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Methods for the Quantitative Determination of Biocides in Tobacco and Tobacco Products

Part VI: A Modified Procedure for Organochlorine, Organophosphorus and Carbamate Insecticide Residues in Tobacco*

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1. INTRODUCTION

The screening procedure for the determination of pesticide residues in tobacco (organochlorine, organophosphorus and carbamate insecticides) published in 1970 (1) has been improved . It was not our aim to develop entirely new methods, but to try a uniform extraction and concentration procedure, which includes compounds soluble and insoluble in water with additional separate purification steps. The improved method is capable of determining altogether about 25 compounds, i. e. 11 organochlorine, 11 organophosphorus/sulfur, and 3 carbamates. Recoveries of added pesticides at the 0.5 ppm level are between 75 and 102 %. Moreover, one can also detect very polar metabolites of some common insecticides.

2. PRINCIPLES OF THE METHOD

The sample is blended with aqueous acetonitrile and filtered. An aliquot is partitioned with methylene chloride. The concentrated extract is investigated for unstable phosphated compounds by FPD — gas chromatography. The remaining solution is purified on alumina with acetonitrile in a "dry-column" technique. One aliquot of the concentrated eluate is subjected to gas chromatography with the flame photometric detector in the P-mode for detection of phosphorus-sulfur pesticides and metabolites. A second aliquot is separated into two fractions on a micro-Florisil column for the determination of organochlorine pesticides after gas chromatography with the microcoulometric system.
Fraction I contains Lindane, 4 Lindane isomers, aldrin, heptachlor, heptachlor epoxide, TDE, DDT and metabolites, toxaphene, polychlorinated biphenyls (PCB) and naphthalenes (PCN). The simultaneous occurrence of DDT, TDE and PCB, PCN requires an additional separation on a silica gel column.
Fraction II contains dieldrin, endrin, endosulfan I, endosulfan II and endosulfan sulfate.

For the detection of carbamate insecticides an aqueous acetonitrile extract is produced in the same manner as for organochlorine and organophosphorus compounds. One half is mixed with diluted acid and partitioned with chloroform. After removing degradation products of carbaryl and propoxur, the extract is hydrolyzed and purified. The free 1-naphthol and isopropoxyphenol are steam-distilled from acid medium and derivatized with trichloroacetyl chloride. The derivatives are separated by gas chromatography with the electron capture detector. The second half of the acetonitrile extract is mixed with diluted acid, filtered and partitioned with chloroform. The concentrated extract is hydrolyzed with diluted alkali and the resulting oxime of methomyl is determined by gas chromatography with the flame photometric detector in the S-mode.

3. METHOD

3.1 *Reagents*

(a) Solvents. — Acetonitrile, methylene chloride, n-hexane, benzene, ethyl acetate and chloroform, Nanograde (Mallinckrodt).

(b) Methyl ethyl ketone. — GR (Merck).

(c) Petroleum ether. — B. p. 40–60° C, GR (Merck).

(d) Triethylamine. — AR (Fluka).

(e) Trichloroacetyl chloride. — For synthesis (Merck).

(f) Extraction solvent. — Acetonitrile: water, 23:12.

(g) Eluting mixture. — Acetonitrile: hexane: methylene chloride, 1:19:80.

(h) Acid mixture. – Dissolve 10 g Na_2SO_4 in 1 L dist. water containing 10 ml H_2SO_4 (95–97 %).

(i) Sodium hydroxide. — 0.002, 0.1, 1.0 N in dist. water.

(j) Calcium hydroxide. — GR (Merck).

(k) Aluminium oxide. — For dry-column chromatography (Woelm).

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Figure 1. Principles of the determination of organochlorine and organophosphorus pesticides.

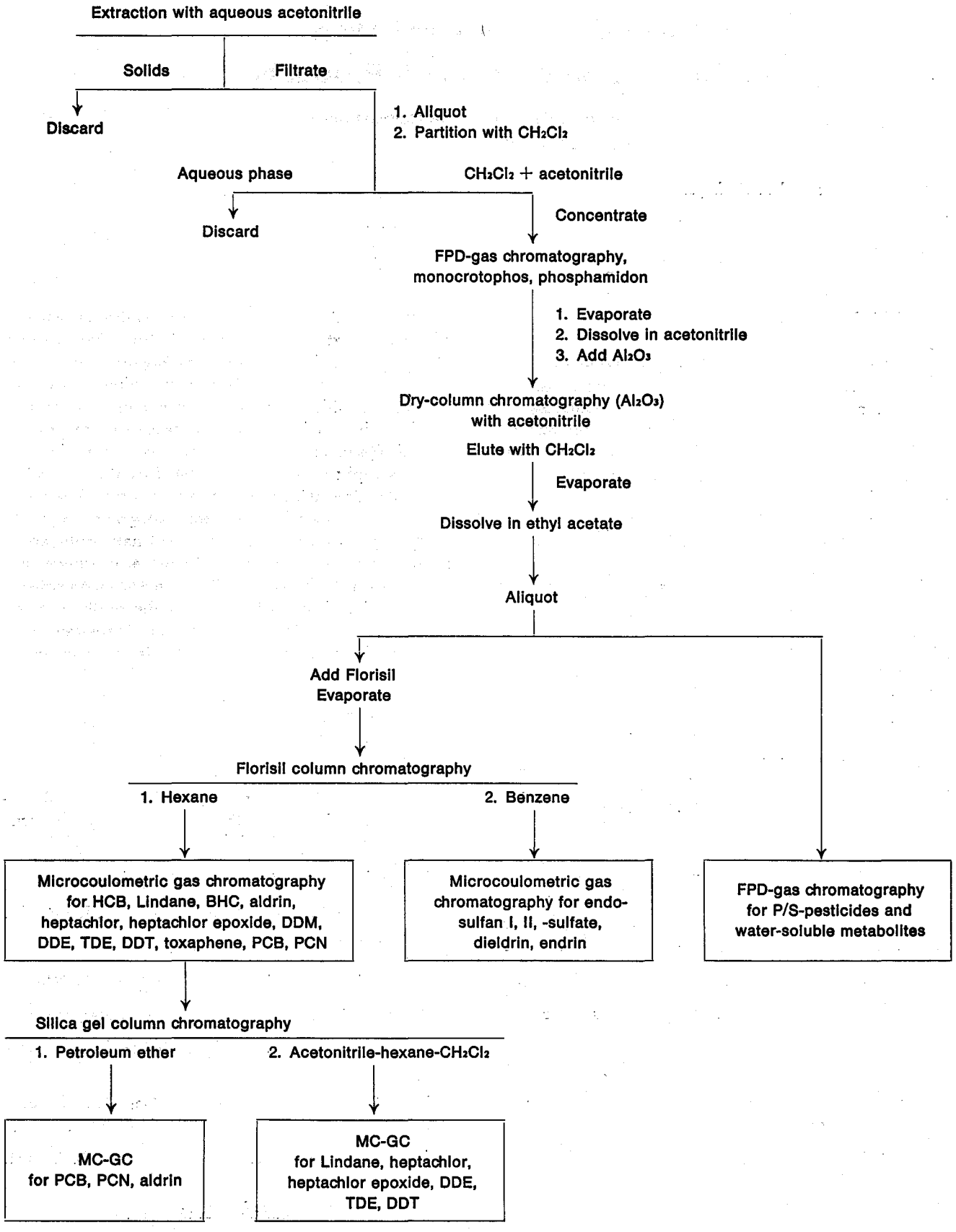
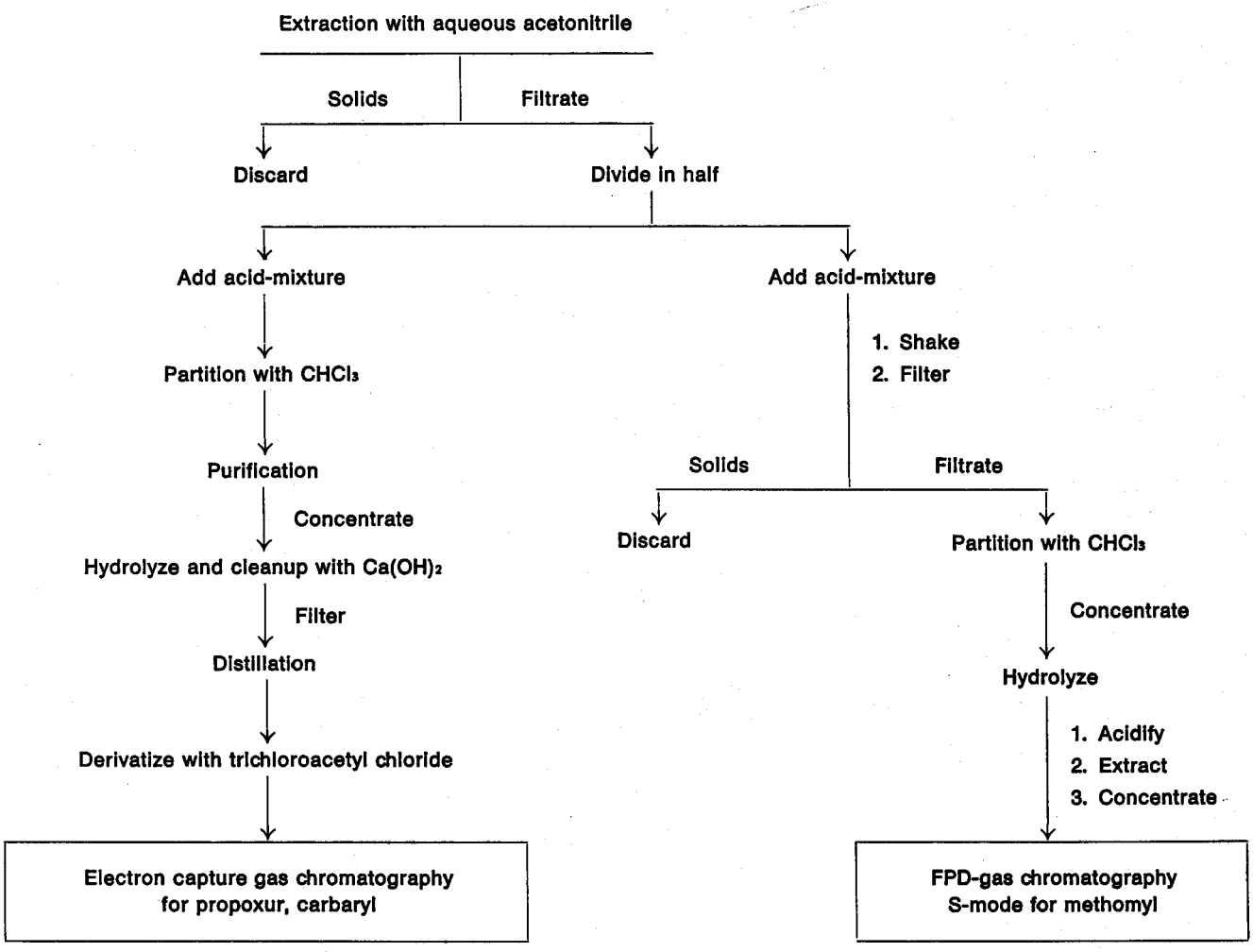


Figure 2. Principles of the determination of carbamates.



(l) Florisil. — PR, 60/100 mesh. Store in oven at 130° C for 5 hours, cool in desiccator and add 0.7 % water. Mix about 1 hour and let equilibrate for 24 hours.

(m) Sand. — Sea sand, acid-washed, calcined (Merck).

(n) Silica gel. — 100 mesh (Mallinckrodt). Treat as follows: Place silica gel to depth of approx. 2 cm in open beaker and heat approx. 7 hours in a 130° C oven. After heating place beaker in desiccator and let cool to room temperature. Quickly weigh 97 g into glass-stoppered bottle and add 3 ml water. Stopper tightly and seal with tape to insure that container is air-tight. Shake well until all water is adsorbed; make sure that no lumps remain. Place sealed container in desiccator and allow to equilibrate 15 hours. Return container to desiccator immediately after use. Desired activity remains for about 5 days.

(o) Pesticide standard solution.

1. Mixture of Lindane, α-, β-, γ-BHC, p,p'-DDE, p,p'-TDE, o,p-DDT, p,p'-DDT. Prepare solution in methyl ethyl ketone, using 2.5, 2.5, 2.5, 2.5, 1.0, 5.0, 0.5, 5.0 ng/µl.

2. Mixture of dieldrin, endrin, endosulfan I, endosulfan II, endosulfan sulfate. Prepare solution in methyl ethyl ketone, using 2.0, 2.0, 1.0, 4.0, 6.0 ng/µl.

3. Mixture of diazinon, dimethoate, malathion, parathion, parathion-methyl. Prepare mixture in ethyl acetate, 5.0 ng/µl, each.

4. Mixture of disulfoton, monocrotophos, phosphamidon. Prepare mixture in ethyl acetate, 5.0 ng/µl, each.

5. Mixture of fensulfothion, azinphos-methyl, azinphos-ethyl. Prepare mixture in ethyl acetate, 5.0 ng/µl, each.

6. Mixture of carbaryl, propoxur. Prepare solution in methyl ethyl ketone, 5.0 ng/µl, each. Take 0.50 ml aliquot and proceed as in "Determination of Carbaryl and Propoxur" [3.3.2.2], beginning with "Add 15 ml 0.1 N NaOH ..." until "Cool the solution", take this hydrolyzed mixture directly to the derivatization step, beginning "Add 2.0 ml benzene ...". The resulting solution contains 1.25 ng/µl carbaryl and propoxur, respectively.

7. Solution of methomyl in methyl ethyl ketone, 5.0 ng/µl. Take an aliquot of 0.5 ml, proceed as in "Determination of Methomyl" [3.3.2.3], beginning "Add 50 ml 0.1 N NaOH . . .". The resulting solution contains 5.0 ng/µl.

3.2 Apparatus

(a) Chromatographic tube. — 22 mm i.d. × 300 mm high, with medium porosity fritted disk, Teflon stopcock and with ST 19/26.

(b) Chromatographic tube. — Same as (a) except 10 mm i.d. × 300 mm high, with ST 14.5/23.

(c) Separatory funnels. – 50 ml, 500 ml, and 1 L pear shape with Teflon stopcocks.

(d) Dropping funnel. — 100 ml, cylindrical, graduated with Teflon stopcock and ST 19/26 outer joint at the outlet.

(e) Steam distillation. — All glass; 100 ml r-b flask, distilling head, 20 cm Liebig condenser.

(f) 1. Gas chromatograph—microcoulometric system. — Laboratory built column oven with short insulated glass line to the pyrolysis furnace, Coulson system; titration cell (halogen) T-300-S; C-250-A microcoulometer connected to an integrator, CRS-204, and 2.5 mV recorder.

2. Gas chromatograph — flame photometric detector. — Perkin Elmer FE 20 H gas chromatograph equipped with a flame photometric detector, Tracor; connected to an integrator, CRS-204, and 2.5 mV recorder.

3. Gas chromatograph — electron capture detector. — Varian 1400 gas chromatograph equipped with a Tritium electron capture detector, 250 mCi source, Varian; connected to an integrator CRS-204, and 2.5 mV recorder.

(g) GLC columns, all glass, 3.2 mm o.d., 2 mm i.d., packed with

1. 11% QF-1 + OV-17, ratio = 1.3, on Gaschrom Q, 80/100. Length, 0.85 m. Conditioning: 80—140° C, 1°/min., with carrier gas, 140—240° C, 1°/min., without carrier gas, keep at 240° C for 18 hours without carrier gas, heat additional 3 hours at 240° C with carrier gas.

2. Packing and conditioning, same as (g, 1), except length, 1.5 m.

3. 1.95% QF-1 + 1.5% OV-17, on Chromosorb W-HP, 100/120. Length, 1.2 m. Conditioning: 40—250° C, 4°/min., with carrier gas, keep at 250° C for 18 hours with carrier gas.

4. 3% FFAP (Varian), on Chromosorb G-DMCS, 80/100. Length, 0.95 m. Conditioning: 40—200° C, 4°/min., with carrier gas, keep at 200° C for 3 hours with carrier gas.

5. 10% DC-200 (12,500 cstk), on Gaschrom Q, 80/100. Length, 1.4 m. Conditioning: 40—250° C, 4°/min., with carrier gas, keep at 250° C for 18 hours with purge of carrier gas.

3.3 Determination

3.3.1 Organochlorine and Organophosphorus Pesticides

3.3.1.1 Extraction: Transfer to a Waring Blendor 20.0 g finely cut fermented tobacco, add 350 ml extraction solvent (f) and about 5 g Hyflo Super Cel and soak for 10 min. Blend at high speed 2 min., filter mixture through a Büchner funnel, using coarse filter paper and gentle suction. Transfer 300 ml filtrate (= 17.1 g sample) to a 1 L separatory funnel, add 10 ml acid mixture (h) and 300 ml methylene chloride (a), mix by gentle shaking for 1 min.; after layers separate, percolate the bottom layer through anhydrous Na_2SO_4. Reextract aqueous layer with 30, 30 ml methylene chloride. Evaporate filtrate to about 5 ml on a rotary vacuum evaporator in a 45° C water bath. Transfer the extract to a graduated tube and concentrate to 5.0 ml in a water bath at 45° C under a stream of clean, dry nitrogen. Inspect an aliquot (2 µl) as under "Residue Detection Methods, Phosphated Pesticides" [3.4.2] for the presence of monocrotophos (Azodrin) and phosphamidon. Evaporate the residual solution to 2 ml and add alumina (k) in small increments, until a free-flowing powder results.

3.3.1.2 Cleanup (alumina dry-column): Pack chromatographic tube (a) with 80 g alumina (k) so it is about 22 cm high after gentle tapping; transfer the extract — alumina powder to the column and top with 1 cm sand (m). Develop the chromatogram with acetonitrile using a solvent head of about 1 cm; the solvent is delivered from a dropping funnel (d) connected with an adapter to the column. Stop solvent flow just before the front reaches the bottom of the column and discard sand plus coloured alumina zone on the top. Elute pesticides with 100 ml methylene chloride. Filter the eluate through a finepored filter, and concentrate to about 5 ml on a rotary vacuum evaporator in a 45° C water bath. Transfer eluate to a 10 ml graduated tube, evaporate just to dryness in a water bath at 40° C under a stream of clean, dry nitrogen and dissolve residue in 2.0 ml ethyl acetate. Take an aliquot of 0.40 ml for the detection of phosphated compounds, "Residue Detection Methods" [3.4.2], reserve residual 1.6 ml for subsequent "Separation of Chlorinated Pesticides".

3.3.1.3 Separation of Chlorinated Pesticides: Concentrate aliquot (1.6 ml) to about 0.5 ml in a water bath at 40° C under a stream of clean, dry nitrogen; add 1 g Florisil (l) and evaporate solvent completely. Pack chromatographic tube (b) with 4 g Florisil (l) with gentle tapping; top Florisil layer with ca. 10 mm anhydrous Na_2SO_4 and pre-wash column with 15 ml n-hexane.

After last of the solvent has just sunk into the Na_2SO_4 layer, transfer extract-Florisil mixture to column and rinse container with small amounts of n-hexane. Elute pesticides with 100 ml n-hexane (a) into a clean 250 ml flask; change receiver and elute with 100 ml benzene (a); solvents are delivered from a dropping funnel connected with an adapter to the column. Concentrate eluates to about 5 ml on a rotary vacuum evaporator in a 35° C water bath, transfer quantitatively to small tubes (10 ml screw-cap tubes with Teflon-lined discs), evaporate just to dryness, and dissolve residues with a known volume of methyl ethyl ketone (b) (usually 1.0 and 0.2 ml for the n-hexane and benzene eluates, respectively). These solutions are ready for gas chromatography, "Residue Detection Methods" [3.4.1], and contain in fraction I (n-hexane) HCB, Lindane, 4 Lindane isomers, aldrin, heptachlor, heptachlor epoxide, TDE, DDT and metabolites, and in fraction II (benzene) dieldrin, endrin, endosulfan I, endosulfan II, endosulfan sulfate.

3.3.1.4 Separation of Polychlorinated Biphenyls and Naphthalenes: Weigh 5 g Celite 545, then 20 g silica gel (n), combine in 250 ml beaker and slurry with 80 ml petroleum ether (c), mixing well. Pour slurry into chromatographic tube (a) with stopcock open and complete transfer with small portions of petroleum ether. Apply air pressure to top of column to settle adsorbent and to force petroleum ether from column; stir adsorbent to remove air bubbles. Continue to apply pressure until solvent level is about 5 mm above surface of gel. (Note: Do not allow column to go dry or to crack at any time; close stopcock when pressure is not being applied.)

Place 250 ml volumetric receiver under column to collect eluate. Take residual solution from fraction I and evaporate just to dryness in a water bath at 40° C under a stream of clean, dry nitrogen; dissolve residue in about 3 ml petroleum ether. Slowly and carefully add sample to column; do not disturb top of column. Complete transfer to column with small portions of petroleum ether, applying slight air pressure after each addition of solvent, until solvent level is about 3 mm above adsorbent. Place dropping funnel (d) containing 250 ml petroleum ether on top of column, open stopcock, and slowly apply slight air pressure until an elution rate of about 5 ml/min. is established. Continue elution until eluate volume is exactly 250 ml. Reduce volume to about 5 ml on a rotary vacuum evaporator in a 35° C water bath and to 0.5 ml after transferring into a small graduated tube under a stream of nitrogen. This solution is subjected to gas chromatography, "Residue Detection Methods" [3.4.1], for the detection of PCB, PCN and aldrin. Now elute column with 200 ml solvent mixture (g); applying slight air pressure. Collect eluate, concentrate on a rotary vacuum evaporator to about 5 ml, transfer to a small graduated tube and evaporate to 1.0 ml. This solution is ready for gas chromatography, "Residue Detection Methods" [3.4.1], for the detection of HCB, Lindane, heptachlor, heptachlor epoxide, DDE, DDT, TDE.

3.3.2 Carbamate Pesticides

3.3.2.1 Extraction: Transfer to a Waring Blendor 10.0 g finely cut fermented tobacco, add 175 ml extraction solvent (f) and about 3 g Hyflo Super Cel and soak for 10 min. Blend at high speed 2 min., filter mixture through a Büchner funnel, using coarse filter and gentle suction. Take an aliquot of 75 ml (= 4.3 g sample) for the "Cleanup of Carbaryl and Propoxur" and a second aliquot of 75 ml for the "Determination of Methomyl" [3.3.2.3].

3.3.2.2 Cleanup of Carbaryl and Propoxur: Transfer the tobacco extract to a 500 ml separatory funnel, add 300 ml acid mixture (h) and 50 ml chloroform, mix by gentle shaking for 1 min.; after layers separate, percolate the bottom layer through anhydrous Na_2SO_4 into a 250 ml separatory funnel. Re-extract aqueous layer with 50, 25 ml chloroform and treat in the same manner as the first extract. Shake the combined chloroform extracts with 25 ml cold 0.002 N NaOH (ca. +5° C) for 15 sec. Immediately after layers have separated, drain chloroform into 250 ml flask and evaporate to about 5 ml on a rotary vacuum evaporator in a 45° C water bath. Concentrate to about 0.5 ml under a stream of nitrogen, add 15 ml 0.1 N NaOH and place the flask in a 60° C water bath for 15 min., pass a stream of nitrogen over the surface of the extract. Swirl the flask frequently to evaporate the chloroform. Cool the solution, add a suspension of 0.2 g $Ca(OH)_2$ + 0.5 g Celite in 2 ml water and shake on a mechanical shaker for 5 min. Filter mixture through a small glass fibre disk, wash filter and container with small portions of 0.1 N NaOH. Transfer filtrate to a 100 ml distilling flask (e), add 35 ml acid mixture (h), and several boiling beads. Distil on a hot plate until 30 ml distillate are received, collecting distillate in a 50 ml graduated cylinder; add 3.0 ml 1 N NaOH and shake well. Transfer aqueous solution to 50 ml separatory funnel, partition with 15 ml n-hexane; after layers have separated, discard n-hexane. Add 2.0 ml benzene, 30 µl trichloroacetyl chloride (e) (from a 50 µl gas-tight syringe) to the aqueous solution in same separatory funnel; shake vigorously for 30 sec. and on a mechanical shaker for 20 min. Let phases separate, drain solvent layer into small screw-cap tube (5 ml), add a small portion of anhydrous Na_2SO_4 and shake. Proceed as in "Residue Detection Methods" [3.4.3.1] for the detection of carbaryl and propoxur.

3.3.2.3 Cleanup of Methomyl: Transfer the 75 ml aliquot to a 500 ml screw-cap flask, add 300.0 ml acid-mixture (h) and 5 g Hyflo Super Cel; shake on a mechanical shaker for 10 min. Filter mixture through a dry fluted filter into a graduated cylinder, and record volume, e.g. 365 ml (97% of the calculated sample equivalent). Extract the filtrate with successive 50, 50 and 25 ml portions of chloroform (a), shaking about 1 minute each time. After each extraction, percolate chloroform through anhydrous Na_2SO_4 into a 250 ml flask; concentrate combined chloroform to about 0.5 ml

on a rotary vacuum evaporator in a water bath at 45° C. Add 50 ml 0.1 N NaOH and place the flask in a 60° C water bath for 15 minutes, pass a stream of nitrogen over the surface of the extract. Swirl the flask frequently to evaporate the chloroform. Cool the solution, transfer to a 100 ml separatory funnel, acidify with 1 N H_2SO_4 and extract with successive 30, 30 and 20 ml portions of ethyl acetate, shaking about 1 minute each time. After each extraction drain solvent phase through anhydrous Na_2SO_4 into a 250 ml flask; make combined extracts alkaline with triethylamine (d) and add 0.2 ml in excess. Concentrate to about 1 ml on a rotary vacuum evaporator in a 45° C water bath; transfer to a 10 ml graduated tube and evaporate solvent to exactly 0.50 ml in a 40° C water bath under a stream of clean nitrogen. This solution is ready for gas chromatography, "Residue Detection Methods" [3.4.3.2].

3.4 Residue Detection Methods

3.4.1 GLC Procedure for Chlorinated Pesticides

Standardization of Equipment. — Set microcoulometric system for the following conditions: inlet, 220° C; furnace, 930—950° C; bias 270 mV; range, 400 ohms; gain, low; synthetic air, 110 ml/min.; recorder chart speed, 0.5 cm/min. GLC conditions; QF-1 + OV-17 column (g, 3): temperature, 180° C; for separation of Lindane isomers, aldrin, heptachlor: 130° C; helium flow, 60 ml/min.; injection port, 220° C.
Standardize system as follows: inject into column 3.0 µl sample solution, using 10 µl syringe; next inject 3.0 µl standard mixture; determine peak areas. Repeat injections until constant areas result for both, sample solution and standard mixture.

Determination. — With by-pass vent open, inject up to 5 µl sample solution into column, and press start button of the integrator; after 1 minute, close vent and record chromatogram. If response is too great, dilute solution. Calculate the $R_{\delta\text{-BHC}}$ (retention time of sample peak / retention time of δ-BHC), inject an appropriate amount of standard mixture.

Calculation.

$$\text{ng pesticide in sample aliquot injected} = \frac{\text{area of sample peak} \times \text{ng standard injected}}{\text{area of standard peak}}.$$

Area of sample and standard peaks should not differ by more than 10 % for quantitative determination.

3.4.2 GLC Procedure for Phosphated Pesticides

Standardization of Equipment. — Set flame photometric system for the following conditions: detector, 180° C; synthetic air, 100 ml/min.; hydrogen, 50 ml/min.; interference filter, 526 mµ; recorder chart speed, 0.5 cm/min. GLC conditions; QF-1 + OV-17 column (g, 1) : temperature, 170° C for separation of disulfoton, monocrotophos, phosphamidon; and for separation of diazinon, dimethoate, parathion-methyl, malathion, parathion; 200° C for separation of disulfoton metabolites; 220° C for separation of fensulfothion, fensulfothion metabolites, fenthion metabolites; 235° C for separation of fensulfothion, azinphos-methyl, azinphos-ethyl, Phosvel, phosalone; DC-200 column (g, 5) : temperature : 160° C for separation of monocrotophos from dimethoate and phosphamidon from parathion; helium flow, 100 ml/min.; injection port, 200° C.
Standardize system as follows: inject into column 2.0 µl sample solution under operating conditions, repeat at least three times. Alternately inject sample solution and standard mixture; when several successive injections produce constant response (response gradually increases to a constant value), column is ready for use.

Detection. — Inject 2.0 µl sample solution into column, push start button of the integrator and close hydrogen shut-off valve; after 1 minute press ignitor button and open hydrogen valve slowly; record chromatogram. Calculate the retention time of sample peak relative to parathion and fensulfothion respectively; inject an appropriate amount of standard mixture.

Calculation.

$$\text{ng pesticide in sample aliquot injected} = \frac{\text{area of sample peak} \times \text{ng standard injected}}{\text{area of standard peak}}.$$

Area of sample and standard peaks should not differ by more than 10 % for quantitative determination.

3.4.3 GLC Procedure for Carbamate Pesticides

3.4.3.1 Carbaryl, Propoxur

Standardization of Equipment. — QF-1 + OV-17 column (g, 2): temperature, 145° C for propoxur; 165° C for carbaryl; nitrogen flow, 55 ml/min.; detector, 200° C; injection port, 180° C; recorder chart speed, 0.5 cm/min. Standardize system as follows: inject into column at appropriate working conditions 2.0 µl standard mixture (0,6), using 10 µl syringe; repeat injections until constant areas result. After each injection raise temperature to 200° C to remove from the column interfering products of the derivatization step.

Determination. — Equilibrate gas chromatographic oven at 145° C and 165° C, respectively; inject into column 2.0 µl sample solution, push start button of the integrator and record chromatogram. About 2 minutes after substance under investigation has emerged from the column, raise temperature up to 200° C; keep at this temperature until last interfering compounds have left the column.
Cool down and proceed as under "Determination". Inject an appropriate amount of standard mixture.

Calculation.

$$\text{ng pesticide in sample aliquot injected} = \frac{\text{area of sample peak} \times \text{ng standard injected}}{\text{area of standard peak}}.$$

Area of sample and standard peaks should not differ by more than 10%, the response must be in the linear region of the electron capture detector.

3.4.3.2 Methomyl

Standardization of Equipment. — FFAP column (g, 4): temperature, 150° C; helium flow, 80 ml/min.; injection port, 180° C; detector, 180° C, hydrogen, 50 ml/min.; synthetic air, 130 ml/min.; interference filter, 394 mμ; recorder chart speed, 0.5 cm/min. Standardize system as follows: inject into column 3.0 μl sample solution, using 10 μl syringe; next inject 3.0 μl standard solution (0,7); determine peak areas. Repeat injections in that sequence until constant areas result for both, sample and standard solutions.

Determination. — Inject 3.0 μl sample solution into column, push start button of the integrator and close hydrogen shut-off valve; after 1 minute press ignitor button and open slowly hydrogen valve; record chromatogram. Inject an appropriate amount of standard solution.

Calculation.

$$\text{ng pesticide in sample aliquot injected} = \frac{\text{area of sample peak} \times \text{ng standard injected}}{\text{area of standard peak}}$$

4. DISCUSSION

The well proved extraction of tobacco with aqueous acetonitrile (1,2), recommended by *Bertuzzi* and co-workers (3), for the extraction of chlorinated compounds from foodstuffs is again the starting point of this procedure. It has been in use in our laboratory since 1965, and an earlier version of our procedure for pesticide residues, based on that extraction, was presented at the Tobacco Chemists' Research Conference in 1968. Our investigations and those of *Storherr* and co-workers (4); *Porter* and co-workers (5); showed that aqueous acetonitrile is not only very efficient for the extraction of chlorinated compounds, but also, for at least the other two great categories of pesticides, the phosphated and carbamate compounds. Partitioning of the acidified acetonitrile extract with methylene chloride proved to be the most efficient concentration step for all kinds of residues, covering the range from nonpolar, i.e., DDT, to very polar, i.e., monocrotophos (Azodrin). This procedure, proposed by *Watts* and co-workers (6), is rather a "freeing from water" than a partitioning; the acetonitrile is removed completely with the methylene chloride. The drawback of this procedure is that during extraction relatively large amounts of extraneous material are removed with the pesticides. However, one of the most important steps in any procedure is the purification or cleanup. For example, the life of the gas chromatographic column and the operation of the detector depends on using pure fractions. The sensitivity of the entire procedure to a specific pesticide depends on a gas chromatographic system that is not contaminated. Along the way developing an efficient cleanup procedure, we investigated finally preparative thin-layer chromatography on alumina with acetonitrile as developing agent. We were encouraged by these promising results and introduced a "dry-column" technique. *Loev* and co-workers (7) stated that "dry-column" chromatography is an improved chromatographic technique by means of which separations comparable to those obtained by thin-layer chromatography can be carried out rapidly, *in a column*, on a preparative scale. A valuable relationship between TLC and "dry-column" chromatography is the direct transferability from the former to the latter. Basically, the "dry-column" procedure is carried out by filling an empty column with adsorbent, depositing the mixture to be separated on top of the column, and developing the chromatogram by allowing solvent to move down the dry column by capillary action and gravity. When the solvent reaches the bottom the separation is complete. Using acetonitrile as solvent the pesticides move with the solvent front, leaving behind the extraneous material. This procedure has the great advantage that both polar and nonpolar pesticides elute into one fraction, with a minimum amount of solvent and in a short time. The eluate is clean enough for the determination of phosphated pesticides and metabolites by flame photometric gas chromatography, and for screening purposes of chlorinated compounds by microcoulometric gas chromatography (Table 1). More detailed analysis is achieved, especially for the detection of endosulfan and very small amounts of dieldrin and endrin, after separation on a micro-Florisil column (Fig. 3 and 4). The presence of PCB, PCN requires subsequent separation on a silica gel column.

A separate procedure was developed for the detection of carbamate insecticides, taking into consideration the greater instability of carbamates and without the necessity to extend the present procedure too much to include nearly "all" pesticides. The extraction and concentration steps using aqueous acetonitrile and chloroform are very similar to those used in the procedure for

Table 1. Efficiency of different cleanup procedures.

	Total residue, mg/g sample	
	Organochlorine fraction	Organophosphorus fraction
Florisil [a]	1.4	—
Celite 545, Nuchar C-190 N, Sea Sorb 43 [b]	—	14.4
Alumina; Florisil [c]	1.0	3.9

a: Mills et al., 1963
CH_3CN-H_2O→petroleum ether→Florisil
Eluant: 100 ml petroleum ether-diethyl ether-dioxan

b: Storherr et al., 1971
CH_3CN-H_2O→methylene chloride→adsorbent mixture
Eluant: 200 ml acetonitrile-benzene

c: Nesemann et al.
CH_3CN-H_2O→methylene chloride→1. alumina→2. Florisil
Eluant: 1. 50 ml acetonitrile, 100 ml methylene chloride
2. 100 ml n-hexane, 100 ml benzene

Figure 3. Gas chromatogram of tobacco extract. After Florisil cleanup, n-hexane fraction. Column temperature: 180° C. Detector: microcoulometric system.

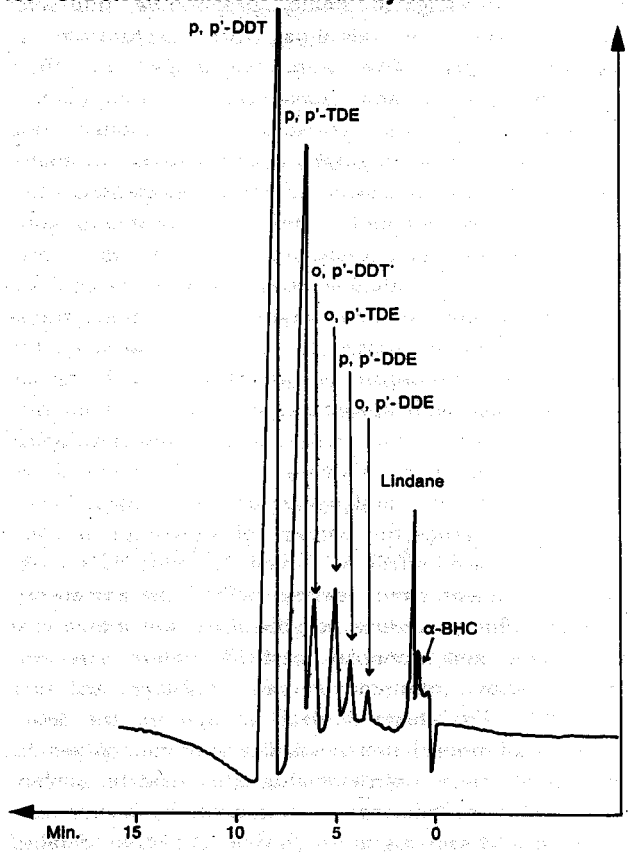


Figure 4. Gas chromatogram of tobacco extract. After Florisil cleanup, benzene fraction. Column temperature: 180° C. Detector: microcoulometric system.

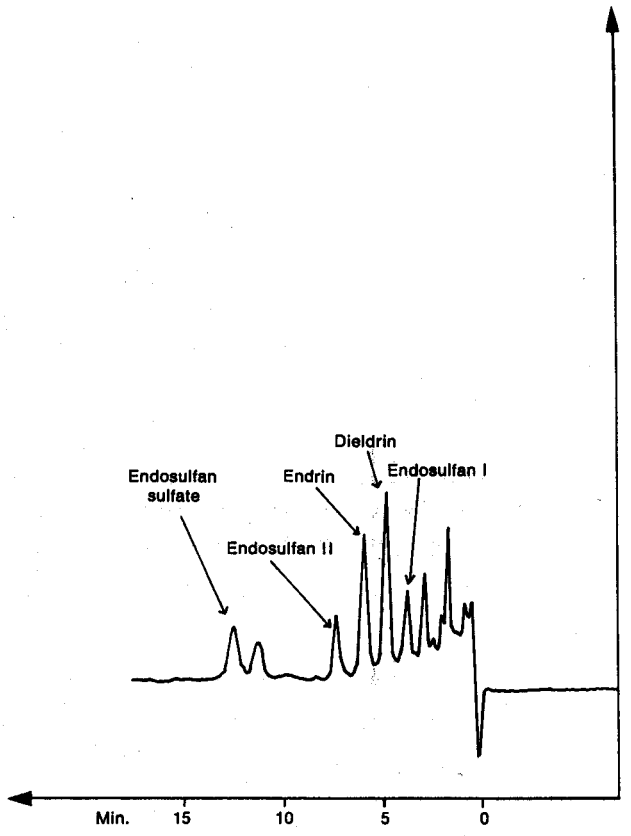


Figure 5. Gas chromatogram of six organochlorine pesticides; standard mixture, 4 ng, each. Separation obtained with routine operational conditions. Column temperature: 180° C. Detector: microcoulometric system.

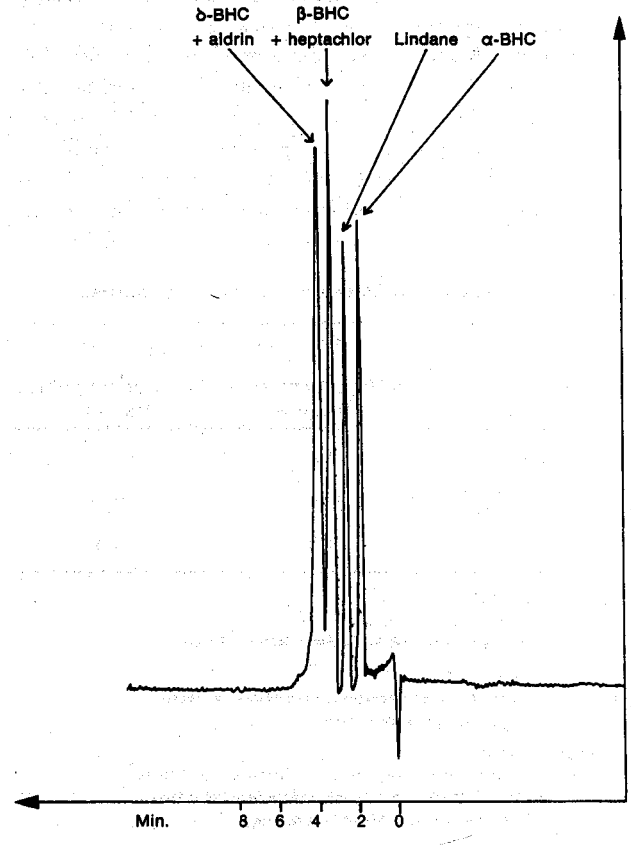


Figure 6. Gas chromatogram of six organochlorine pesticides; standard mixture, 4 ng, each. Separation at reduced column temperature. Column temperature: 128° C. Detector: microcoulometric system.

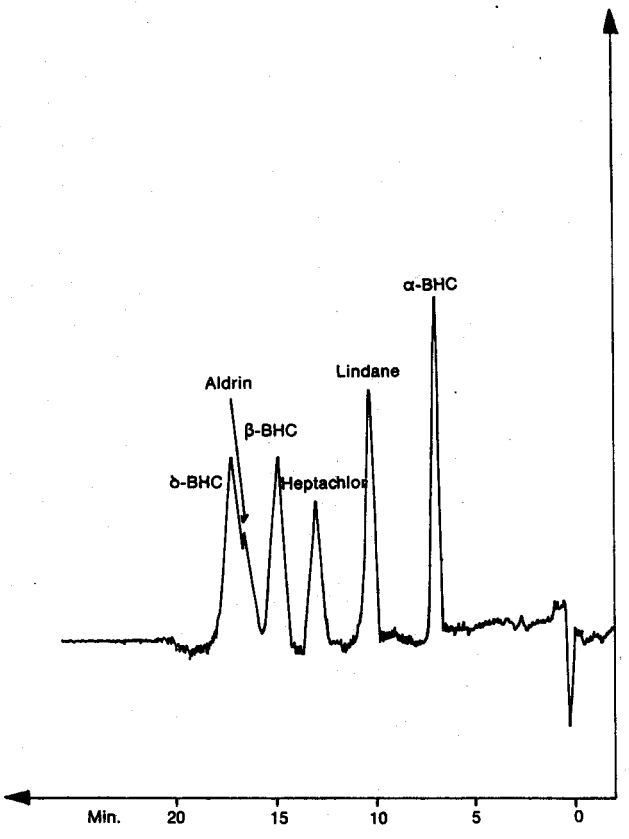


Figure 7. Gas chromatogram of tobacco extract. After Florisil cleanup, n-hexane fraction. Separation at reduced column temperature. Column temperature: 128° C. Detector: microcoulometric system.

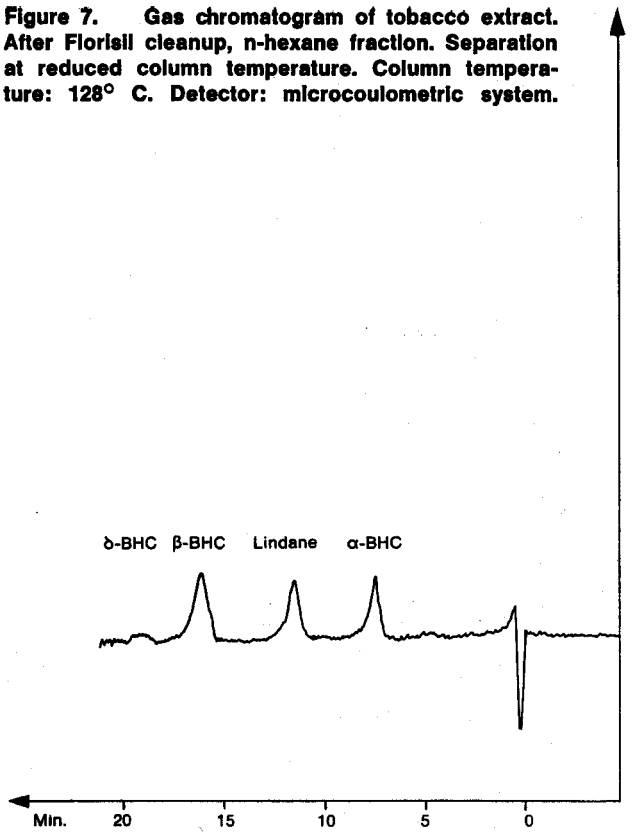
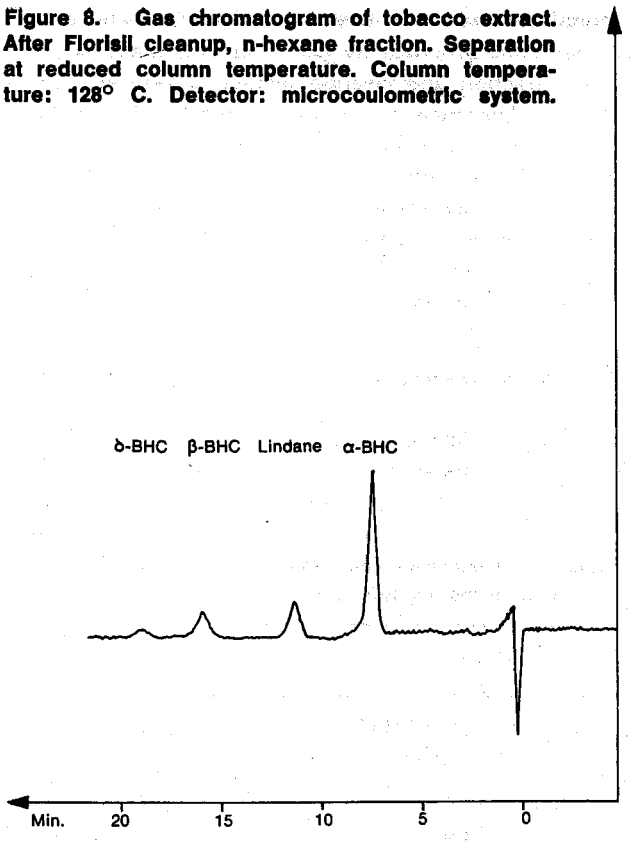


Figure 8. Gas chromatogram of tobacco extract. After Florisil cleanup, n-hexane fraction. Separation at reduced column temperature. Column temperature: 128° C. Detector: microcoulometric system.



chlorinated and phosphated pesticides. Only distillation of the purified, hydrolyzed tobacco extract gave a sufficient cleanup for the following detection of alkylphenol and naphthol, resulting from propoxur and carbaryl, as trichloroacetyl derivatives with electron capture gas chromatography (Fig. 9–11).

The determination of methomyl requires very little cleanup, because of the possibility to detect methomyl as the methylthiopropionaldehyde oxime via the sulfur response with the flame photometric detector. This procedure, published by *Pease* and co-workers (8), is not very sensitive, but specific, and does not suffer by interferences from tobacco.

In general, the microcoulometric system is about 10 to 20 times less sensitive to chlorinated compounds than the electron capture detector, but highly specific for halogen. The lack of sensitivity, which can be overcome by a more concentrated sample solution, is outweighed by the advantage of specificity with regard to the great number of non-halogen compounds, to which the electron capture detector responds.

One problem in work on pesticide residues is the identification of compounds.

Possible errors result in routine work from identifying pesticides only by measuring the retention time and calculating the relative retention values after gas chromatography on one column. Rarely confirmation is carried out on a second or third different column, especially in the determination of the well known chlorinated insecticides. In the presence of Lindane isomers, the α-, β-, δ- and ε-isomers, the β- and δ-isomers become confused with heptachlor and aldrin, respectively.

A separation is achieved only at very low column temperature (Fig. 5 and 6). Nowadays a number of the analysed leaf tobacco has varying amounts of these isomers (Fig. 7 and 8). The possible misinterpretation is especially critical if it is concerned with banned pesticides such as aldrin and heptachlor.

Table 2. Recovery and sensitivity of pesticides added to tobacco at the 0.5 ppm level.

Pesticide added	Recovery, %	Sensitivity, ppm
α-BHC	88	0.02
Lindane (γ-BHC)	88	0.02
β-BHC	88	0.02
δ-BHC	88	0.02
p,p'-DDE	95	0.02
o,p'-DDT	98	0.03
p,p'-DDT	101	0.04
Dieldrin [a]	86	0.03
Endrin [a]	88	0.03
Endosulfan I	96	0.03
Endosulfan II	97	0.05
Endosulfan sulfate	96	0.1
p,p'-TDE	96	0.04

a: Fortified with 0.1 ppm.

SUMMARY

The screening procedure for the determination of pesticide residues in tobacco (organochlorine, organophosphorus and carbamate insecticides) published in 1970 has been improved by introduction of the dry-column chromatography as cleanup procedure. A new

procedure has been developed for the detection of carbaryl and propoxur by gas chromatography.
The extraction and cleanup method developed can be used to determine 11 organochlorine, 11 organophosphorus and 3 carbamate insecticides. The procedure can be applied to determine further insecticides and, in addition, fungicides, herbicides and metabolites.
The recoveries of added pesticides at 0.5 ppm level are between 75 and 102%. The coefficient of variation detected with parathion is 4.4%.

ZUSAMMENFASSUNG

Die 1970 publizierte Methode zur Bestimmung von Pestizidrückständen auf Tabak (Screening-Methode für Organochlor-, Organophosphor- und Carbamatinsektizide) wurde durch Einführung der Trockensäulenchromatographie als Reinigungsverfahren verbessert. Außerdem wurde eine gaschromatographische Methode zur Bestimmung von Carbaryl und Propoxur entwickelt.
Mit dieser Methode können 11 Organochlor-, 11 Organophosphor- und 3 Carbamatinsektizide nebeneinander bestimmt werden. Die Anwendbarkeit für weitere Insektizide sowie Fungizide, Herbizide und Metabolite ist gegeben.
Die Rückgewinnung zugesetzter Pestizide im 0.5-ppm-Bereich liegt zwischen 75–102%. Der Variationskoeffizient der Ergebnisse mit dieser Methode, bestimmt an Parathion, beträgt 4,4%.

Table 3. Recoveries and sensitivities of pesticides added to tobacco at the 0.5 ppm level.

Pesticide added	Recovery, %	Sensitivity, ppm
Azinphos-ethyl (Ethyl Guthion)	102	0.07
Azinphos-methyl (Guthion)	96	0.2
Diazinon	90	0.01
Dimethoate	98	0.02
Disulfoton (Di-Syston)	84	0.03
Fensulfothion (Dasanit)	97	0.01
Malathion	86	0.03
Monocrotophos (Azodrin)	75	0.1
Parathion-ethyl	84	0.02
Parathion-methyl	86	0.03
Phosphamidon I	81	0.07
Phosphamidon II	101	0.07

Table 4. Recoveries and sensitivities of pesticides added to tobacco at the 0.5 ppm level.

Pesticide added	Recovery, %	Sensitivity, ppm
Carbaryl (Sevin)	84	0.01
Methomyl (Lannate)	102	0.07
Propoxur (Baygon)	86	0.01

Table 5. Reproducibility of the results, determination of field-incurred parathion in tobacco.

Analysis	Parathion, ppm		
	I[a]	II	III
1	0.66	0.64	0.59
2	0.66	0.63	0.59
3	0.60	0.67	0.62
4	0.60	0.67	0.63
5	0.63	0.65	0.61
$\bar{x}$	0.63	0.65	0.61
Coefficient of variation, %		4.4	

a: I, II, III = chemists performing analyses.

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RESUME

Le procédé de «screening» pour la détermination des résidus de pesticides dans le tabac (organochlorine, organophosphore et insecticides au carbamate), publié en 1970, a été amélioré par l'introduction de la chromatographie à colonne solide comme moyen de purification. On a aussi mis un nouveau procédé au point pour la détection de carbaryle et de propoxure par chromatographie en phase gazeuse.
Les méthodes d'extraction et de purification qui ont été mises au point peuvent être utilisées pour déterminer 11 organochlorines, 11 organophosphores et 3 insecticides au carbamate. Ce procédé peut être appliqué pour la détermination d'autres insecticides et aussi pour des fungicides, des herbicides et des métabolites.
Les pesticides additionnés jusqu'à un taux de 0,5 ppm sont récupérés de 75% à 102%. Au parathion on observe un coëfficient de variation de 4,4%.

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Figure 9. Gas chromatograms A, control sample; B, control sample with 0.5 ppm carbaryl added. Column temperature: 165° C. Detector: electron capture.

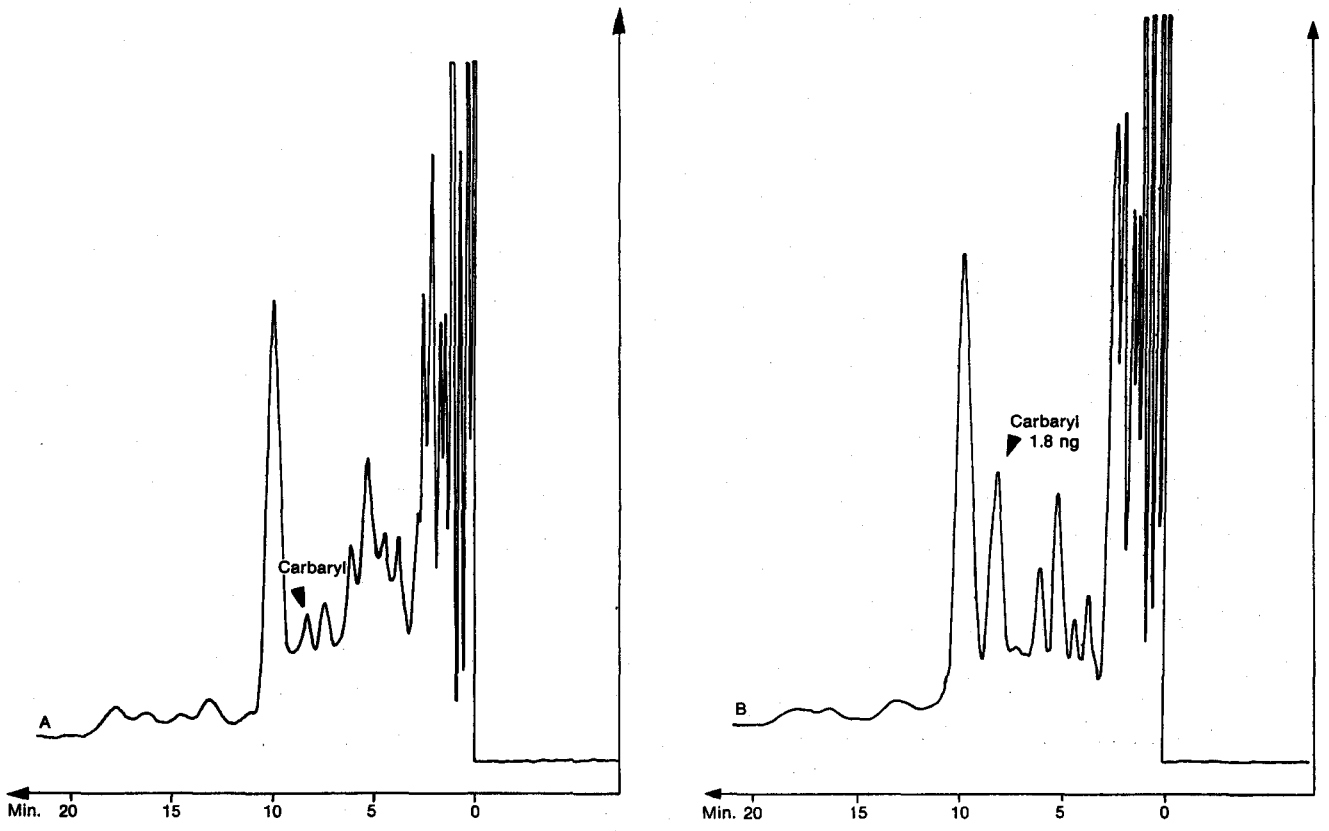

Figure 10. Gas chromatograms A, control sample; B, control sample with 0.5 ppm propoxur added. Column temperature: 145° C. Detector: electron capture.

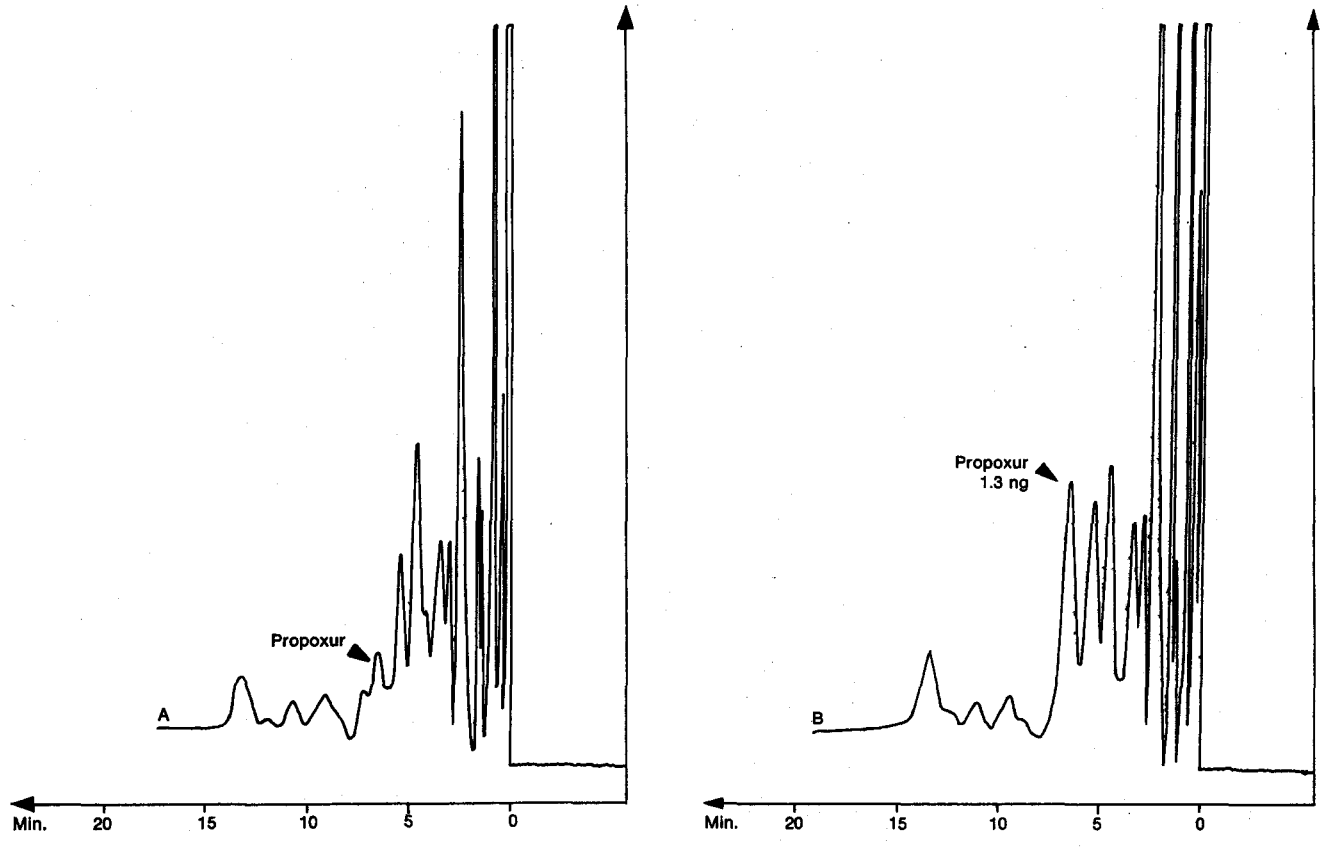

Figure 11. Gas chromatograms A, control sample; B, control sample with 0.5 ppm methomyl added. Column temperature: 150° C. Detector: flame photometric, S-mode.