# THIN-LAYER CHROMATOGRAPHIC SEPARATION, IDENTIFICATION AND ESTIMATION OF RESIDUES OF SOME CARBAMATE AND ALLIED PESTICIDES IN SOIL AND WATER

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Thin-layer chromatographic methods for the determination of chlorophenoxy acid[1] and triazine[2] herbicides in soil and water have been reported. This work has now been extended to cover herbicides of the carbamate, urea and uracil classes; two carbamate insecticides have also been included in the study because of their similar structure and chromatographic properties.

The chemical nature of the ultimate residues of herbicides and insecticides of carbamate or similar structure is not at present fully understood. The compounds themselves are known to have toxic properties affecting the thyroid, nervous system and blood of animals.

Carbamates can be separated, identified and estimated quantitatively by paper[3], thin-layer[4] and gas–liquid chromatography[5,6], but little work has been done on the quantitative measurement of total carbamate residues in crops. Of the methods available for this work, colorimetric methods although quite sensitive, lack selectivity and are time consuming; paper chromatography is time consuming and not particularly selective; and gas–liquid chromatography, although selective and sensitive, is very expensive and can only be applied to a small number of carbamates, unless conversion to more suitable derivatives is carried out[7,8].

## EXPERIMENTAL

### *Compounds studied*

The compounds that were studied may be placed in three distinct classes as follows:

(1) *Ureas*, RNH·CO·NR′R″, where R = phenyl or Cl-substituted phenyl, and R′ and R″ = alkyl or alkoxy groups, *e.g.*:

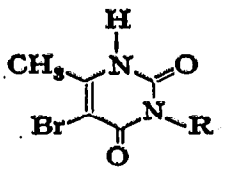
fenuron — R = Ph, R′ = R″ = Me
monuron — R = *p*-ClPh, R′ = R″ = Me
diuron — R = 3,4-di-ClPh, R′ = R″ = Me
linuron — R = 3,4-di-ClPh, R′ = Me, R″ = OMe.

(2) *Carbamates*, RNH·COOR′, where (a) R = aryl and R′ = alkyl, *e.g.*:

propham — R = Ph, R′ = isopropyl
chlorpropham — R = 3-ClPh, R′ = isopropyl
barban — R = 3-ClPh, R′ = $-CH_2-C\equiv C-CH_2Cl$

or (b) R = Me and R′ = aryl or substituted aryl, *e.g.*:
carbaryl (Sevin): R = Me, R′ = 1-naphthyl
arprocarb (Unden): R = Me, R′ = 2-isopropoxyphenyl

(3) *Uracils*,



, *e.g.*:

isocil — R = isopropyl
bromacil — R = 1-methylpropyl.

*Thin-layer separations*

Various solvent systems were tried on 250 $\mu$ layers of both silica gel and alumina. Separation between the classes was relatively easy but only a few solvent systems showed any separation between the individual members of each class, see Table I. All the compounds except the two uracils were visualized by spraying with a solution of *p*-$NMe_2PhCHO$ in EtOH (0.5%) followed by 2*N* aq. $H_2SO_4$ and heating at 150° for

TABLE I

SEPARATION OF CARBAMATE-TYPE PESTICIDES BY THIN-LAYER CHROMATOGRAPHY
Stationary phase: silica gel.
Solvent systems: 1 = Hexane–acetone (9:1); 2 = hexane–acetone (7:3); 3 = chloroform–nitromethane (5:5); 4 = chloroform; 5 = chloroform–acetone (9:1); 6 = chloroform–acetone (7:3); 7 = ether; 8 = dichloromethane.

| *Pesticide* | $R_F \times 100$ *in solvent system* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
| Fenuron | 10 | 64 | 28 | 12 | 58 | 78 | 25 | 41 |
| Monuron | 10 | 46 | 38 | 12 | 59 | 77 | 20 | 50 |
| Diuron | 20 | 51 | 52 | 14 | 64 | 80 | 23 | 57 |
| Linuron | 34 | 43 | 86 | 58 | 89 | 91 | 74 | 92 |
| Propham | 60 | 77 | 100 | 72 | 93 | 100 | 100 | 86 |
| Chlorpropham | 60 | 77 | 100 | 78 | 92 | 100 | 100 | 89 |
| Barban | 40 | 65 | 100 | 80 | 92 | 94 | 100 | 86 |
| Carbaryl | 20 | 59 | 79 | 47 | 94 | 89 | 81 | 61 |
| Arprocarb | 25 | 55 | 71 | 42 | — | — | 85 | 51 |
| Isocil | 20 | 51 | 46 | — | 63 | 81 | — | — |
| Bromacil | 22 | 56 | 54 | — | 69 | 84 | — | — |

20 min. The above applies for silica gel, but on alumina the O-aryl carbamates did not show up. These may be detected either by Brilliant Green in acetone followed by bromination, possibly coupled with a further spray of dichlorofluorescein and visualization under ultra-violet, or by a spray of *p*-nitrobenzene diazonium fluoborate in methanol (freshly prepared) followed by 2 *N* aq. NaOH and heating at 150° for 20 min.

The uracils were the most difficult group to detect, but a Brilliant Green spray followed by bromination, 2*N* aq. $H_2SO_4$, dichlorofluorescein and visualization under

ultra-violet did show them up. It was found best to use an ethanolic spray of sulphuric acid on alumina, since an aqueous spray damaged the surface.

Overspraying after heat treatment also improved visualization in the case of *p*-$NMe_2PhCHO$, and a final spray of 2*N* aq. NaOH was found very effective in this case.

The use of the fluorescent whiteners known as the Calcofluors proved effective in the visualization of all the carbamates, uracils and ureas. They are sensitive in most cases to 2 $\mu$g although they lack sensitivity in the cases of arprocarb, carbaryl and barban. Calcofluor R White when built into the chromatoplate in a concentration of 0.02 % can be used to distinguish between the ureas and uracils, and the carbamates themselves, as shown in Table II.

TABLE II

THE VISUALIZATION OF URACILS, UREAS AND CARBAMATES ON CHROMATOPLATES CONTAINING CALCOFLUOR R WHITE

QF = Quenched fluorescence; F = fluorescence.

| *Compound* | *Effect of U.V. light* | | *Sensitivity* ($\mu$g) |
|---|---|---|---|
| | *2540 Å* | *3600 Å* | |
| Uracils and ureas | | | |
| Isocil | QF | — | 1.0 |
| Bromacil | QF | — | 1.0 |
| Monuron | QF | — | 2.0 |
| Fenuron | QF | — | 2.0 |
| Diuron | QF | — | 2.0 |
| Linuron | QF | — | 1.0 |
| Carbamates | | | |
| Propham | — | F | 3.0 |
| Chlorpropham | — | F | 4.0 |
| Arprocarb | — | F | 5.0 |
| Carbaryl | — | F | 5.0 |
| Barban | — | F | 5.0 |

*Effect on the $R_F$ of changes in the pH of the chromatoplates*

Since the compounds contained potentially basic groupings, it was considered worthwhile to investigate the variation of $R_F$ value with change in pH of the absorbent layer. Silica gel plates were prepared with 1*N* solutions of either sodium hydroxide or oxalic acid instead of water. Separations were not improved, but the basic character of the ureas was shown up by the reduced $R_F$ values on the acidic layers, and the increased $R_F$ values on the basic layers, silica gel itself being somewhat acidic. The N-phenyl carbamates showed similar basic properties, but the O-aryl carbamates showed little variation of $R_F$ with change in pH.

The addition of acid or base (glacial acetic or diethanolamine to the extent of 2 % by volume) to the solvent mixture had a similar effect on the $R_F$ values, but again gave little or no improvement in separation.

Since barban contained an acetylenic grouping it was considered interesting to study its $R_F$ behaviour on plates with silver nitrate incorporated into the absorbent layer. Plates with 2–10 % silver nitrate in silica gel were prepared, but no retardation

of the main component of barban was observed. However, on one of these plates technical barban was resolved into six components.

*Quantitative measurement*

In order to obtain the greatest variation in spot size with weight, it is necessary that the initially applied sample be kept in as small a spot as possible. To this end various solvents were tried for their spreading capacities, *i.e.* how large an area the sample will occupy when spotted under standard conditions. Spreading capacities increased in the following order: hexane < petroleum ether < $CCl_4$ < benzene < $CH_2Cl_2$ < $CHCl_3$. Bearing in mind the low affinity of the compounds for hydrocarbons, carbon tetrachloride was chosen as the solvent for sample application. Amounts ranging from 1 to 10 $\mu$g of the compounds were applied to the plate (sample dissolved in 40 $\mu$l $CCl_4$ and 10 $\mu$l applied). After development and visualization the spots were ringed either in daylight or under ultra-violet light where applicable.

The silica gel within the area of the spot was removed and a sheet of transparent graph paper was placed in contact with the absorbent layer and then a sheet of light-sensitive diazo paper placed in contact with the latter, the sheets being kept in close contact by a second glass plate underneath. Illumination for 20 sec and development in an ammonia tank for a few minutes showed up the spots on the diazo paper with superimposed squares[9], allowing the areas to be readily estimated, see Fig. 1. A linear

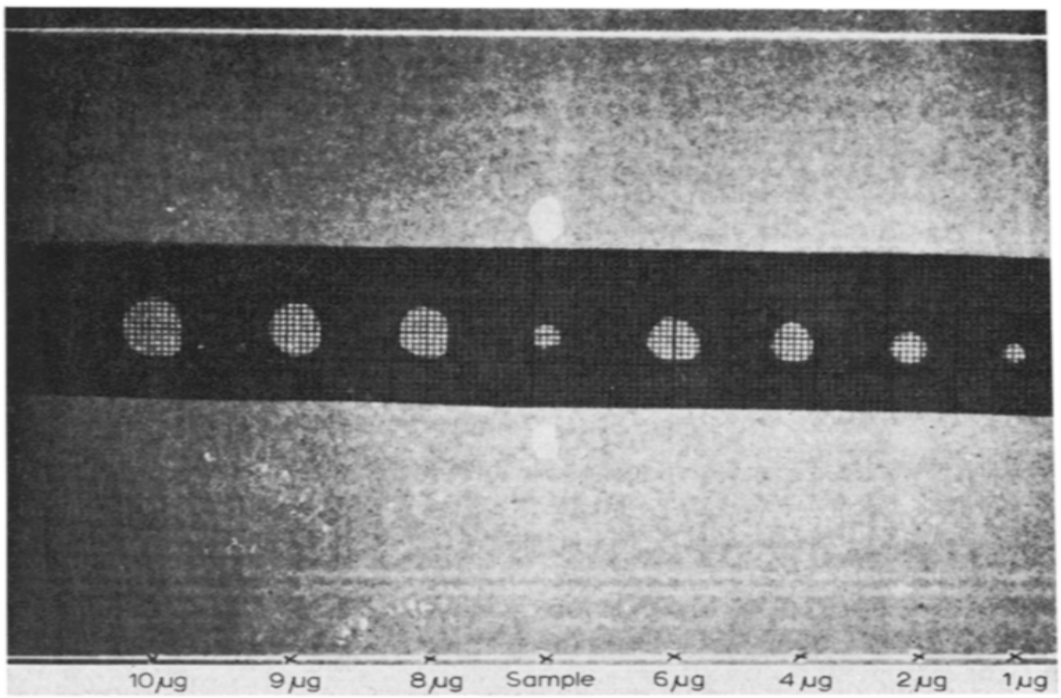



Fig. 1. Estimation of herbicides by area measurement.

relationship is shown to exist between the logarithm of the weight of compound in the spot and the square root of the area of the spot, see Fig. 2. Thus recoveries may be estimated by reference to a calibration chart.

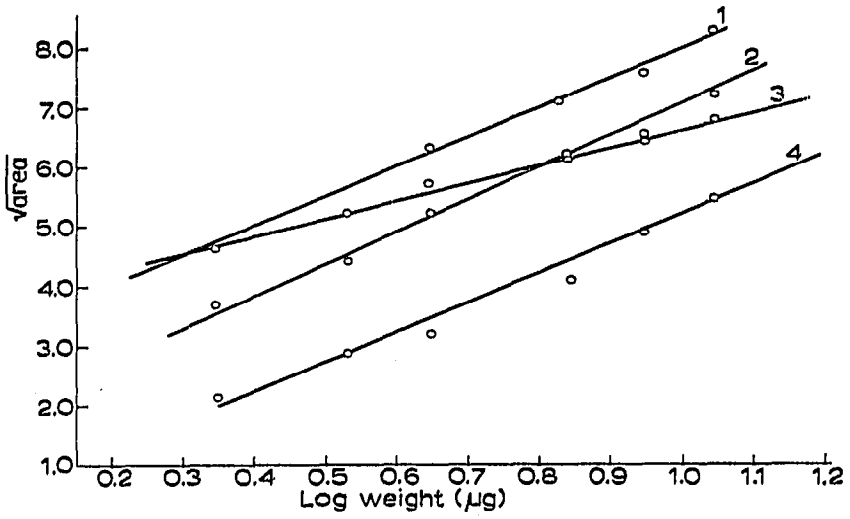



Fig. 2. Spot area–weight relationship for herbicides. 1 = Diuron; 2 = linuron; 3 = propham; 4 = fenuron.

## RESIDUE ANALYSIS PROCEDURES

### *Water samples*

All the compounds studied have been recovered qualitatively from water at a 0.1 p.p.m. level, by extraction from neutral solution with dichloromethane (2 × 25 ml). Calibration charts were drawn for fenuron, diuron, linuron, propham and carbaryl and recoveries appeared virtually quantitative. Development of the plate was in hexane containing 30% acetone, followed by a Brilliant Green spray and bromination, and then a dichlorofluorescein spray, allowing the spots to be ringed under ultra-violet light. Compounds having high $R_F$ values were more difficult to ring accurately due to the more diffuse nature of the spots as compared to those with lower $R_F$ values. Interference from co-extractives was negligible in the case of those samples examined, *i.e.* tap water and a sample of river water. Other visualization reagents may be employed to advantage for some of the compounds. *p*-Dimethylaminobenzaldehyde can be used with most of the compounds, and *p*-nitrobenzene diazonium fluoborate for carbaryl and arprocarb. Chloroform–nitromethane as solvent was not suitable for use with Brilliant Green as a visualization reagent. This solvent also gave very slight variations in spot size with weight, thus requiring very accurate ringing if a meaningful result was to be obtained. It is thus considered that chloroform–nitromethane (1:1) should only be used as a developing solvent for identification, and hexane–acetone mixtures for quantitative estimations.

### *Soil samples*

These presented far more difficulties than water samples, as would be expected. Extraction of soil samples (50 g) with both ether (3 × 50 ml) and acetone (3 × 50 ml) was carried out for comparison purposes. A considerable amount of material was extracted and applied to the plate, which was developed with hexane–acetone (70:30) and visualised. The Brilliant Green and bromination treatment and the spray of *p*-nitrobenzene diazonium fluoborate in methanol both fogged the plate over the whole $R_F$ range. However the Calcofluor spray proved to be effective for the extracts

of the sandy soil although not for the clay soils. Table III gives the recoveries obtained from water and from sandy soil containing 0.1 p.p.m. of the uracils and ureas.

TABLE III

RECOVERY OF URACILS AND UREAS FROM SANDY SOIL, CLAY SOIL AND WATER

| *Herbicide* | *Added level (p.p.m.)* | *% recovery* | | |
|---|---|---|---|---|
| | | *Water* | *Sandy soil* | *Clay soil* |
| Isocil | 0.1 | 95 | 80 | — |
| Bromacil | 0.1 | 95 | 72 | — |
| Monuron | 0.1 | 95 | 76 | 70 |
| Fenuron | 0.1 | 95 | 84 | 60 |
| Diuron | 0.1 | 95 | 70 | 60 |
| Linuron | 0.1 | 95 | 76 | 76 |
| Propham | 0.1 | 95 | — | — |
| Chlorpropham | 0.1 | 95 | — | — |
| Arprocarb | 0.1 | 95 | — | — |

Quantitative estimation of residues from clay soils was made very difficult due to the considerable interference from co-extracted materials. A suitable clean-up procedure for these extracts had to be found before any quantitative work could begin.

*Clean-up procedure*

Triazine herbicides have been cleaned-up[2] by utilizing their basic properties *i.e.* washing the acidified extract with solvent. This technique has now been tried for the most basic of the compounds, *i.e.* fenuron, diuron, monuron, linuron and propham. Only fenuron exhibited sufficient basicity to be retained in the acidic layer, the rest being quantitatively extracted into the organic layer under identical conditions to those used for the triazine herbicides. It was thus considered that a column chromatographic technique would have to be developed.

Alumina containing 5 % by weight of water was used as the stationary phase. The ureas were tested on this phase with various mobile phases. After carbon tetrachloride (50 ml) had been passed through the column (10 $\mu$g) monuron, diuron and linuron were eluted with $CCl_4$–$CH_2Cl_2$ (1:1) (50 ml), and fenuron, the most polar of the four ureas, was eluted with $CH_2Cl_2$ (50 ml) afterwards. It has also been shown that linuron may be eluted with petroleum ether (40°–60°)–ether (1:1) (50 ml), and that fenuron, monuron and diuron may be afterwards eluted with ether alone (25 ml). Propham, barban and carbaryl have also been eluted with petroleum ether–ether mixtures. The results in Table III are for clay soil extracts cleaned up by the above method.

SUMMARY

Some ureas, uracils and carbamates have been extracted from water and from sandy and clay soils and identified and quantitatively estimated by thin-layer chromatography. Several indicators are used in the visualization of the pesticides on the thin-layer chromatoplates and a novel area measurement technique is introduced.

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