Determination of Linuron in Soil by Application of an Automated Diazotization and Coupling Procedure

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The Technicon AutoAnalyzer system has improved the analytical precision and reduced tedious manual work in various types of laboratories. Some papers on the application of this system in the pesticide residue field have also been published. In a recent report Ott and Gunther (1) have summarized most of these applications.

This paper, describing a semi-automated method for the determination of linuron (3,4-dichlorophenyl-N'-methyl-N'-methoxy urea) in soils, is based on a manual method of Bock et al. (2). Linuron is extracted from the soil by Soxhlet extraction with petroleum ether and, after evaporation of the solvent, hydrolysed to 3,4-dichloro aniline, which is then diazotized and coupled with N-(1-naphthyl) ethylene diamine. This diazotization and coupling procedure, as used in the Averell and Norris method (3) for determination of parathion, forms the basis of at least 25 different pesticide methods. By automation of this part of the linuron analysis, we hoped it would be possible to determine many other pesticides with the AutoAnalyzer by using the same manifold system. In fact, we have also used the system shown in Figure 1 for the determination of parathion, paraoxon, parathion-methyl, azinphos-methyl, propham, and chlorpropham. The manual pretreatment necessary will, of course, depend on the nature of the compound in question, e. g. whether it is a nitro compound, a carbamate or a urea derivative.

Method

Apparatus

Technicon AutoAnalyzer with manifold system shown in Figure 1.

Mechanical hand mixer. The "bamix" from General Electric-ESGE, Switzerland is very convenient.

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Soxhlet extractors

Kuderna-Danish concentrators and reflux condensers to fit test tubes of approx. 15 ml. capacity with B 14 ground glass joints.

Reagents

Petroleum ether, redistilled b.p. 35 - 50°C

1 N HCl containing 0.5 ml. Brij 35 per liter

Sodium nitrite, 0.25%, in water

Ammonium sulfamate, 2.5%, in water

N-(1-naphthyl) ethylene diamine dihydrochloride, (Product no. 6237, E. Merck, Darmstadt, Germany), 1%, in water. Kept in a glass bottle the solution may be stored in the refrigerator for 14 days.

3, 4-Dichloroaniline standard solutions. Dissolve 3, 4-dichloroaniline in 1 N HCl and prepare appropriate working solutions by dilution with 1 N HCl.

Procedure

Mix equal parts by weight of soil and anhydrous sodium sulfate in a glass jar by means of a mechanical hand mixer. Weigh 25 g of the mixture into a Soxhlet-tube and extract it overnight with petroleum ether. Adjust the volume of the extract to 100 ml. with petroleum ether. Evaporate an aliquote of the extract, (5, 25, or 50 ml., according to the amount of linuron expected), in a Kuderna-Danish concentrator. Remove the last few millilitres of solvent by placing the open test tube of the concentrator in a hood overnight or by blowing a stream of air over the partly-tilted test tube by means of a fan. At this stage, the test tube often contains heavily colored residues which, however, do not interfere with the linuron determination. Add 4 ml. of 1 N HCl and 2-4 grains of granulated pumice stone to avoid bumping, and boil under reflux for two hours. After completion of the hydrolysis, cool the contents by immersing the test tube in cold water. The colored residues mentioned above will now solidify. Filter the solution through a filter paper of fine pore size directly into a 2 ml. sample cup. Close the cup and store it for the subsequent automatic analysis.

For preparation of the standard curve run samples of

standard solutions of 3,4-dichloro aniline containing 0.325, 0.813, 1.63, 3.25, and 4.88 µg. per ml. (corresponding to 2, 5, 10, 20, and 30 µg. of linuron respectively in a 4 ml. volum), into 2 ml. sample cups randomly intermixed with the pretreated soil samples, and with the range expander in 2X position. From time to time, check the manual part of the procedure by evaporating standard solutions of linuron in petroleum ether to dryness, and continue as described above from "Add 4 ml. of 1 N HCl and .....".

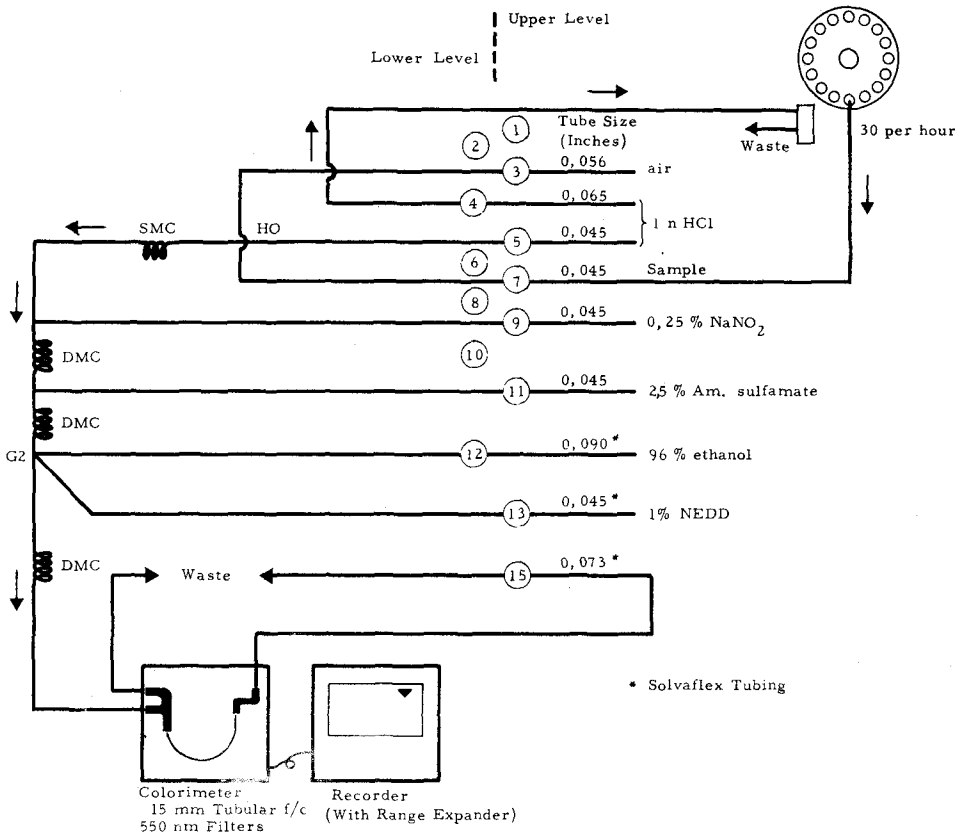

Figure 1. Manifold system for diazotization and coupling procedure.

Results and Discussion

Bock et al. hydrolyzed linuron to 3, 4-dichloro aniline with sodium hydroxide solution. We prefer to perform this step in acid medium, even if this requires a longer time for hydrolysis. Not only are the number of manipulations reduced, but we also avoid the losses of 3, 4-dichloro aniline which we have observed during alkaline hydrolysis. These losses are due to formation of the steam-volatile free amine. As the amine reaching the reflux condenser is not completely washed down during the subsequent acid addition through the condenser, amine which has built up in the condenser sometimes contaminates later runs. This is especially noticeable in blanks and samples of low linuron content.

Further, when adapting the manual method to the AutoAnalyzer system, we found it advantageous to use ethanol instead of acetone as a means of preventing precipitation of the diazo dye. Acetone is detrimental to the pump tubings.

In order to demonstrate the precision and to establish the limit of detectability (4) of the combined manual and automated method, linuron was added to petroleum ether extracts of three different types of linuron-free soil. This was performed by adding a standard solution, in an amount corresponding to 1.6 ppm of soil, directly to 50 ml. of extracts contained in the Kuderna-Danish concentrators. The results are presented in Table 1.

Table 1

Recovery experiments in which linuron was added to linuron-free soil extracts

Soil type	Number of analysis	Added ppm	Found ppm	Recovery % *	Stand. dev.	Limit of detectability *
Sand	15	0	0.060	-	±0.01	0.03
"	15	1.6	1.610	96.9	±0.06	
Peat	15	0	0.093	-	±0.03	0.08
"	15	1.6	1.622	95.6	±0.08	
Silt loam	15	0	0.110	-	±0.02	0.07
"	15	1.6	1.741	101.9	±0.06	

* Corrected for blank-values

To establish the effectiveness of the extraction procedure, linuron was added to bulk samples of three soils. The way in which a small quantity of a substance is added to a large sample is generally considered important. If, in this case, a small volume of a relatively concentrated standard solution of linuron is added to a great volume of moist soil, it may be difficult to obtain a sufficiently homogeneous sample and to evaporate the organic solvent completely. Organic solvents remaining in the soil may hold the linuron in a more readily extractable state than is the case in soil taken under field conditions. Drying of the soil before addition of the standard solution would improve the homogenation process as well as the subsequent removal of organic solvent, but could alter the adsorption properties of the soil. In our experiments, a very dilute aqueous solution of linuron, (25 mg./liter), was added to a part of the soil. After careful mixing, the slurry was intimately mixed with sufficient of the bulk soil to give the appropriate linuron content.

Some characteristics of the three different soil types used in these experiments are summarized in Table 2, and the results from our recovery runs are shown in Table 3.

Table 2

Characteristics of the soil types used

	Sand	Peat	Silt loam
pH	5.4	5.2	5.0
Moisture content, % (after fortification)	17.2	63.3	31.5
Loss on ignition, %	3.6	61.8	10.8
Cation-exchange capacity, m.e./100 g. (sum exchangeable K^+, Na^+, Ca^{++}, Mg^{++}, and H^+)	7.4	85.7	23.7

Table 3

Recovery experiments in which linuron was added to linuron-free soil samples

Soil type	Procedure *	Number of analysis	Added ppm	Found ppm	Recovery %	Stand. dev. ppm
Sand	I	10	0	0.045		±0.01
"	I	10	1.5	1.297	83.3	±0.13
"	II	10	1.5	1.295	83.3	±0,16
"	III	5	2.0	1.662	80.9	±0.07
Peat	I	10	0	0.089		±0.02
"	I	10	1.5	1.148	70.6	±0.12
"	II	10	1.5	0.765	45.1	±0.09
"	III	5	2.0	0.684	29.8	±0.02
Silt loam	I	10	0	0.096		±0.01
" "	I	10	1.5	1.079	65.5	±0.13
" "	II	10	1.5	1.169	70.9	±0.14
" "	III	5	2.0	0.426	21.3	±0.07

As contrasted with Bock et al., none of the soil types studied gave complete recovery when extracting the moist soil. By mixing the soil samples with anhydrous sodium sulfate as described under "Procedure", the recovery from peat soil could be improved to some extent, possibly due to a drier and more free-flowing sample, which thus renders the linuron more accessible to the petroleum ether. Drying the soil at 110°C before addition of the linuron had a markedly deleterious effect on the recovery, particularly from peat and loam.

The discrepancy between our findings and those of Bock et al. may possibly be explained by differences in the soil types or in the method of adding the linuron to the soil samples.

* I : as described under procedure
II : as I, but without addition of Na_2SO_4
III : as I, but soil dried at 110°C before addition of linuron

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