Journal of Chromatography, 89 (1974) 177–183

CHROM. 7107

DETERMINATION OF BHC ISOMERS IN SOILS BY GAS–LIQUID AND THIN-LAYER CHROMATOGRAPHY AFTER EXTRACTION WITH LIGHT PETROLEUM

H. MAHEL'OVÁ, M. SACKMAUEROVÁ, A. SZOKOLAY and J. KOVÁČ

Research Institute of Hygiene and Research Institute of Agrochemical Technology, Bratislava (Czechoslovakia)

(First received August 8th, 1973; revised manuscript received October 4th, 1973)

SUMMARY

The determination of residues of BHC* isomers in soils by means of both gas–liquid and thin-layer chromatography and the conditions for their extraction in the presence of both DDT and DDE were studied. A method for the deactivation of soil by water using active silica was developed. By this means, a constant powdery consistency was obtained, which permitted the use of a specific extraction solvent (low-boiling light petroleum) under conditions of Soxhlet extraction. In contrast to extraction procedures using the *n*-hexane–acetone system as solvent, the present method markedly reduces the proportion of coextracted materials, so that in addition to DDT and DDE, the α-, β-, γ- and δ-isomers of BHC can also be determined simultaneously in clay soils. The yield given by the proposed method of Soxhlet extraction ranges between 81.8 and 105.8% according to the type of insecticide.

INTRODUCTION

Recently, many workers[1–6] have studied the problems of the determination of organochlorine pesticide residues in soils. The effectiveness of the extraction procedure is a fundamental requirement and therefore a great deal of attention has been paid to the study of the optimal conditions of extraction. The organochlorine insecticides are in an adsorbed state, bound to the soil particles. The stability of such binding depends both on the adsorptive capacity and on the physical and physico-chemical properties of the soil[7–12]. In certain types of soil, the insecticide–soil particle bond is to a certain extent differentiated, owing to the polarity of the compound. An air-dried soil can be considered as a polar adsorbent, the active surface being dependent on the size of the soil particles. Hence adequate extraction of an insecticide from the active surfaces of soil particles is dependent on the deactivation of the active centres of the soil sample by water or by molecules of the solvent during the extraction procedure. The selection of a suitable extraction solvent, or solvent system, is, therefore, of great

---

* BHC = 1,2,3,4,5,6-Hexachlorocyclohexane.

importance with respect to the deactivation of the soil by water before the extraction procedure.

Although previous workers have examined a wide range of chlorinated insecticides, they did not investigate the BHC isomers. This problem has recently been considered from the viewpoint of ecological studies[13]. When choosing analytical methods, we therefore selected such procedures that would enable us to identify and determine these substances in the presence of DDT and DDE by gas–liquid chromatographic (GLC) and thin-layer chromatographic (TLC) methods.

In order to increase the selectivity of the extraction and also to suppress the hydrolysis of DDT to DDE under the conditions of Soxhlet extraction, we chose low-boiling light petroleum instead of a mixture (*n*-hexane–acetone) as the solvent and also used Siloxid*, which was required for the formation of a powdery consistency of soil samples deactivated by water.

EXPERIMENTAL

Black soil silt loam, pH 6.2, containing 2.8% of organic compounds, that had been treated with both DDT and BHC until 1969, was used for extraction. The soil samples were air dried and sieved through an 18-mesh sieve. Samples weighing 20 g were processed. It was first necessary to establish the volume of distilled water to be added to the sample in order to deactivate the active centres of the soil sample before extracting the residues with the organic solvent. The best yield was recorded after the addition of 25% of water. After incubation for 24 h, the water-treated soil samples were further processed. The excess of water was removed from the soil particles by adding Siloxid to the sample in order to obtain a homogeneous, powdery mixture. This mixture was then extracted for 12 h with 250 ml of low-boiling light petroleum (boiling range 35–50°) in a Soxhlet extractor.

The soil samples were also extracted with the aid of a mechanical shaker. The mixture was shaken three times with light petroleum for 1-h periods and the separate extracts were filtered into 500-ml flasks through a layer of anhydrous sodium sulphate. The light petroleum was then evaporated off by means of a rotary vacuum evaporator to leave a volume of 5–10 ml.

In addition, extraction of mixtures of soil with Hyflo Supercel according to Williams[1] was carried out in a Soxhlet apparatus using *n*-hexane–acetone (49:51) as solvent.

After the extraction had been completed, the total extract was concentrated to a small volume (about 5 ml) by means of a rotary vacuum evaporator. The extract was then purified by column adsorptive chromatography. A 2 × 40 cm chromatographic column was prepared from layers of anhydrous sodium sulphate (1.5 cm), Celite 545 (4 g), a mixture of Celite (8 g) plus fuming sulphuric acid (6 ml, containing 10% of free SO_3) and anhydrous sodium sulphate (1.5 cm). The column was packed with degreased cotton-wool. The concentrated extract was quantitatively pipetted into the column and the residues were eluted with light petroleum. Approximately 250 ml of eluate were collected and evaporated just to dryness, and the residue was dissolved immediately in 1 ml of *n*-hexane.

---

* Siloxid is an active silicon oxide (Tonaso, Neštěmice, Czechoslovakia).

The residues were determined on a Perkin-Elmer F-7 gas chromatograph equipped with an electron capture detector, under the following conditions: temperature of column and detector, 190°; temperature of injection space, 210°; carrier gas, nitrogen at a flow-rate of 60–80 ml/min; column filled with 1.5% OV-17 plus 2% QF-1 on Chromosorb, 80–100 mesh. Portions of 1 μg of the purified eluate were injected into the apparatus, and the results were read off from a calibration graph.

In addition to GLC, TLC on standard Silufol* plates was also used to determine the residues.

RESULTS

Table I shows the dependence of the yields of the chlorinated insecticide residues from a sample of clay soil (five determinations carried out by GLC) on the amount

TABLE I

DEPENDENCE OF THE YIELDS OF CHLORINATED INSECTICIDE RESIDUES FROM BLACK SOIL SILT LOAM ON THE AMOUNT OF WATER ADDED

Insecticide	*Yield (mg/kg)*			
	Water added (%)			
	0	*20*	*25*	*40*
α-BHC	0.000	0.04	0.006	0.006
β-BHC	0.000	0.006	0.013	0.014
γ-BHC	0.005	0.010	0.028	0.030
δ-BHC	0.000	0.001	0.004	0.002
p,p'-DDE	0.016	0.032	0.033	0.020
p,p'-DDT	0.036	0.075	0.092	0.070

TABLE II

COMPARISON OF THREE DIFFERENT EXTRACTION PROCEDURES FOR EXTRACTION OF CHLORINATED INSECTICIDE RESIDUES FROM BLACK SOIL SILT LOAM

Extraction with light petroleum of a homogeneous mixture of soil with Siloxid according to the following purification methods: (1) in the Soxhlet apparatus; (2) in a mechanical shaker. Extraction with n-hexane–acetone according to Williams[1] of a homogeneous mixture of soil with Celite in the Soxhlet apparatus: (3) extract purified on a column with Al_2O_3; (4) extract purified by shaking into two different solvents. u = Undeterminable.

Insecticide	*Insecticide concentration (mg/kg)*			
	1	*2*	*3*	*4*
α-BHC	0.006	0.004	u	u
β-BHC	0.013	0.009	u	u
γ-BHC	0.028	0.029	u	u
δ-BHC	0.004	0.002	u	u
p,p'-DDT	0.033	0.023	0.026	0.027
p,p'-DDE	0.092	0.067	0.078	0.062

---

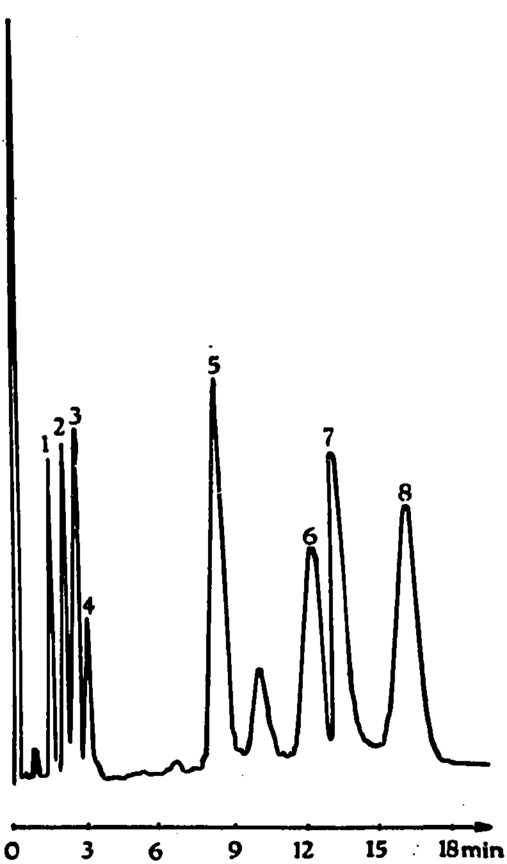
* Silufol is a registered product with a standardized silica gel layer (Chema, Horni Počernice, Czechoslovakia).

of water added to the soil sample in order to deactivate it. The homogeneous mixture of soil with Siloxid was extracted in the Soxhlet apparatus 24 h after the water had been added. It can be seen that maximal amount of residues was extracted after the addition of 25% of water. This amount of water ensures the optimal humidity of the soil, and the addition of more water gives no further improvement.

From Table I, it is clear that the efficiency of the extraction with light petroleum without deactivating the soil by water is insufficient.

The proposed method of deactivation by 25% of water was used to compare three different extraction procedures from the point of view of the yields of BHC isomers, DDT and DDE obtained. Ten determinations by GLC were carried out.

From Table II, it is clear that it is possible to isolate efficiently residues of DDT, DDE and BHC isomers using low-boiling light petroleum as the extraction solvent by both Soxhlet extraction as well as by mechanical shaking, the only requirements being deactivation of the soil by water and a powdery consistency of the sample (achieved by adding Siloxid). The use of a non-polar solvent markedly increased the



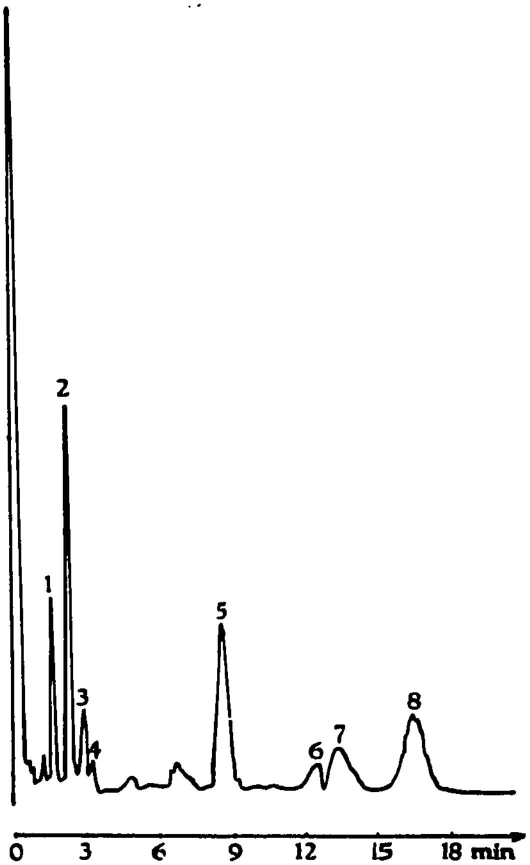


Fig. 1. Separation of standard mixtures. 1, α-BHC; 2, γ-BHC; 3, β-BHC; 4, δ-BHC; 5, *p,p'*-DDE; 6, *o,p'*-DDT; 7, *p,p'*-DDD; 8, *p,p'*-DDT.

Fig. 2. Gas chromatogram of a soil sample after extraction with light petroleum. 1, α-BHC; 2, γ-BHC; 3, β-BHC; 4, δ-BHC; 5, *p,p'*-DDE; 6, *o,p'*-DDT; 7, *p,p'*-DDD; 8, *p,p'*-DDT.

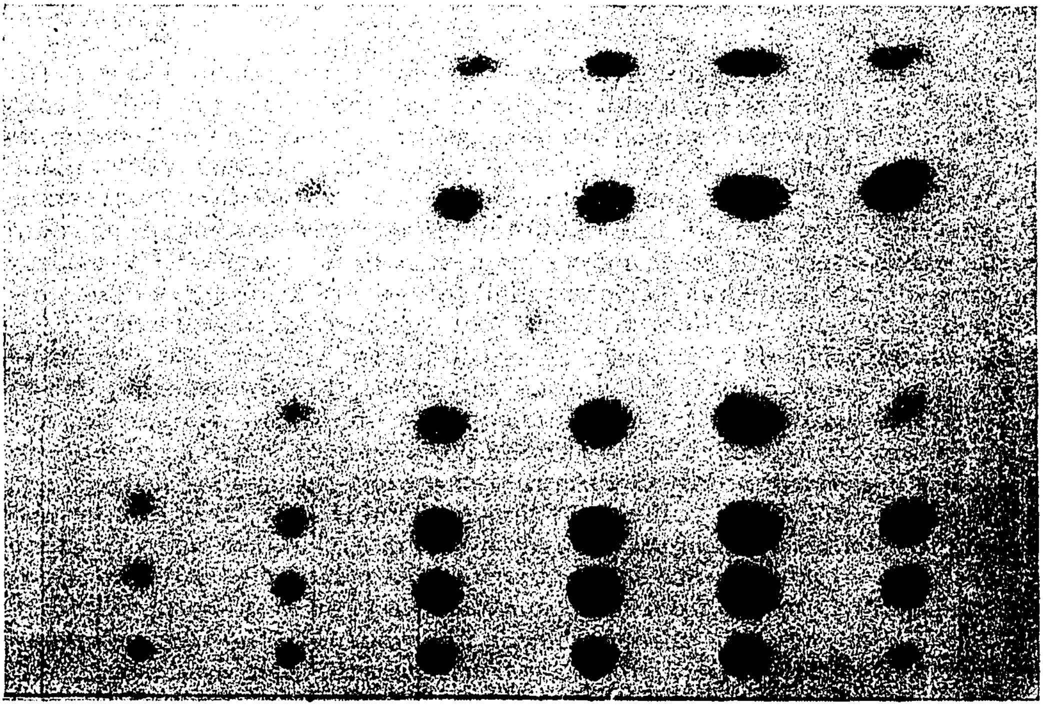


Fig. 3. TLC of a cleaned extract from a soil sample compared with insecticide standards. The first five runs demonstrate the mixture of standards upwards from below: δ-, β-, γ-, α-BHC, *p,p'*-DDT and *p,p'*-DDE with increasing concentrations from 0.05 to 0.7 μg. The final run shows the adequate eluate.

specificity of the extraction; thus, when purifying the sample, all of the substances examined can be determined simultaneously by both GLC and TLC. Fig. 1 shows the separation of standard mixtures by GLC, and Fig. 2 is the GC trace of a soil sample submitted to extraction. Fig. 3 is a TLC record on a Silufol plate of the same soil sample as in Fig. 2.

The low-boiling light petroleum excludes, in fact, all possibility of the hydrolysis of DDT and DDE under the conditions of Soxhlet extraction. It also decreases the proportion of interfering substances coextracted, which are commonly present in the *n*-hexane–acetone system (Fig. 4).

It also follows from Fig. 4 that because of the coincidence of the more polar interfering coextracted substances, BHC isomers cannot be determined by the *n*-hexane–acetone system according to Williams. This solvent system can be used only when *p,p'*-DDT and/or *p,p'*-DDE are determined in soils by GLC.

Determination of yields and of the precision of the method

The yield given by the method was verified by adding known amounts of insecticides into soil samples using two different extraction procedures under identical conditions. The results of the model experiments are shown in Table III.

The precision of the method expressed as the coefficient of variation (based on ten parallel GLC determinations) ranged between 4.3 and 11.9%.

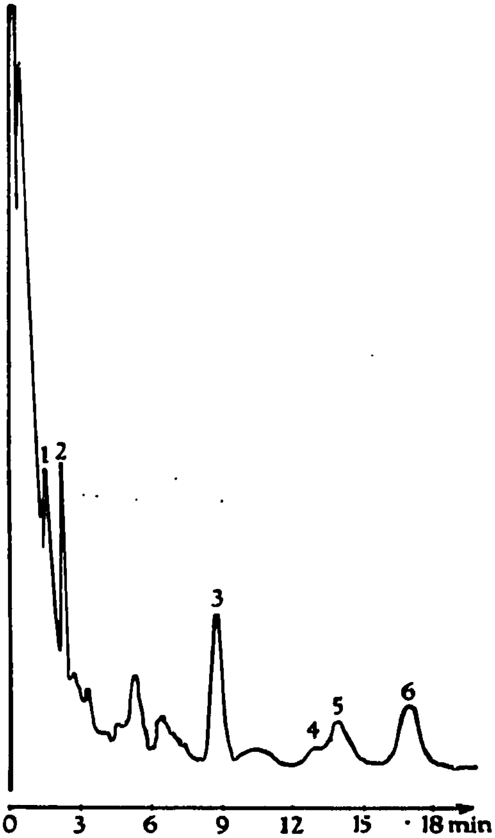


Fig. 4. Gas chromatogram of a soil sample after extraction with *n*-hexane–acetone (49:51) according to Williams[1]. 1, α-BHC; 2, γ-BHC; 3, *p,p'*-DDE; 4, *o,p'*-DDT; 5, *p,p'*-DDD; 6, *p,p'*-DDT.

TABLE III

YIELDS OF CHLORINATED INSECTICIDES FROM BLACK SOIL SILT LOAM

Standard solution added.

The values given represent the sum of insecticides added to the sample and insecticide present in soil before adding standard solution.

Insecticide	*Amount (mg/kg) added*	*Soxhlet extraction*		*Extraction in a mechanical shaker*	
		Found *(mg/kg)*	Yield *(%)*	Found *(mg/kg)*	Yield *(%)*
α-BHC	0.1	0.088	81.8	0.080	73.8
β-BHC	0.1	0.113	99.7	0.087	74.1
γ-BHC	0.1	0.115	86.8	0.115	87.3
δ-BHC	0.1	0.088	84.4	0.086	81.8
p,p'-DDE	0.1	0.139	105.8	0.108	74.8
p,p'-DDT	0.1	0.197	104.5	0.167	75.3

DISCUSSION

The potential use of light petroleum as a non-polar extraction solvent for water-deactivated soil is considerable, because it fulfils the conditions of good solubility of chlorinated insecticides. However, from the point of view of the interaction of a non-polar solvent with the insecticide molecules, in a heterogeneous system with the small contact surfaces of humid soil agglomerates, it appears not to be very suitable. A better interaction was reached by adding Siloxid and, in this way, enlarging the contact surface of the soil particle–extraction solvent interface. The method of soil deactivation by water allows the use of low-boiling light petroleum as a highly specific extraction solvent.

The favourable influence of water on soil has also been demonstrated by Williams[1], who studied the conditions of extraction. He enlarged the contact surface of soil particles with the solvent by adding Celite to the soil. The *n*-hexane–acetone solvent mixture was found to be suitable for determinations of DDT and/or DDE residues by the GLC method only. Because of the coincidence of more polar interfering coextracted substances, however, it cannot be recommended for the simultaneous determination of BHC isomers in soils (Table II, Fig. 3). The interfering influence of coextracted substances results from the decrease in the specificity of the extraction by the polar component of the solvent system. The considerable proportion of coextracted substances present (when purification is carried out on Al_2O_3) makes it impossible to determine DDT, DDE and BHC isomers by the TLC method.

The best yields were recorded with extraction in the Soxhlet apparatus (Tables II and III). Extraction in the mechanical shaker gave lower yields but they were, however, still satisfactory with respect to the level of determination. From the point of view of practical work, the latter method seems to be more advantageous: the extraction time required is 3 h compared with 12 h required for the Soxhlet extraction.

A good yield by the proposed method depends on the evaporation of the eluate just to dryness, otherwise the yield of the BHC isomers becomes even lower[14].

It is necessary to make parallel determinations of residues by TLC in order to confirm the precision of the determination and to eliminate artifacts that coincide with the substances examined. Highly sensitive Silufol standard plates with a standardized silica gel layer[15] were selected for this purpose.

REFERENCES

1 I. H. Williams, *J. Ass. Offic. Anal. Chem.*, 51 (1968) 715.
2 M. Chiba, *Residue Rev.*, 30 (1969) 63.
3 M. Chiba and H. V. Morley, *J. Agr. Food Chem.*, 16 (1968) 916.
4 E. P. Lichtenstein, G. R. Myrdal and K. R. Schulz, *J. Econ. Entomol.*, 52 (1959) 118.
5 E. P. Lichtenstein, G. R. Myrdal and K. R. Schulz, *J. Econ. Entomol.*, 52 (1959) 124.
6 K. I. Benyon and K. E. Elgar, *Analyst (London)*, 91 (1966) 143.
7 S. Pennington and C. E. Meloan, *J. Chromatogr.*, 27 (1967) 250.
8 R. G. Nash and E. A. Woolson, *Science*, 157 (1967) 924.
9 R. S. Adams, *Soil Sci. Soc. Amer., Proc.*, 35 (1971) 78.
10 K. W. Snelling, *Agron. J.*, 61 (1969) 72.
11 J. K. Oddson, *Soil Sci. Soc. Amer. Proc.*, 34 (1970) 412.
12 C. S. Helling, *Advan. Agron.*, 23 (1972) 104.
13 *Internationales Lindan Symposium, Vienna, June 1972* Centre International d'Etudes du Lindone, Brussels.
14 J. Uhnak, M. Sackmauerová, A. Szokolay and A. Madarič, *Chem. Zvesti*, 27 (1973) 128.
15 A. Szokolay and A. Madarič, *J. Chromatogr.*, 42 (1969) 509.