographic tubes, fitted with stopcocks 22 mm id×250 mm, having a 250 ml reservoir at the upper end.

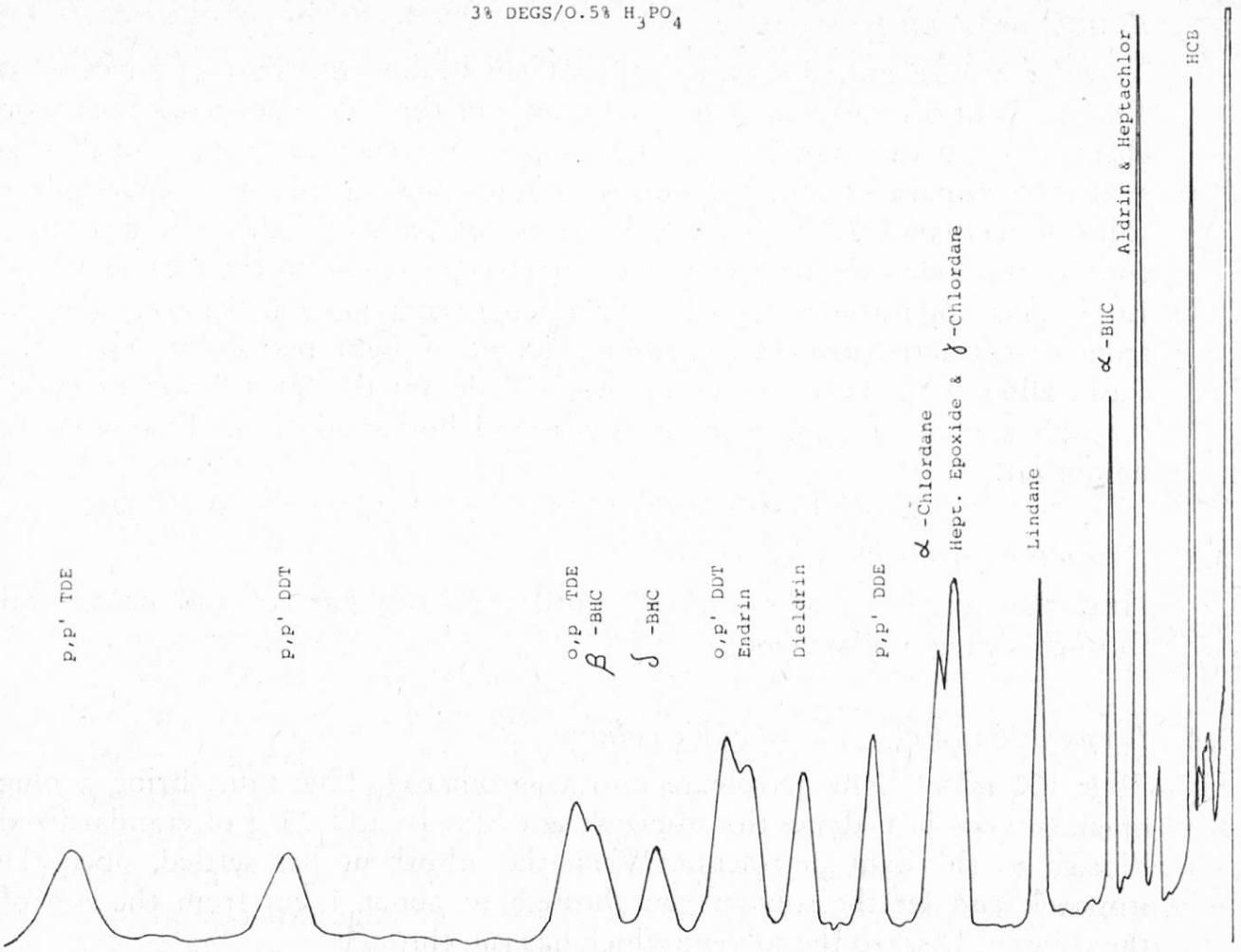


Fig. 2. Separation of 17 chlorinated pesticide compounds on a 2 % DEGS column stabilised with 0,5 % phosphoric acid.

4.7 Various glassware.

4.8 Microsyringe for GLC: recommended is the Hamilton automatic syringe, type CR 700—20.

5. *Procedure*

5.1 *Extraction of fat from meat and fish*

Cut 25 g of the sample into very small pieces, preferably with a meat chopper. Grind with sufficient anhydrous sodium sulfate to form a coarse powder. Transfer to a 300 ml conical flask, add 75 ml of pure light petroleum and let boil under reflux for 10 minutes. Let cool and pour the supernatant liquid into a 500 ml round bottomed flask. Repeat the refluxing with 75 ml of light petroleum three times and evaporate combined extracts in the rotative evaporator in order to obtain fat.

5.2 *Extraction of fat from cheese*

Transfer a 30 g grated sample to a 250 ml beaker and heat in an oven at 100 ° C. Pour off clear fat. This procedure sometimes does not work for cream cheese. In that case, cut 25 g of the sample into small pieces and transfer to a Soxhlet apparatus. Extract during 3 hours with a mixture of methylene chloride-methanol 2:1, v/v. Avoid the use of paper thimbles, because they contain too much electron capturing material. Evaporate the extract in the Rotavapor apparatus until all solvent has been removed. Separate the fat from co-extracted protein by adding 20 ml of light petroleum. Swirl the flask, allow it to stand for 15 minutes and decant the fat solution carefully through a plug of glass wool into a round bottomed flask. Evaporate to obtain fat.

5.3 *Extraction of fat from butter*

Heat the sample to about 60 ° C until the fat separates and decant fat through a plug of glass wool.

5.4 *Preparation of chromatographic column*

Pour 100 ml of light petroleum into a chromatographic tube. Bring a plug of glass wool just above the stopcock and slowly add 25 g of standardized Florisil to the light petroleum. When the adsorbent has settled, open the stopcock and let the solvent run through to about 1 cm from the top of the column. Discard the solvent which has run through.

5.5 *Clean-up of vegetable oils, animal fats and extracted fats from meat, fish, cheese and butter*

Bring 0,5—1 g of the sample, accurately weighed, in a small beaker and dissolve it in 10 ml of light petroleum. Transfer this fat solution quantitatively to the Florisil column, using a few ml portions of light petroleum as a rinse. Let the supernatant liquid run through the column into a 1 L round bottomed flask and elute the pesticides with 300 ml of the eluting mixture, mentioned under 3.3. The speed of the elution should not exceed 5 ml per minute.

Run a blank in the same way, starting with 10 ml of light petroleum.

5.6 *Clean-up of other fatty foods-preparation of the sample*

5.6.1 *Blank value*

Bring 10 ml of distilled water in a 250 ml glass beaker.

5.6.2 *Fresh milk and unsweetened condensed milk*

Weigh 10 g of the sample in a 250 ml glass beaker.

5.6.3 *Sweetened condensed milk*

Mix 10 g of the sample thoroughly with 5 ml of distilled water.

5.6.4 *Milk powder and infant feeding on milk basis*

Dissolve 3 g of the sample completely in 10 ml of distilled water with a temperature of about 40 ° C. Avoid formation of lumps.

5.6.5 *Chocolate, cocoa powder and egg yolk powder*

Mix 2—3 g thoroughly with 10 ml of hot distilled water.

5.6.6 *Whole egg*

Mix 5 g of the homogenised sample thoroughly with 5 ml of distilled water.

5.7 *One step extraction and clean-up*

Add in small portions 25 g of Florisil to the blank, fresh milk or sample solutions as prepared under 5.6.1—5.6.6. During these additions mix thoroughly by stirring with a glass rod until a homogeneous free flowing powder is obtained. Prepare a chromatographic column as described unter 5.4, but add sufficient light petroleum to form a 12 cm layer over the Florisil. Transfer the mixture of sample and Florisil to the column by pouring it slowly into the supernatant solvent. When the powder has settled, let the solvent run through into a 1 liter round bottomed flask and perform elution of the pesticides as described unter 5.5.

Concentrate the eluate in the Rotavapor apparatus to less than 1 ml, but not to dryness! Remove the remaining solvent by means of an air current. Wash the whole interior of the flask repeatedly with small portions of light petroleum. Transfer the washings quantitatively to a 5 ml volumetric flask. Make up to volume and shake well to mix.

5.8 *Gas chromatography*

5.8.1 *Tentative identification*

a) Average recommended operation conditions for the gas chromatographic columns:

— 1,5 % OV-17/1,95 % QF-1: column temperature 210 ° C, injector temperature 230 ° C, temperature of detector: when containing a tritium source may not exceed 210 ° C. Ni63-electron capture detectors may be heated to 250 ° C or higher. For the phosphorus detector, whether of the flame photometric or thermionic type, consult the instrument manual. Carrier gas flow 50 ml of nitrogen/minute.

— 2 % DEGS + 0,5 % $H_3PO_4$: column temperature 190 ° C, carrier gas flow 40 ml $N_2$/min.

Injector and detector temperature should be the same as for the OV-17/QF-1 column.

N.B. The separation characteristics of a column are difficult to reproduce. It is, therefore, quite possible that in certain cases a higher or a lower column temperature and/or flow rate may give a better separation than under the conditions specified above.
Laboratories having only a single column apparatus should choose the OV-17/QF-1 column.

b) *Selecting the appropriate sensitivity*

It ist not possible to give definite instructions concerning the parameters associated with optional performance, because these are different for each instrument. Reponse values for individual pesticides are not only dependent on detector performance, but also on the state of the column. See under 4.2. Consult the instrument manual for supplying the right voltage to the electron capture detector and select a sensitivity at which 25 picograms of lindane produce at least a 50 % full scale deflection. This sensitivity which is necessary for the determination of ppb levels of chlorinated pesticides can already be achieved at a moderately low attenuator setting without obtaining a noisy baseline. If this is not the case, the performance of the instrument is not optimal.
The sensitivity of the phosphorus detector is satisfactory, if 1 nanogram of bromophos produces not less than a 50 % scale deflection without appreciable noise.
Inject a suitable aliquot usually 5 microlitres of the blank into the gas chromatograph, using a micro-syringe. Wait till all possible present peaks have emerged. Ideally, the blank should only produce a solvent peak, but this is virtually impossible to achieve, especially during chromatography with the electron capture detector.
Subsequently, inject a same volume of the concentrated sample extract and wait again till all the peaks are eluted. Compare the two chromatograms. If the chromatogram of the sample is qualitatively and quantitatively identical with that of the blank, the analysis may be discontinued. However, if very low amounts (especially of organophosphorus compounds) are to be detected and the peaks on both chromatograms are too small to permit a conclusive comparison, proceed as follows: bring suitable aliquots of both blank and sample in calibrated tubes and evaporate the solvent by means of an air current until a 10-fold concentration is achieved. Inject aliquots of both concentrates into the gas chromatograph and compare again.
Peaks in the sample extract should be considered significant if their size exceeds 10 times that of the blank value. Tentatively identify those peaks as follows:

*Chlorinated pesticides-electron capture mode*

Inject 25 picograms of aldrin and calculate the retention times of the peaks in the sample relative to that of aldrin.

*Table 1*

*Relative retention times (RRT) of chlorinated pesticides (Aldrin = 1,00) and their degradation products*

| Pesticide | 1,5 % OV-17/1,95 % QF-1 column temperature 210 °C | 2 % DEGS + 0,5 % $H_3PO_4$ column temperature 190 °C |
|---|---|---|
| HCB | 0,45 | 0,40 |
| Pentachlorobenzene | 0,23 | 0,25 |
| alpha-BHC | 0,49 | 1,19 |
| alpha-PCCH | 0,40 | 1,10 |
| gamma-BHC | 0,66 | 1,90 |
| gamma-PCCH | 0,27 | 0,34 |
| beta-BHC | 0,75 | 5,90 |
| delta-BHC | 0,86 | 5,40 |
| delta-PCCH | 0,32 | 0,82 |
| epsilon-BHC | 0,97 | 5,28 |
| aldrin | 1,00 | 1,00 |
| heptachlor | 0,80 | 1,00 |
| heptachlor epoxide | 1,51 | 2,76 |
| gamma-chlordane | 1,64 | 2,70 |
| alpha-chlordane | 1,80 | 2,95 |
| dieldrin | 2,36 | 4,20 |
| p,p'DDE | 2,20 | 3,50 |
| o,p'TDE (DDD) | 2,53 | 6,20 |
| p,p'TDE (DDD) | 3,26 | 10,70 |
| o,p'DDT | 3,07 | 4,85 |
| p,p'DDT | 3,95 | 8,85 |
| Endrin | 2,81 | 4,70 |
| Thiodan A | 1,97 | 3,10 |
| Thiodan B | 3,62 | 10,0 |
| Methoxychlor | 8,25 | — |
| Kelthane | 1,50 | 3,50 |
| PCNB | 0,68 | 0,84 |
| Pentachloroaniline | 0,86 | 2,10 |
| Methylpentachloro-phenyl sulfide | 1,08 | 1,35 |
| Toxaphene | irregular deviation of the baseline from 0,70—7,0 Maxima at 2,30, 3,25, 3,50, 4,20 and 5,80 | ditto, but response more ill-defined, less characteristic maxima |

*Table 2. Relative retention times (RRT) of phosphated pesticides*

| Pesticide | 1,5 % OV-17/1,95 % QF-1 column temperature 210 °C | | 2 % DEGS + 0,5 % $H_3PO_4$ column temperature 190 °C | |
|---|---|---|---|---|
| | RRT | RRT | RRT | RRT |
| | Aldrin = 1,00 | Parathion = 1,00 | Aldrin = 1,00 | Parathion = 1,00 |
| Ronnel (Fenchlorphos) | 1,07 | 0,66 | 1,75 | 0,37 |
| Bromophos | 1,56 | 0,90 | 2,78 | 0,65 |
| Iodofenphos | 2,73 | 1,54 | 5,37 | 1,20 |
| Ethion | 4,43 | 2,40 | 6,8 | 1,50 |
| Dursban | 1,37 | 0,77 | 1,75 | 0,42 |
| Phenkapton | 8,2 | 4,70 | 16,8 | 3,24 |

*Phosphated pesticides-phosphorus detector*

Similarly, use the peak given by 0,5 nanogram of parathion for calculating the relative retention times.

For this purpose, measure rentention times from the leading edge of the solvent peak to the top of the peak(s) with a millimeter ruler.

Refer to the lists of relative retention times which will indicate the pesticides and other contaminants which are probably present (see table 1 and 2).

This enumeration is not limitative: a few other pesticides and also polychlorinated naphthalenes might appear in the eluate, but only the elution pattern of the above mentioned compounds has been studied up to now.

It should be borne in mind that even relative rentention times are only approximative values. If, for example, a peak is observed on OV-17/QF-1 which has a relative retention time of 0,80, it is *probably* heptachlor.

To confirm this, inject not only a heptachlor standard, but standards of other pesticides with very close retention times (beta and delta-BHC) as well.

Perform tentative identification by comparison of retention times on both columns. In case of doubt, add a small quantity of the suspected pesticide to an aliquot of the sample solution and inject again to check if known and unknown coincide.

If PCB's are detected, as is nearly always the case where fish is analysed, they should be separated from simultaneously present chlorinated pesticides.

For this purpose, the column chromatography on silicic acid of *Armour* and *Burke* (2) is recommended.

5.8.2 *Gas chromatography — Quantitative analysis*

Quantitation of peaks representing chlorinated pesticides or PCB's may only be performed if they fall within the linear range of the electron capture detector response.

This range is different for each pesticide and is also dependent on the type of detector. Generally speaking, the linear range of 63Ni detectors is more narrow than that of the devices equipped with a tritium source.

Check, therefore, if the quantity of the chlorinated pesticide in the injected sample aliquot falls within linear range. If this is not the case, prepare a suitable dilution and inject again. Compare the size of the residue peak with the size of a peak from a known quantity of the appropriate standard. Sufficient accuracy is achieved when simply using peak height (expressed in mm) for quantitation. For best results, make sure that the quantities of substance in standard and unknown are about equal.

5.9 *Thin-layer chromatography (TLC) — Confirmation of identity*

5.9.1 *TLC of chlorinated pesticides*

Transfer a suitable aliquot, i. e. a volume containing enough pesticide to give a spot within range of 0,025—0,25 microgram, of the sample solution to a small glass or porcelain dish. Do the same with an equal portion of the blank. Leave to evaporate at room temperature until a few drops are left. With the aid of micropipettes of 50 microliter bring the concentrates quantitatively to a plate of aluminium oxide E (4.3), using a few drops of light petroleum to rinse the dishes. Apply standard solutions to give spots of 0,025, 0,05, 0,10, 0,15, 0,20 and 0,30 microgram. For best results keep size of spotted sample aliquots and standards as small as possible.

Develop the chromatogram by ascending migration in a pre-saturated tank using light petroleum as a mobile phase. When the mobile phase has reached the front line, remove the plate from the tank and let the adherent solvent evaporate.

Spray abundantly with the chromogenic reagent 3.10. Under spraying will result in poor sensitivity. After spraying, wait 10 minutes and examine the plate carefully. If, at this time, brown or black spots have appeared, these are not due to pesticides. Sometimes, both blank and sample exhibit a yellow brownish zone at Rf 0,70. Mark position of the spot(s) with a pencil and place the chromatogram under the ultra-violet source 4.4. Irradiate for 10 minutes. Remove the plate from the lamp and spray lightly with distilled water until the chromatogram is just moistened. Expose the

*Table 3. Relative retention times (RRT) of Polychlorinated Biphenyls measured on the 2 % DEGS + 0,5 % $H_3PO_4$ column; Column Temperature 190 °C*

| RRT | 0,6 | 0,7 | 0,9 | 1,0 | 1,1 | 1,2 | 1,3 | 1,5 | 1,6 | 1,8 | 1,9 | 2,0 | 2,3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aroclor | | | | | | | | | | | | | |
| 1242 | | 3,5 | 10,4 | 2,3 | 6,9 | 20,6 | 8,6 | 13,9 | 7,2 | 3,8 | | | 11,0 |
| 1248 | | | 3,4 | | | 8,1 | 1,6 | 11,0 | 11,4 | 6,1 | | 4,9 | 26,5 |
| 1254 | | 1,3 | 2,3 | 1,7 | 4,8 | 1,8 | 5,7 | | 3,4 | 1,6 | | 0,9 | 16,9 |
| 1260 | | | | | | | | | | | | 1,3 | 1,8 |
| 1262 | | | | | | | | | | | | 0,4 | 0,3 |

*Table 4. Relative retention times (RRT) of Polychlorinated Biphenyls measured on the 1,5 % OV-17/1,95 % QF-1 column; Column Temperature 210 °C*

| RRT | 0,4 | 0,5 | 0,6 | 0,7 | 0,8 | 0,9 | 1,0 | 1,2 | 1,4 | 1,6 | 1,8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Aroclor | | | | | | | | | | | |
| 1242 | 3,7 | 9,0 | 5,8 | 21,6 | 9,6 | 7,6 | 7,7 | 8,7 | 6,9 | 10,8 | 8,1 |
| 1248 | | | 3,0 | 1,6 | 9,7 | 3,3 | 3,8 | 10,5 | 9,3 | 17,7 | 13,2 |
| 1254 | | | 2,1 | 1,2 | 4,0 | 2,5 | 4,3 | 2,8 | 1,9 | 7,5 | 8,6 |
| 1260 | | | | | | | | | | 1,4 | 2,4 |
| 1262 | | | | | | | | | | 0,4 | 0,6 |

plate again to the U.V. lamp. The pesticides should now appear as violet black spots in 1—2 minutes.

If the visibility of the spots is not satisfying, irradiate for another 10 minutes, moisten again and continue irradiation for 1—2 minutes until even the lowest concentration of the standards is clearly discernible.

It is sometimes difficult to distinguish the pesticides with the higher Rf-values, because of masking effects due to impurities. In that case, repeat chromatography in the following system:

aluminium oxide E impregnated with 5 % viscous paraffin, mobile phase: acetonitrile — methanol — water — acetone 40 — 30 — 20 — 10 v/v. Impregnate the ready made plates by ascending migration in a 5 % paraffin solution in a closed tank. Store the impregnated plates in a closed container. Prior to chromatography, saturate the mobile phase with paraffin.

*(Aldrin = 1,0) and surface of every peak in percentage of total peak area as* Surface of every peak in percentage of total peak area

| 2,8 | 2,9 | 3,4 | 3,7 | 4,0 | 4,3 | 4,7 | 5,3 | 6,0 | 6,3 | 7,3 | 7,9 | 8,4 | 10,6 | 15,7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5,0 | | | | | | 7,5 | | | | | | | | |
| 12,2 | 2,9 | 2,7 | | 8,9 | | | | | | | | | | |
| 4,6 | 5,9 | 13 | 0,2 | 13,5 | 4,3 | | 14,7 | | | 1,3 | 1,1 | 0,7 | | |
| | 5,0 | 5,6 | 2,6 | 12,1 | | 16,6 | 12,6 | 4,3 | 3,7 | | 15,4 | 4,5 | 9,0 | 5,3 |
| | 3,5 | 3,3 | 2,7 | 6,8 | 1,6 | 16,1 | 6,6 | 6,8 | 5,2 | 0,6 | 17,9 | 13,7 | 5,6 | 8,9 |

*(Aldrin = 1,0) and surface of every peak in percentage of total peak area as* Surface of every peak in percentage of total peak area

| 2,3 | 2,5 | 2,8 | 3,2 | 3,6 | 4,1 | 4,4 | 5,4 | 5,8 | 6,5 | 7,0 | 8,5 | 11,4 | 13,0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4,9 | 6,6 | 8,2 | 1,7 | 5,7 | | | | | | | | | |
| 5,3 | 9,8 | 13,2 | 9,5 | 9,9 | 11,3 | 2,2 | 2,2 | 1,5 | 1,1 | | | | |
| | 6,7 | 6,1 | 10,0 | 7,4 | 11,5 | 13,2 | 7,7 | 3,0 | 15,8 | | 13,4 | | 4,0 |
| | 4,4 | 4,1 | 6,1 | 5,4 | 6,0 | 13,5 | 6,8 | 7,0 | 15,3 | 1,8 | 17,8 | 2,4 | 5,8 |

In this system the order of migration is completely reversed. Compounds such as DDE, HCB and aldrin will show low Rf-values and will be more easily recognisable.

*Approximate Rf-values of chlorinated pesticides and their degradation products.*

| Adsorbent $Al_2O_3E$ | | Mobile phase: light petroleum | |
|---|---|---|---|
| delta-BHC | 0,00 | delta-PCCH | 0,35 |
| beta-BHC | 0,03 | alpha-PCCH | 0,08 |
| epsilon-BHC | 0,11 | Aldrin | 0,72 |
| gamma-BHC | 0,20 | Heptachlor | 0,60 |
| alpha-BHC | 0,31 | Heptachlor epoxide | 0,31 |
| alpha-Chlordan | 0,45 | Dieldrin | 0,13 |
| gamma-Chlordan | 0,39 | Endrin | 0,16 |
| p,p'DDE | 0,65 | Methoxychlor | 0,00 |

| | | | |
|---|---|---|---|
| o,p'TDE | 0,26 | Endosulfan | 0,02 |
| p,p'TDE | 0,23 | | 0,21 |
| o,p'DDT | 0,57 | Kelthane | 0,00 |
| p,p'DDT | 0,48 | | 0,60 |
| HCB | 0,77 | Toxaphen | 0,00—0,60 (streak) |
| Pentachlorobenzene | 0,76 | PCB | |
| PCNB | 0,68 | all Aroclors yield not clearly | |
| Pentachloroanilin | 0,21 | separated spots between 0,65—0,75 | |
| Methylpentachlorophenyl-sulfide | 0,72 | | |
| gamma-PCCH | 0,50 | | |

It should be borne in mind that organophosphorus compounds containing chlorine or bromine are also revealed by the photochemical treatment.

Their Rf-values in the system for the chlorinated pesticides are:

| | | | |
|---|---|---|---|
| Fenchlorvos (Ronnel) | 0,19 | Dursban | 0,10 |
| Iodofenphos | 0.15 | Phenkapton | 0,09 |
| Bromophos | 0.17 | | |

Estimate the approximate quantity of the pesticide(s) in the sample aliquot by comparison with the spots given by different quantities of the reference substance(s).

The limit of detection is about 25 nanograms.

N.B. For a successful revelation of the chlorinated pesticides, the laboratory atmosphere should not contain traces of hydrochloric acid, chlorine or sulphurous compounds. Even vapours of halogenated solvents, such as chloroform, cause considerable background darkening and consequently lower the limit of detection.
In general, a sensitivity of 25 nanogram is sufficient for routine work. If lower levels are to be detected, the aluminium oxide layers should be prewashed by ascending migration with acetone-$H_2O$ 1:1.
On the washed plates it is possible to distinguish quantities 5—10 nanograms.

5.9.2 *TLC of phosphated pesticides — enzymatic revelation method (13)*

The application of the sample aliquots is performed as described under 5.9.1, but the volume applied should represent a quantity of pesticide in the range of 5—30 nanograms. Spot reference solutions accordingly.
Adsorbent: $SiO_2$ G ready made plates 20×20 cm coated on glass.
Mobile phase: Light petroleum-methylene chloride — ethyl ether 8 : 2 : 2 v/v.
When the mobile phase has reached the front line, remove the plate from the tank and let the adherent solvent evaporate.
Expose the chromatogram during about 30 seconds to bromine vapour, by placing it in an all glass tank, previously saturated with the vapours of

this chemical. During this operation the organothiophosphates are converted to their oxygen analogues which are active esterase inhibitors.
Subsequently, position the plate in a strong draft of air for about 20 minutes to remove excess bromine.
Prepare a 1 % solution of lyophilised human serum (3.11) in distilled water. Spray the chromatogram thoroughly with this solution and allow the reaction between pesticides and esterase to proceed at 37 ° C during 30 minutes.
Prepare the substrate solution just prior to spraying by dissolving 5 mg of beta-naphthyl acetate (3.12) in 2 ml of ethanol. Add a solution of 20 mg Fast Blue salt (3.13) in 20 ml of 25 percent aqueous ethanol, mix and spray on the chromatogram. Hydrolysis of the substrate is accelerated by incubating the plate at 37 ° C in a humid atmosphere.
Within 10 minutes the spotted quantities of the pesticides become visible as white spots on a reddish-violet background due to the reaction between the liberated naphthol and the Fast Blue salt.
The limit of detection by this enzymatic revelation procedure is often better than 5 nanograms.

*Rf-values of non-polar thiophosphate esters*

$SiO_2$ G/Light petroleum — diethyl ether — methylene chloride 8 : 2 : 2 v/v.

| | |
|---|---|
| Fenchlorphos (Ronnel) | 0,84 |
| Iodofenphos | 0,80 |
| Bromophos | 0,82 |
| Ethion | 0,75 |
| Dursban | 0,87 |
| Phenkapton | 0,80 |

It is clear that the thin-layer chromatography of these pesticides is not very satisfying. However, separation by gas chromatography is excellent and it is most unlikely that residues of several of these compounds would occur together in foodstuffs.

## *Discussion*

A primary consideration in selecting this method, was it's simplicity and reliability. Even a relatively untrained technician can easily analyse 6 samples during one day, including gas chromatography. As extraction and clean-up are performed in one step, the risk of errors in manipulation is reduced to a minimum. The procedure described provides recoveries of 80—100 percent for all chlorinated pesticides and PCB's commonly encountered in fatty foodstuffs. It has also been found to be suitable for the determination of bromophos in maize oil and for monitoring kelthane and ethion residue levels in essential oils of citrus fruits.

Thiodane is the only pesticide listed that is unsufficiently recovered by this method. The yield of recovery experiments was not better than 60 percent. However, this pesticide it not very stable and, therefore, rarely encountered in fatty foods. The lower detection limit of the chlorinated pesticides is approximately 0,001 ppm, with the exception of toxaphene which can only be determined at levels not lower than 0,2 ppm. The non-polar phosphated pesticides and PCB's can be detected if they exceed the level of 0,05 ppm, but only when the concentration of the sample extract to be subjected to gas chromatography represents not less than 1 g of fat pro ml.

The selection of the two gas chromatographic columns OV-17/QF-1 and DEGS was based on the fact that the elution pattern on these stationary phases is really different which gives already a high degree of reliability to the tentative identification procedure (14). Moreover, both columns give a maximum number of quantifiable peaks when used for multi-residue analysis.


## *Summary*

A rapid, one step method for the determination of residues of chlorinated pesticides, PCB's and 6 organothiophosphates in fatty foods is described. Fats and oils are simply cleaned up by placing a quantity not exceeding 1 g on top of a chromatographic column composed of partly deactivated Florisil. For the analysis of milk, dairy products, eggs, egg yolk powder, cocoa powder and chocolate an adsorption clean-up is performed by mixing a certain quantity of the sample (in presence of water) with enough Florisil to form a free-flowing powder. During this procedure the sample is evenly coated on the adsorbent particles and becomes accessible to extraction solvents. The powder thus obtained is brought on top of a chromatographic column of partly deactivated Florisil immersed in light petroleum. Subsequently, the pesticides are eluted with 300 ml of methylene chloride-light petroleum (20 + 80) and the eluate is evaporated, after which the residues are taken up in a small volume of light petroleum.

Tentative identification and quantitation of the residues is achieved by gas chromatography on two high efficiency columns: the mixed liquid phase 1,5 % OV-17 + 1,95 % QF-1 and the polyester DEGS at a 2 % loading, stabilised with 0,5 % phosphoric acid.

Confirmatory analysis is carried out by thin-layer chromatography. Chlorinated compounds are revealed by photochemical reaction with silver nitrate, whereas detection of organothiophosphates is achieved by an esterase inhibition procedure.

The lower limit of detection of chlorinated pesticides is approximately 0,001 ppm, with the exception of toxaphene which can only be determined at levels not lower than 0,2 ppm.

The non-polar phosphated pesticides and PCB's can be detected if their levels exceed 0,05 ppm, provided that the sample extract to be subjected to gas chromatography represents not less than 1 g of fat pro ml.



## *Résumé*

Les auteurs décrivent une méthode qui, comportant une seule opération de purification, permet le dosage rapide des résidus de pesticides organochlorés, de 6 organo-

thiophosphates et des PCB, dans les produits alimentaires riches en graisse. Les graisses et les huiles sont simplement purifiées par passage, d'une quantité n'excédant pas 1 g, à travers une colonne de florisil partiellement desactivé. Le lait, les produits laitiers, les œufs, la poudre de jaune d'œuf, la poudre de chocolat, le chocolat, etc. dispersés dans une certaine quantité d'eau, sont mélangés avec une quantité suffisante de florisil pour obtenir une poudre fluide. Au cours de cette opération l'échantillon est réparti de façon uniforme sur les particules de l'adsorbant ce qui le rend accessible aux solvants d'extraction. La poudre, ainsi obtenue, est versée au sommet d'une colonne de florisil partiellement desactivée et immergée dans de l'éther de pétrole. Les pesticides sont ensuite élués au moyen de 300 ml d'un mélange de chlorure de méthylène et d'éther de pétrol (20 + 80). Après évaporation de l'éluat, le résidu est repris par un petit volume d'éther de pétrole.

Les essais d'identification et le dosage des résidus sont faits par chromatographie à gaz (CPG) sur deux types de colonnes à haute efficacité dont les phases liquides sont la phase mixte 1,5 % OV-17 + 1,95 % QF-1 et la phase à 2 % de polyester DEGS, stabilisée avec 0,5 % d'acide phosphorique.

L'analyse de confirmation est réalisée par chromatographie sur couche mince (CCM). Les combinaisons organochlorées sont révélées par une réaction photochimique avec le nitrate d'argent, tandis que les organothiophosphates sont détectés par une technique d'inhibition enzymatique (esterase).

Pour les pesticides organochlorés la limite inférieure de détection par CPG est approximativement de 0,001 ppm à l'exception du toxaphene, qui ne peut être dosé qu'à une teneur égale ou supérieure à 0,2 ppm.

Les pesticides organophosphorés non-polaires et les PCB sont détectables si leur teneur excède 0,05 ppm à la condition toutefois que l'extrait, prêt à l'injection, ne corresponde pas à moins d'un gramme de graisse par ml.


## *Zusammenfassung*

Es wird ein rasches Einstufenverfahren für die Bestimmung der Rückstände chlorierter Pestizide, PCB's und 6 Organothiophosphaten in fettreichen Lebensmitteln beschrieben. Fette und Oele werden einfach gereinigt, indem eine Menge von nicht mehr als 1 g durch eine chromatographische Säule aus teilweise aktiviertem Florisil geführt wird. Für die Analyse von Milch, Milchprodukten, Eiern, Trockeneidotter, Kakaopulver und Schokolade wird eine Adsorptionsreinigung durchgeführt, indem eine gewisse Menge der Probe (in Anwesenheit von Wasser) mit so viel Florisil gemischt wird, daß ein leicht fließendes Pulver entsteht. Während dieses Vorganges wird die Probe auf die adsorbierenden Teilchen homogen angelagert und ist folglich der Lösungsmittelextraktion zugänglich. Das so erhaltene Pulver wird auf eine chromatographische Säule aus teilweise aktiviertem Florisil in Petroläther gebracht. Anschließend werden die Rückstände mit 300 ml Methylenchlorid-Petroläther (20 + 80) eluiert, das Eluat eingedampft, und die Rückstände dann in einem kleinen Volumen Petroläther aufgenommen.

Nachweis und quantitative Erfassung der Rückstände wird gaschromatographisch auf zwei Säulen hohen Trennungsgrades erreicht: die gemischte flüssige Phase 1,5 % OV-17 + 1,95 % QF-1 und die Phase aus dem Polyester DEGS zu 2 %, mit 0,5 % Phosphorsäure stabilisiert.

Die Analyse wird mittels der Dünnschichtchromatographie bestätigt. Chlorierte Verbindungen werden mittels einer photochemischen Reaktion mit Silbernitrat sichtbar ge-

macht, während die Organothiophosphate mittels eines Esterase-Inhibitionsverfahrens nachgewiesen werden.

Die Nachweisgrenze für die chlorierten Pestizide ist etwa 0,001 ppm, mit der Ausnahme des Toxaphens, welches nur bei Gehalten von nicht weniger als 0,2 ppm bestimmt werden kann.

Die nicht-polaren, phosphatierten Rückstände und die PCB's können nachgewiesen werden, wenn ihre Gehalte 0,05 ppm überschreiten, aber unter der Bedingung, daß der Probenextrakt für die Gaschromatographie nicht weniger als 1 g Fett pro ml entspricht.

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