Suitability of Thin-layer Chromatography, Gas Chromatography and Bioassay for the Determination of Aldrin, Dieldrin and DDT Residues in Different Soils Without Cleanup

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Until recently, methods used for residue analysis for soil were largely those established for food analysis with minor modification, and no specific methods were available for soil (1). Although some progress has been made recently in extraction methods (2, 3) there are many undeveloped areas in pesticide methodology with soil.

In this study, the suitability and limitations of three analytical techniques, thin-layer chromatography (TLC), gas chromatography (GLC) and insect bioassay are compared for the determination of aldrin, dieldrin and DDT residues in different soils without cleanup. Analysis without cleanup has certain advantages because cleanup procedures are usually time consuming and may cause a loss of extracted pesticides (4). Determination without cleanup was thought to be possible with soil because its organic content is usually low.

Three soil types (sandy-loam, clay and muck) of known pesticide spray history were extracted with five solvent systems. Extracts were evaluated using the above 3 analytical techniques in parallel in a similar manner to that used for foods by Phillips et al. (5).

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Only field-treated soils of known pesticide history were used for comparison purposes. Although absolute amounts of residues present were not known for comparison purposes this was not important since the same extract was used to evaluate all 3 systems. Cleanup was avoided except where partial cleanup was effected inadvertently by solvent transference. Thus, the simplified experimental design allowed a direct evaluation of the analytical techniques and of the effect of co-extractives.

GLC was used as a "yardstick" to evaluate the other two techniques. Use of GLC with soil extracts that have not been cleaned-up appears to present few problems (2) but little has been done using TLC. It was hoped that TLC might provide a "fingerprint" as to soil type and also provide a rapid screening technique for residues in soil similar to that used previously for wheat extracts (6). Bioassay, while nonspecific, measures the availability of all material toxic to the insect in contrast to GLC which responds to individual compounds. Results obtained by these two techniques were compared to see if differences could be correlated with different soil types.

Materials and Methods

Soils

Samples of top soil of three general types (Matilda sandy loam, muck and Rideau clay) each with a known history of organochlorine insecticide treatments, were collected from the Experimental Farm, Canada Department of Agriculture, Ottawa. All samples were air-dried at room temperature and screened through a 10-mesh sieve before extraction.

Solvent and Extraction

Solvents used were distilled in glass grade (Burdick and Jackson lab., Inc.) and all were checked for impurities before use. Acetonitrile, acetonitrile/acetone (1/1, v/v), acetone/n-hexane (1/9, v/v), acetone/n-hexane (1/1, v/v) and methanol/methylene chloride (1/1, v/v) were used as extraction solvents. Many of these systems were known to be relatively inefficient for the extraction of aldrin and dieldrin

from a sandy-loam soil (2) but since other soil types were being used, other pesticides were being measured, and other analytical techniques being evaluated a cross-spectrum of solvents was evaluated.

Post extraction procedures, such as partitioning, washing and concentration are those described by Chiba and Morley, since they have been shown to give quantitative recoveries of extracted pesticides (4).

Analytical Techniques

Thin-layer chromatography. Thin-layer chromatography was carried out by using 5% acetone in n-hexane as a developing solvent (6). The amount and position of background on TLC (related to the amount of coextractives) was observed under ultraviolet (UV) light, then the plate was sprayed with ammonical silver nitrate as the chromogenic reagent (6).

Gas chromatography. An Aerograph Hi-Fi Model 600 with an electron-capture detector was used. Experimental parameters were as follows: Column temp. 173°C; injection temp. 164°C; detector temp. 173°C; N_2 gas 15 p.s.i. (20 ml. per minute); column a mixture of DC-11 and QF-1 (3 to 2) 4% on chromosorb W Regular (60-80 mesh), 2 feet x 1/8 inches aluminum; injection volume 2 µl.

Bioassay. Solvent extracts of soils were assayed with Drosophila using a dry-film technique (7), and direct bioassay (8).

Results and Discussion

Thin-layer Chromatography

The use of this technique in parallel screening showed that background interference varied markedly for different extraction systems and soil types and, as expected, was almost directly related to the amount of co-extractives, which was expressed in weight (mg. per g. of original soil) and shown in Table 1. When the equivalent of 2 g. of sandy loam or clay soil was spotted, the lower limits of detectability for p,p'-DDE, p,p'-DDT, aldrin and dieldrin were 0.1 mmg., 0.2 mmg., 0.5 mmg. and 1.0 mmg. respectively. Thus, a convenient screening of these pesticides can be made without cleanup. With muck soil, however, Rf values and lower limits of detectability varied markedly owing to the background interference from the coex-

tractives and screening can not be achieved with the above conditions.

TABLE 1

Results of gas chromatographic analysis and bioassay of pesticide residues in three types of soils of known field history obtained with five solvent systems.

Soil[a]	Solvent systems[b]	GLC[c], p.p.m.				Bio-assay scoring[e]	Co-extractives[f]
		HHDN[d]	HEOD[d]	DDE[d]	DDT[d]		
1	A[g]	0.82	0.72	ND[h]	0.68	1	0.18
	B	0.57	0.51	ND	0.35	4	0.06
	C	0.51	0.47	ND	0.29	2	0.07
	D	0.50	0.45	ND	0.36	2	0.06
	E	0.45	0.39	ND	0.24	5	0.07
2	A	0.61	0.79	0.42	7.16	5[i]	3.95
	C	0.51	0.58	0.43	8.27	4	2.02
	B	0.57	0.47	0.34	5.17	1	0.89
	E	0.48	0.47	0.42	7.11	1	0.94
	D	0.33	0.25	0.27	4.04	1	0.42
3	C	0.01	ND[h]	0.12	0.21	2	0.14
	A	0.01	ND	0.08	0.18	5	0.29
	E	0.01	ND	0.04	0.18	1	0.08
	B	0.01	ND	0.10	0.11	2	0.04
	D	0.01	ND	0.10	0.09	4	0.04

[a] 1: Matilda sandy loam, treated with aldrin.
2: Muck, treated with aldrin and DDT.
3: Rideau clay, no pesticide treatment.

[b] A: methanol/methylene chloride (1/1), B: acetonitrile/acetone (1/1), C: acetone/n-hexane (1/1), D: acetonitrile, E: acetone/n-hexane (1/9).

[c] Average of 3 determinations.

[d] HHDN: aldrin, HEOD: dieldrin, DDE: p,p'-DDE, DDT: o,p'-DDT plus p,p'-DDT.

[e] 1 shows the highest mortality.

[f] 20 ml of each extract equivalent to 20 g of soil sample evaporated to constant weight.

[g] GLC determination was made after transference to n-hexane, but bioassay was made without transference.

[h] Not detectable.

[i] After transference from methylene chloride to n-hexane the extract showed the highest mortality.

With muck soil, extracts which showed UV quenching gave a poor response with $AgNO_3$ reagent and vice versa. In general, the best background was obtained from acetonitrile and the poorest from methanol/methylene chloride system. Under UV light, at least four yellowish fluorescent spots were observed from all extracts, and two extra pinkish spots from methanol/methylene chloride system. No direct correlation was found between TLC background and GLC background. Although TLC was not employed for the quantitative examination it is undoubtedly of great potential use for preliminary cleanup of extracts for analysis by GLC, since most of the co-extractives that are electron capturing stayed at the origin.

Gas-Liquid Chromatography.

The background of the gas chromatograms without cleanup showed no interference at the relatively high residue levels that occurred (Table 1). This is understandable since most soil extracts had to be diluted 10 to 25 times in order to approximate the pesticide levels of the standards, i.e. final concentrations were of the order 0.1-0.04 g. of soil/ml. This dilution resulted in corresponding dilution of co-extractives. If this dilution was not required, preliminary cleanup may be necessary before GLC analysis. In general, results by GLC were different from those obtained by TLC and bioassay.

Bioassay.

Measurable differences in resultant toxicity and hence in the order of scoring occurred between soil types and solvent extracts, which appear to be associated with amounts of co-extractives in samples (Table 1). The mortality scores with muck soils were inversely related to the amount of co-extractives; the more co-extractives the less mortality, and bioassay scores were not related very closely to GLC estimates of residue contents (Table 1). It appears from the results that there may be threshold amounts of co-extractives that interfere with the biological assay of residues in different soils. For example, the results obtained after transfer from methylene chloride

to n-hexane with muck soil reversed to show the highest mortality in the group, and was then related more closely to the results of GLC. This finding suggests that bioassay of extracts without cleanup was impracticable or at least inaccurate because of the influence of co-extractives. This points out the necessity of studying effective cleanup techniques if bioassay is to be employed for absolute quantitation.

The use of bioassay for absolute determination of residues in samples of unknown history frequently relies upon chemical separation and identification. However, since the significance of pesticide residues lies in their biological availability and toxicological status, it seemed worthwhile to attempt to arrive at some sort of "toxicity index" by which the results of chemical analysis could be evaluated. Results of dry-film and direct bioassay which are expressed in dieldrin-equivalent figures were compared with GLC to try to assess the potential usefulness of these techniques (Table 2).

The quantitative accuracy of dry-film bioassay has been shown to depend partly upon the extraction efficiency and selectivity of the solvent system, but in this case comparison of the results by GLC and bioassay was made using the same solvent extract, so that discrepancies in the results between GLC and bioassay must be due to other factors. The amount and nature of the co-extractives appears to be one of the most important factors, since they are known to play an important part in toxicity measurement (9). Direct bioassay should eliminate some of these variables, but some natural soil constituents are toxic, and availability of the insecticide residue varies in different soil types (10). Muck soils showed wide divergence in results and since the direct bioassay showed slightly higher results than dry-film, this cannot be attributed to availability only, but must also, involve masking extractives.

TABLE 2

Comparison of Results by Bioassay and GLC

Soil	Bioassay		GLC	Ratio	
	Total Toxicity Expressed as Dieldrin content (p.p.m.). Determined by Mortality[a]		Same Extract as Bioassay[b]	GLC/Bioassay	
	Direct	Extract[b]	Aldrin Plus Dieldrin, p.p.m.	Direct	Extract
Matilda sandy loam	1.2	0.8	0.9	0.8	1.2
Muck	0.5	0.3	1.1	2.2	3.6
Rideau clay[c]	0.8	1.2	1.4	1.7	1.2

[a] Compared with dieldrin standard.

[b] Solvent used was acetone/n-hexane, 1/1.

[c] Different from the clay soil listed in Table 1. Coextractives with the above solvent was 0.38 mg/g.

Further work is required in order to form definite conclusions, but the best hope for quantitative analysis appears to be for dry-film bioassay in which cleanup of extracts, and separation and identification of toxic residues has been effected. TLC would appear to be the most promising approach for this purpose.

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