

Validity of Using Soil Fortification with Dieldrin to Measure Solvent Extraction Efficiency

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Four different types of soil with 13 to 18% moisture content when field-collected were treated in that condition with an aqueous suspension of dieldrin-¹⁴C (field soils). Samples of the same soils were air-dried, treated with dieldrin-¹⁴C in pentane, and stored either in the air-dried state (dry soil) or after addition of 20% water (moist soil). All samples were stored for 6 to 8 months and subsequently extracted in the air-dried condition with various solvents. Extraction efficiencies of solvents were

similar for the field and moist soils; much higher recoveries were obtained from the dry soils. Addition of 20% water to the soils immediately before extraction with 1-to-1 hexane-acetone or hexane-2-propanol gave similar recoveries (92 to 98%) from all soil treatments. Thus fortification of air-dried soils with dieldrin should not be used for measuring true recovery rates from field samples extracted in the air-dried state.

The abilities of various solvents to extract organochlorine pesticide residues from soils have been expressed usually as percentage recovery of a specific pesticide from a fortified sample of dry soil (Decker *et al.*, 1965; Duffy and Wong, 1967; Saha and McDonald, 1967; Saha and Stewart, 1967; Stewart *et al.*, 1965). Such fortification studies do not give an accurate measure of the abilities of solvents to extract field-applied pesticides (Chiba and Morley, 1968). They added dieldrin-¹⁴C to an air-dried sandy loam soil and recovered 91 to 96% of the added dieldrin by five different solvent systems 2 days after fortification of the soil. The same solvents extracted only 36 to 67% of dieldrin present in the same soil as a result of contamination in the field.

The use of radioactive labeled compounds is perhaps the only way to determine in absolute terms the efficiency of a given process (Gunther, 1962; Wheeler and Frear, 1966). Saha (1968a) used dieldrin-¹⁴C to compare the recoveries of dieldrin from soil by various solvents. In this study dieldrin-¹⁴C was added to an air-dried soil, which was then held for 4 months either in the air-dried state or with the addition of 20% water. Mixtures of hexane-2-propanol or hexane-acetone extracted less dieldrin (58 to 65%) from the soil stored in the presence of water than from the dry soil (74 to 85%). Although the moist soil was stored at approximately field moisture levels, the dieldrin was not applied to the soil in the moist condition. This raises some doubt about the validity of the results obtained with the moist soil (Saha, 1968a) and the results may not reflect true recovery of dieldrin, had it been applied to the soil under field moisture conditions.

The object of the investigation reported here was to study the efficiencies of several solvents to extract dieldrin-¹⁴C from different types of soils treated in field moisture conditions with dieldrin-¹⁴C, and compare these results with those obtained from the same soils fortified with dieldrin-¹⁴C in the air-dry state but stored in the presence or absence of water.

EXPERIMENTAL

The dieldrin-¹⁴C (specific activity 72 mc. per mmole) was purchased from the Radiochemical Centre, Amersham,

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England. The material was analyzed by thin-layer and gas-liquid chromatography and was 99% pure.

Soil Treatment. The soil samples (each about 10 pounds) were collected from farms in southwestern Saskatchewan. The soil types used in this study were sandy loam, clay loam, silty clay, and heavy clay. Partial analyses are given in Table I.

Field Soil. An aqueous suspension of dieldrin-¹⁴C was prepared by adding 4000 millimicrocuries of dieldrin-¹⁴C in 1 ml. of acetone to 10 ml. of water containing 1% Triton X-100. About 230 grams of each soil (about 200 grams air-dry weight) as received from field was spread in a 1-inch layer on aluminum foil. The aqueous suspension of dieldrin-¹⁴C was then applied onto the soil with a 125-ml. reagent sprayer bottle, thoroughly mixed, and stored in a stoppered bottle for 6 to 8 months at room temperature. This treated soil is referred to as field soil.

Dry and Moist Soil. About 5 pounds of each soil was air-dried at room temperature, pulverized, screened through a 20-mesh screen, and mixed thoroughly. Two hundred grams of air-dry soil was added to 4000 millimicrocuries of dieldrin-¹⁴C in 150 ml. of pentane. The solvent was removed in a flash evaporator and the treated soil was mixed thoroughly by tumbling for 8 hours. One hundred grams of this treated soil was stored in a stoppered bottle for 6 to 8 months (referred to as dry soil below). Twenty milliliters of water was added to the remaining 100 grams of soil, mixed as thoroughly as possible, and stored at room temperature in a stoppered bottle for 6 to 8 months. This treated soil is referred to as moist soil.

All soils were air-dried prior to the extraction experiments, irrespective of their previous history.

Table I. Partial Analyses of Soils Used

Soil Type	% of Oven-Dried Wt. after Organic Matter Removal			
	Organic matter, %C × 1.724	Sand, 0.05 mm.	Silt, 0.05-0.002 mm.	Clay, 0.002 mm.
Heavy clay	2.64	5.9	26.9	67.1
Silty clay	3.26	6.0	43.7	50.3
Clay loam	2.96	24.1	43.6	32.4
Sandy loam	2.90	62.1	19.3	18.6

Solvents. Hexane, 2-propanol, acetone, methanol, and chloroform were nanograde solvents (Burdick and Jackson Laboratories, Inc.). Dimethylformamide and other reagents were Fisher analytical reagent grade.

Extraction Methods. Mechanical Shaker. An accurately weighed amount (about 2 grams) of air-dried soil was shaken in a mechanical shaker for 1 hour with 10 ml. of the extraction solvent or solvent mixture and allowed to stand for 10 minutes. The supernatant liquid was filtered and the residue re-extracted twice in the same way with 10 ml. of solvent each time but shaking for 10 minutes only. The combined filtrate was concentrated to about 15 ml. in a film evaporator at room temperature, and transferred to a 25-ml. volumetric flask. The radioactivity present in a 1-ml. aliquot of the extract was counted in duplicate in a scintillation counter.

To study the effect of the addition of water on the extraction of dieldrin- ^{14}C , 0.4 ml. of water was added to 2 grams of soil and mixed thoroughly. The wet soil was then extracted with solvent in the same way as the air-dried soil.

Soxhlet. Two grams of soil was extracted in a micro-Soxhlet extractor with 15 ml. of chloroform-methanol (1 to 1) for 8 hours. The heating was adjusted to take about 3 minutes to complete one cycle. The extract was transferred to a 25-ml. volumetric flask and made up to the mark with the same solvent mixture and 1-ml. aliquots were counted in a scintillation counter in duplicate.

DMF Extraction. The method for extraction with dimethylformamide was that used by Chiba and Morley (1968). Ten grams of soil was kept in contact with 20 ml. of DMF for 19 hours and extracted in a high speed blender for 5 minutes. The mixture was filtered under suction using Whatman No. 1 filter paper with a 1-cm. pad of Super Cel. The container was rinsed with 2×5 ml. of DMF and the soil, filter paper, and pad were returned to the blender and re-extracted in the same way. The combined filtrate was partitioned into petroleum ether and water. The petroleum ether layer was dried (Na_2SO_4), concentrated to 10 to 15 ml., and transferred to a 25-ml. volumetric flask. One-milliliter aliquots were counted in duplicate.

All extractions were carried out in duplicate.

Determination of Total Radioactivity in Soil. The total amount of radioactivity present in the soil was determined by the Van Slyke wet combustion method. The apparatus used was similar to that used by Smith *et al.* (1964) for combustion of samples containing ^{14}C -labeled pesticides. One hundred milligrams of soil was mixed with 800 mg. of potassium iodide-potassium dichromate (2 to 1) and the mixture heated with 15 ml. of Van Slyke-Folch wet combustion reagent (Van Slyke *et al.*, 1951). The evolved $^{14}\text{CO}_2$ was trapped in a mixture of phenethylamine-ethanol-toluene (1:2:1) solution (4 ml.) and counted in a scintillation counter after the addition of 10 ml. of toluene containing 0.4% PPO.

Radioactivity was determined in a Beckman Model LS-100 scintillation counter, using a channel ratio and quenching curve method. Counting efficiencies were between 80 and 90%. The statistical error involved in counting radioactivity was 2 to 3%.

RESULTS AND DISCUSSION

The cost of dieldrin- ^{14}C excluded its use in field experiments. To simulate field application conditions an aqueous suspension of dieldrin- ^{14}C was sprayed on field-collected soils as they were received. The conditions under which these treated soils were stored were not the same as those to which field-applied dieldrin is exposed. This difference

should not affect the adsorption of dieldrin on soil surface or any possible interaction of dieldrin with any component of soil. Since these soils were not sterile, biodegradation of dieldrin could occur under laboratory conditions in the same way as under field conditions. Any difference in biodegradation under these two conditions is likely to be quantitative and not qualitative. Matsumura and Boush (1967) found a few soil microorganisms that were very active in degrading dieldrin. But very little degradation of dieldrin occurred in soil. Only 1 to 6% of added dieldrin- ^{14}C was converted into water-soluble metabolites by six soil samples out of 36 soils examined by these investigators. A clay loam and a muck soil were examined in this laboratory for biodegradation of dieldrin- ^{14}C and about 95% of the activity was identified as unchanged dieldrin (Saha, 1968b).

Field-applied dieldrin might volatilize or undergo photodecomposition. Dieldrin is converted into its hexacyclo isomer under ultraviolet irradiation in solution (Rosen *et al.*, 1966). An unidentified product was found in grass that had been sprayed with dieldrin and exposed to sunlight for several months (Roburn, 1963). Photodecomposition of dieldrin on the soil surface has not yet been reported, although the possibility cannot be ignored. However, the extent of this degradation would be less when dieldrin is incorporated into the soil, as in the present study.

It appears that biodegradation of dieldrin or its photodecomposition in soil would have little effect on the extractability of dieldrin-derived residues from soil. Since the results in Table II are expressed as percentage of total dieldrin present in the soil at the time of extraction, the question of volatilization of field-applied dieldrin should not affect these figures. It is thus assumed that the results obtained with the field soils would very closely represent the true abilities of the various solvents to extract field-applied dieldrin.

All solvents and methods had nearly the same efficiency (Table II) in extracting dieldrin from the moist soils and from the field soils, for all four types of soils studied here. Any discrepancy in the percentage recovery of dieldrin- ^{14}C from soils treated under these two conditions can be accounted for by the ± 2 to 3% statistical error involved in counting radioactivity with a scintillation counter. However, mixtures of hexane-2-propanol and hexane-acetone gave much higher recoveries from the dry soils than from the moist or field soils. In some cases about twice as much dieldrin was extracted from the dry soils, compared with the moist or field soils. These results are in agreement with those obtained in the previous study (Saha, 1968a). In the present study all soils were extracted in the air-dry state. The presence of water in soil during, or after, treatment with dieldrin has a considerable effect on the extractability. This difference is observed when soils are extracted in the air-dry condition. Addition of water to soil immediately before extraction with hexane-2-propanol or hexane-acetone enhances dieldrin recovery from soil and similar amounts are then extracted from dry, moist, or field soils by these solvents. It appears that the process of air drying of wet soils containing dieldrin makes the dieldrin more strongly bound to the dried soil and less accessible to solvents.

Fortification of air-dried soils with dieldrin should not be used to measure true recovery rates from field samples. For extraction methods involving air-dried field samples and solvents like hexane-2-propanol (Duffy and Wong, 1967; Saha and McDonald, 1967) or hexane-acetone (Decker *et al.*, 1965), the true recoveries can be obtained in two ways. Dieldrin can be added either to soils at field-moisture level

Table II. Recoveries of Dieldrin-¹⁴C from Different Soil Types by Various Solvents

% Recovery^a of Dieldrin-¹⁴C (Based on Total Radioactivity Present in Soil as Determined by Oxidation to ¹⁴CO₂ by Van Slyke Method)

Solvent	Heavy Clay			Silty Clay			Clay Loam			Sandy Loam		
	Dry ^b	Moist ^c	Field ^d	Dry ^b	Moist ^c	Field ^d	Dry ^b	Moist ^c	Field ^d	Dry ^b	Moist ^c	Field ^d
Hexane-2-propanol (1:1)	97.0	67.1	65.3	92.0	57.0	59.0	85.3	59.6	57.0	86.0	43.1	40.0
Hexane-2-propanol (2:1)	92.0	64.2	62.8	92.5	56.7	56.9	84.6	57.0	54.7	80.3	41.0	39.5
Hexane-acetone (9:1)	98.0	64.7	64.0	96.5	66.1	67.4	86.0	62.2	65.0	93.9	53.6	55.0
Hexane-acetone (1:1)	98.8	66.6	65.5	99.3	74.0	75.6	95.4	74.6	74.6	96.3	54.1	51.5
20% water + hexane-2-propanol (1:1)	95.2	93.7	95.7	95.6	94.8	93.2	91.3	92.0	91.7	96.0	94.2	95.0
20% water + hexane-acetone (1:1)	98.0	95.0	94.2	99.4	95.0	98.0	94.1	92.0	93.1	98.1	96.0	95.2
Dimethylformamide	84.8	86.0	84.0	83.0	84.5	86.0	91.0	80.0	80.8	91.9	78.0	78.1
Chloroform-methanol (1:1) in Soxhlet	99.8	99.3	99.0	100.0	98.8	100.0	100.0	101.0	99.3	99.6	100.0	99.3

^a Average of duplicate analyses. DMF extractions carried out by Chiba and Morley (1968) method in high speed blender. Chloroform-methanol extractions in Soxhlet, other extractions in mechanical shaker.

^b Air-dry soil treated with dieldrin-¹⁴C and left at room temperature for 6 to 8 months.

^c Same as dry soil but stored with 20% water and air-dried before extraction.

^d Aqueous suspension of dieldrin-¹⁴C sprayed on field-collected soil as received but extracted in air-dry condition.

or to air-dried soil which is then stored in the presence of water. In either case these treated soils should be extracted in the air-dried condition to give true extraction efficiencies of such methods, which are usually low (Table II). For the evaluation of recovery rates where field samples are extracted by these solvents without air drying, fortification of air-dried soils could give meaningful results.

Hexane-2-propanol was less efficient in extracting dieldrin from all types of soils than hexane-acetone. In general, it was more difficult to extract dieldrin from sandy loam soil than from heavy clay soil by these solvents. Dimethylformamide, considered by Chiba and Morley (1968) to be a good solvent for extracting dieldrin from sandy loam soil, had about 78% extraction efficiency for the particular sandy loam soil used in the present study. It also extracted more residue from soils with higher clay content. It was, however, a far better solvent than the commonly used hexane-2-propanol or hexane-acetone mixtures.

About 92 to 96% dieldrin was extracted by hexane-2-propanol (1 to 1) from field soils when 20% water was added to the soil immediately before extraction, as compared to 40 to 63% recovery for extractions from air-dried samples. Hexane-acetone (1 to 1) appeared to extract a little more dieldrin (93 to 98%) from soils containing 20% water at the time of extraction. Addition of 20% water to soil and extraction with hexane-2-propanol (1 to 1) or with hexane-acetone (1 to 1) recovered more than 90% of dieldrin present in all types of soils from sandy loam to heavy clay. Exhaustive extraction in a Soxhlet with chloroform-methanol (1 to 1) mixture was required to extract 99% or more of dieldrin.

Soxhlet extraction with chloroform-methanol is not recommended for routine determination of dieldrin in soil, as extraction in Soxhlet gives appreciable co-extractives (Chiba and Morley, 1968) which can interfere in electron-capture gas chromatographic determination of dieldrin. Extraction of soils, as received from the field, is also undesirable because of the difficulty in getting a representative sample from wet

soils. Therefore it would be preferable to air-dry field samples, pulverize, and mix thoroughly to take a representative sample for analysis. About 20% water should be added to the soil and the dieldrin should then be extracted with 1-to-1 hexane-2-propanol or hexane-acetone. Such an extraction method would give reasonably good extraction efficiency (92 to 98%) and less co-extractives.

The results obtained in this study apply only to dieldrin. Similar studies with other organochlorine pesticides are in progress.

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