Quantitative Extraction of Root-Absorbed Dieldrin from the Aerial Parts of Forage Crops

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A blending procedure widely used to extract insecticide residues from fresh plant materials is inefficient when applied to crops containing solely internal accumulations of dieldrin. The repeated blending of corn, alfalfa, orchard grass, and wheat in *n*-hexane–isopropyl alcohol (2 to 1, 8 to 10 ml. per gram) resulted in the extraction of only 50 to 90% of the total insecticide found. An additional extraction with chloroform-methanol (1 to 1) in a Soxhlet apparatus completely removed dieldrin. Factors other than the actual processing technique may affect the efficiency of dieldrin extraction by blending when this chemical is present within plant tissues. The extraction of insecticides from plant materials, whether surface or internal residues, is often the weakest link in the entire analytical procedure. Only by the use of labeled compounds is it possible to determine the absolute efficiency of extraction procedures.

Reports of the absorption and translocation of chlorinated insecticides into vegetable crops are numerous. Marth (*6*) has reviewed the literature concerning the presence of these compounds in plants and their products. Many vegetable crops have been shown to contain measurable levels of these chemicals when grown in soil containing chloro-organic insecticides. Furthermore, several crops used as forage contain internal insecticidal accumulations when grown on substrates treated with these chemicals (*2, 5, 10, 11*).

These chlorinated insecticides are generally extracted in one of three ways. If they are present on the surface of fruits, they may be removed by tumbling the fruits in a nonpolar solvent. Soxhlet extraction is often employed to remove these insecticides from dry, finely ground plant materials. Blending is the most commonly used method, particularly for the extraction of insecticides from fresh plant materials.

While the presence of internal residues of chlorinated hydrocarbon insecticides is well established, there has been little concern over whether the extraction methods outlined quantitatively extract these chemicals from the interior of plant materials. Extraction efficiencies of insecticidal residues are commonly measured by addition of known amounts of the chemical to an untreated sample of the crop prior to the extraction procedure and determination of the recovery; to a treated sample and determination of the percentage recovery of the added compound by difference; and to the extract of an untreated sample prior to analysis. Despite the fact that such fortification procedures provide data concerning only the accuracy of the analytical technique and not extraction efficiency, many investigators use these methods to measure the effectiveness of the extraction of both internal and external pesticides.

Thornburg (*9*) has suggested the use of a "weathered residue study" in an attempt to gain some measure of extraction efficiencies without sample fortification. Three subsamples were used: extracted using the routine method; stripping performed three times; and three times the normal volume of solvent used. If all three methods yielded the same results, the routine procedure was considered trustworthy and might be used. Although this procedure avoids samples fortification, it still gives no absolute measure of extraction efficiency, since a constant error might be present in all procedures.

Klein *et al.* (*3*) have discussed the extraction of chlorinated insecticides present as surface residues. The investigators made two applications, 10 days apart, of radioactive methoxychlor [1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane] to spinach plants. During processing it was observed that extraction of an air-dry sample in a Soxhlet extractor using ethyl ether for 18 hours removed 89% of the label. The radioactive count on the material in the Soxhlet thimble remained the same, however, after 28 hours of additional refluxing. Changing the solvent to benzene and continuing the extraction reduced the measurable radioactivity by only 15% after 9 hours, and further refluxing in isopropyl alcohol–benzene mixture for 5 hours reduced the count by only one third. The authors described this phenomenon as "fixation." Using labeled methoxychlor, Klein *et al.* (*3*) were able to achieve an absolute measure of their true extraction efficiency. This is one of the few examples in the literature of such a direct measure of extraction efficiency.

The problem of obtaining quantitative extraction of solely internal chlorinated insecticides became apparent when plants grown in substrates containing radioactive dieldrin were extracted by a commonly used method. Corn, orchard grass, and wheat were grown for 2 to 4 weeks in sand or in liquid culture to which ^{14}C-dieldrin had been added. The plant materials were extracted

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by blending in *n*-hexane–isopropyl alcohol (2 to 1). The solvent was decanted from the plant tissues and the blending was repeated using fresh solvent. Radioactivity measurements were then made on the plant material remaining from the blending to determine how well the dieldrin had been extracted. The presence of significant radioactivity (12 to 25% of the quantity originally present) made it desirable to develop a method that would remove essentially all of the internal dieldrin. An additional extraction of plant materials using chloroform-methanol in a Soxhlet apparatus effectively removed 99% of the remaining labeled insecticide from these tissues.

Corn (*Zea mays* L. var. Pennsylvania 354 MF), alfalfa (*Medicago sativa* L. clone C-91), orchard grass (*Dactylis glomerata* L. var. Potomac), and wheat (*Triticum aestivum* var. Pennel Red) were the species used. The corn, orchard grass, and wheat were grown from seed; and the alfalfa plants were 2- to 3-week-old rooted cuttings.

Substrate Preparation

Sand and soil (a mixture containing 3 parts by weight of Gilpin shale loam soil, and one part each of peat moss and sand) were the substrates. Quantities of dieldrin dissolved in acetone, from 0.5 to 25 p.p.m. based on substrate weight, were added to the substrates and the acetone was allowed to evaporate at room temperature. The dieldrin-treated sand or soil was thoroughly and vigorously mixed in a small Patterson-Kelley mixer to ensure a homogenous distribution of the insecticide. The dieldrin content of the treated substrates was measured before and after the plants were grown in them.

Each 4-inch plastic pot contained 20 wheat seeds, approximately 100 orchard grass seeds (110 mg.), one corn seed, or one rooted alfalfa cutting. The pots were filled to within 1 cm. of the top with substrate. Wheat and corn were harvested 3 weeks after planting, unless otherwise noted. Alfalfa was harvested after 4 weeks of growth and orchard grass after 5 to 6 weeks. When harvested, the plants were approximately 6 to 12 inches in height and each sample weighed from 10 to 30 grams (fresh weight) depending upon the species. In general, three pots of plants constituted one sample.

The plants grown in sand were watered from the bottom with Hoagland's No. 1 nutrient solution at frequent intervals. The soil-grown plants were watered with tap water and occasionally with half-strength Hoagland's No. 1 solution.

The plants were maintained in a growth room on a 16-hour photoperiod with temperature of 30° ± 2° C. during the light period and 23° ± 2° C. during the dark period. Approximately 1900 foot-candles of mixed fluorescent and incandescent light reached the substrate surfaces.

Insecticide

Unlabeled dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene) was purified by recrystallization from methanol. The purity was determined by gas and thin layer chromatographic analyses (described below).

The ^{14}C- and ^{36}Cl-dieldrin (provided by the Shell Development Co.) were purified by thin-layer chromatography (described below). Radioautography was used to detect the radioactive dieldrin and any labeled impurities.

Plant Extraction and Cleanup

The plants were harvested by cutting them approximately 1 inch above the surface of the substrate. Dieldrin was immediately extracted from the plant samples by a double-extraction technique described briefly by Mumma *et al.* (*8*).

1. The fresh plant samples were finely chopped, either in a Hobart food chopper (Model 8141), or if small enough, by hand with mincing shears.

2. The entire sample or a representative subsample was placed in a Waring Blendor with mixed extracting solvent (redistilled *n*-hexane–isopropyl alcohol, 2 to 1). The ratio of solvent to tissue was approximately 8 to 10 ml. per gram. Blending was performed for 3 minutes and the solvent decanted. Fresh solvent was added and the blending repeated. The fluid extract was separated from the slurry of plant material by filtration. After the extracting solvent had drained from the residual plant material, the filter paper containing the extracted tissues was placed in a Soxhlet apparatus and extracted with chloroform-methanol (1 to 1) overnight.

3. The *n*-hexane–isopropyl alcohol extracts were washed two to three times with 1% aqueous sodium chloride to remove the alcohol. The *n*-hexane extract remaining was stored over anhydrous sodium sulfate at 0° C.

4. The chloroform-methanol extracts obtained by the Soxhlet extraction of the blended tissues were concentrated under vacuum through the use of a rotary evaporator. After the chloroform had evaporated, the aqueous methanol remaining was transferred quantitatively to a separatory funnel. The evaporator flask was rinsed with *n*-hexane and then with aqueous sodium chloride solution. The rinsings were added to the funnel and the entire mixture was shaken vigorously for approximately 30 seconds. When the layers separated, the water was drawn off and the *n*-hexane phase collected. The aqueous phase was re-extracted with fresh *n*-hexane. The *n*-hexane extracts were combined and stored over anhydrous sodium sulfate at 0° C.

The *n*-hexane extracts were cleaned up by passing them through a Nuchar-activated carbon-alumina-Celite column described by Giang and Schechter (*1*). Ten grams of the adsorbent mixture were used and the insecticide was eluted with 200 ml. of 1% acetone in *n*-hexane. The eluates were transferred to Kuderna-Danish evaporators, concentrated to a suitable volume, and stored until they were analyzed.

Chromatography

Gas-liquid chromatography as previously described (*8*) was employed for the quantitative determination of dieldrin in all extracts.

The sensitivity of the gas chromatographic method for dieldrin was 0.003 p.p.m. In order to determine the effectiveness of the analytical procedure (excluding extraction), known quantities of dieldrin were added to extracts of blank samples. Recoveries of this insecticide averaged 95%.

Thin layer chromatography (*4*) was used to purify crude preparations of dieldrin and to aid in the identification of dieldrin which was extracted from plant tissues. The adsorbent used was aluminum oxide G (Warner-Chilcott Co.); and 2% acetone in *n*-heptane was the developing solvent (*4*). Detection methods were radioautography and silver nitrate spray (*4*).

Mass Spectrometry

The identity of the compounds detected as dieldrin by chromatography was confirmed by mass spectrometry. The compounds separated by GLC and TLC were collected and their mass spectra determined as described by Mumma and Kantner (*7*).

Results and Discussion

The results presented illustrate that extraction of insecticides by blending, a procedure routinely used with fresh plant materials, is not quantitative when applied to plants containing only internal insecticides; and that many variables due to the kind of plant material and how it was grown affect extraction efficiencies.

Figure 1 presents extraction efficiencies of the *n*-hexane–isopropyl alcohol blending for wheat, corn, alfalfa, and orchard grass grown in either sand or soil. These data represent the average values from a total of approximately 300 samples and 600 analyses statistically analyzed and found to be significant at the 1% level. The absolute amounts found were between 0 and 125 μg. Wheat and orchard grass, grown in sand, are more efficiently extracted than either corn or alfalfa. Approximately 80% of the total dieldrin found in wheat and in orchard grass was extracted by the *n*-hexane–isopropyl alcohol blending procedures and 20% was removed by chloroform-methanol. In plants grown in soil, however, orchard grass was the only crop with which the extraction by blending was even 80% effective. In both sand and in soil, the efficiency of the blending procedure, when applied to corn and alfalfa, was low. In the case of corn, 65 to 70% of the total dieldrin found was extracted with *n*-hexane–isopropyl alcohol and in the case of alfalfa only 55 to 60% was extracted by these solvents.

Figure 2 illustrates the extraction efficiencies of the *n*-hexane–isopropyl alcohol mixture in relation to the plant level of dieldrin. If the concentration in wheat was less than 0.1 p.p.m. when grown in sand and less than 0.3 p.p.m. when grown in soil, the extraction efficiency of the blending procedure was low relative to the efficiency when applied to plants containing higher levels of this chemical. A similar observation was made for corn in sand. The extraction efficiency of the blending procedure increased and leveled off as the plant dieldrin level increased. There appeared to be no similar change in extraction efficiency with alfalfa or orchard grass. The extraction efficiency of dieldrin from the wheat grown in sand was not significantly different between plant levels higher than 0.1 p.p.m.; all other figures were significant at the 1% level.

For both wheat and orchard grass the extraction efficiency by blending appeared to be lower with each subsequent cutting of the same plants (Figure 3). The left portion of Figure 3 illustrates the efficiency for three cuttings of the same wheat plants. The effectiveness of the blending procedure decreased with each successive cutting. Statistical analyses of these data showed that all of the data in Figure 3 were significant at the 1% level, except the difference between the first and second cutting of wheat; the trend is of interest, however. The data for orchard grass are presented on the right-hand portion of Figure 3. Again, the trend is toward less efficient extraction with each successive cutting of the same plants.

The factors responsible for the relatively poor extraction of dieldrin from crop materials by the blending procedure are unknown. One might be the incomplete penetration of the extraction solvents. Further, there may be a physical or chemical combination between the dieldrin which was not extractable with *n*-hexane–isopropyl alcohol and some constituent of the

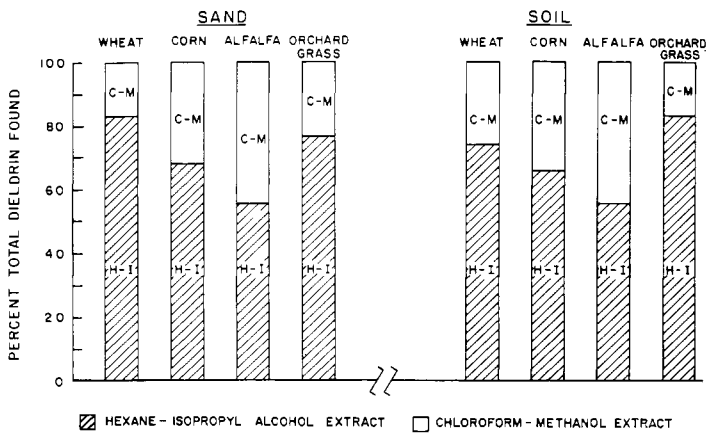

Figure 1. Efficiency of dieldrin extraction by blending as applied to four plant species grown in sand and in soil

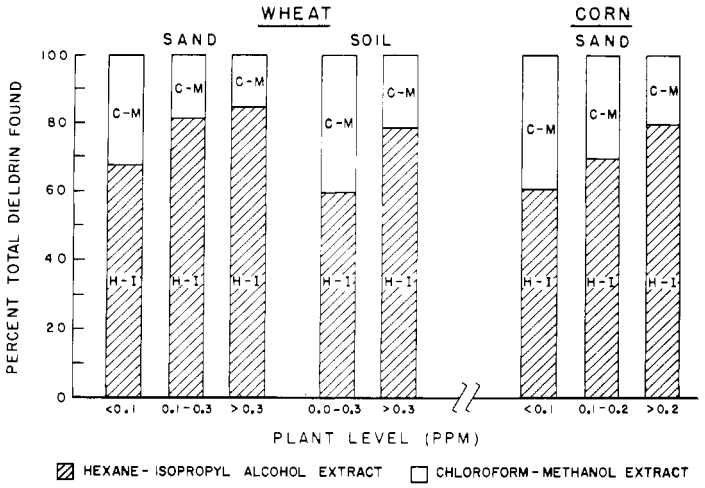

Figure 2. Efficiency of dieldrin extraction by blending as related to plant levels of insecticide

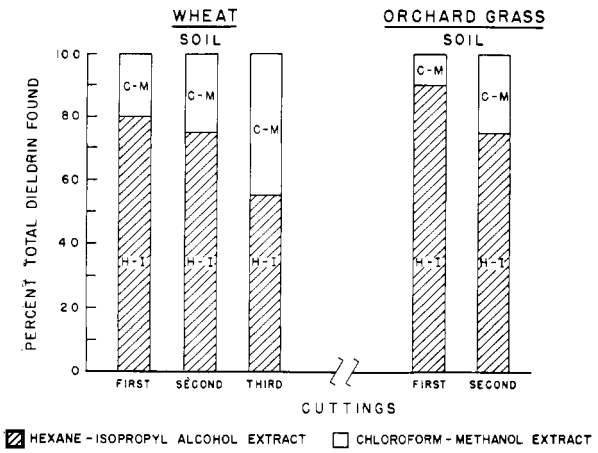

Figure 3. Efficiency of dieldrin extraction by blending as related to number of cuttings taken from same plants

plant tissue. Since this remaining dieldrin was removed by a subsequent extraction with chloroform–methanol, an excellent surfactant lipid solvent, an association may exist between a portion of the dieldrin inside the plant and the plant surfactant lipids.

An examination of the lipids extracted from fresh alfalfa and wheat by the two solvent systems gives added credibility to this postulate. The relative percentage of phospholipids in the chloroform–methanol solvent was much higher than in the *n*-hexane–isopropyl alcohol solvent. In fact, the chloroform–methanol extract contained approximately 25 to 50% of the total phospholipids, which correlates roughly with the amount of pesticide extracted. While these data suggest an association of dieldrin with phospholipids, other possibilities have not been eliminated.

It is hoped that these data may stimulate further research in the area of extraction techniques. Further, it appears urgent that currently used methods be re-evaluated utilizing labeled compounds. This is the only way to obtain an absolute measure of extraction efficiency.

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