Rapid Microdetermination of Lindane in Rice[1]

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With the advent of gas chromatography with electron-capture detection, the microdetermination of halogenated pesticides has been greatly improved in sensitivity. Goodwin et al. (1), who first described a practical procedure, simplified the analysis by extracting with acetone followed by partitioning into hexane in such a manner that no evaporative treatment was necessary. The hexane extract was then injected into the chromatograph without cleanup.

The need for cleanup of plant extracts containing chlorophyll has been demonstrated by Burke and Giuffrida (2) who showed that p,p' DDT in particular degrades appreciably in the presence of chlorophyll during passage through the chromatograph under the conditions employed. Deleterious effects on other halogenated pesticides besides DDT dictate that the removal of the green pigments in plant extracts is essential for accuracy.

Regarding other likely losses that may occur during processing of a sample, Burke, in a recent paper (3), points out the

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possibility of loss of the more volatile pesticides such as lindane during evaporation of extracts at ambient or elevated temperatures. The advantages of Goodwin's procedure in this regard and in its overall simplicity are apparent.

The purpose of this paper is to report an extremely rapid and simple technique for the determination of lindane in the rice plant and in the paddy soil and water. The technique combines the simplicity of the acetone-hexane extraction and the effectiveness of florisil as a chlorophyll adsorbent. No evaporative treatment is required and a minimum amount of solvent is employed.

Method

Apparatus and Reagents: Perkin-Elmer Model 154D with electron-capture detector. The instrument settings were as follows: Column and detector temperature -- 160°C, injector temperature -- approximately 200°C, amplifier attenuation -- 32, range -- 1, detector power supply -- 9 volts DC, nitrogen flow rate -- 100 ml/min., recorder range -- 1 MV (Leeds and Northrup Model G). The column consisted of a 1 meter pyrex U-shape tube, i.d. 5/32 in. packed with 5% QFI on Chromosorb W 60-80 mesh.

Reagents include redistilled acetone and hexane, 2% (w/v) sodium sulfate and florisil adsorbent (purchased from Varian Aerograph Co., Walnut Creek, California).

Extraction and Cleanup:

Plant Tissue: Blend in a Waring blender 25 g of fresh tissue with a volume of acetone which when added to the

moisture of the sample totals 100 ml. Using a moisture value of 85% and considering a 2 ml volume contraction that occurs with acetone-water mixtures, 81 ml is generally added. Blend at low speed for 30 seconds and a minute at high speed to dissolve all pigments. Filter through glass wool in a glass funnel. Avoid contact with plastic materials.

Pipette 20 ml of filtrate to a 100 ml volumetric flask, introduce 5 ml hexane and mix thoroughly. Add 2% (w/v) sodium sulfate until the total volume reaches halfway up the neck of the flask. Mix thoroughly and allow to stand for 30 minutes. Transfer 2 ml of the hexane layer to a 2-3/8 in. x 5/8 in. vial and add 0.4 g florisil. Stopper with a clean cork, shake vigorously and allow to settle. Repeat shaking if necessary. Any chlorophyll in the extract will be completely adsorbed. Each ml of extract represents a mg of sample. Carotenoids will remain in solution. Cleanup is unnecessary for any non-chlorophyll containing material such as roots, grain or bran.

Soil: Weigh 25 g of sample and transfer to a 250 ml volumetric flask with 50 ml acetone using small amounts at a time. Press out large particles, carefully bring to boil with mixing, digest for 2 minutes and cool. Add 5 ml hexane, mix thoroughly and then introduce 2% sodium sulfate until the total volume reaches halfway up the neck of the flask. Mix thoroughly and allow to stand 30 minutes. The hexane super-

natant is injected into the chromatograph without cleanup. Each µl of extract represents 5 mg of soil.

Water or Dew: Transfer 1 to 4 ml of sample to a small vial, 2-3/8 in. x 5/8 in., add 0.2 g sodium sulfate and 1 ml hexane. Stopper with a clean cork and shake vigorously for 30 seconds. Allow to stand 30 minutes. No cleanup is necessary. Each µl of extract represents 1 to 4 µl of sample.

Chromatography: Inject 1 to 5 µl of chlorophyll-free extract containing no more than 5 nanograms of lindane. Retention time for lindane is 2.9 minutes. If present, the alpha isomer appears at 2.2 and the delta at 4.0 minutes. Sensitivity may be varied by changing attenuation provided the baseline is stable. Measure peak height and refer to the standard curve to obtain nanograms in the injected sample.

Standard Curve: Inject 1 to 5 µl of a 1 ppm solution of lindane and plot peak heights against nanograms. Similar curves may be prepared for the alpha and delta isomers. Nearly straight line relationship exists for each isomer.

Results and Discussion

Extraction: The high degree of solubility of lindane in acetone, one in two parts acetone (4) makes it the solvent of choice for extraction. After the addition of a definite volume of hexane to the extract, lindane is completely partitioned into the hexane by decreasing the concentration of acetone with 2% sodium sulfate. Obviously the recovery of both the hexane and lindane is important.

Figure 1 shows the recovery of hexane as the concentration of acetone is decreased by the addition of 2% sodium sulfate. At least 40 ml of the sulfate must be added to 20 ml of acetone to effect complete separation of the 5 ml of added hexane.

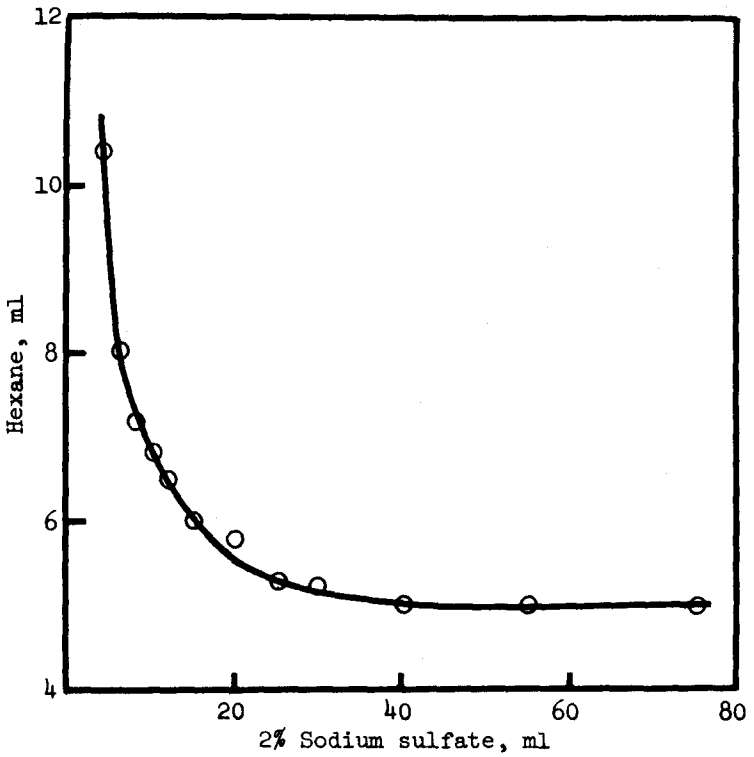

Fig. 1. Recovery of hexane from a (20+5) ml acetone-hexane mixture upon the addition of 2% sodium sulfate.

The complete partitioning of lindane into hexane in the presence of approximately 20% acetone was established in a separate test in which 100% recovery of lindane was obtained.

The prescribed extraction procedure results in a concentrated hexane extract 1 ml of which represents the extract of a gram of fresh tissue. An extract equivalent to twice as much sample may be conveniently obtained by using 40 ml of acetone extract and 5 ml of hexane in a 200 ml volumetric flask. An eight-fold reduction in extract volume is thus achieved without evaporation. A 5 µl sample injection would then represent 10 mg of tissue.

The use of a volumetric flask as suggested by Gutenmann and Lisk (5) is convenient as the small volume of concentrated extract rises to the narrow neck of the flask thus facilitating withdrawal. With green tissue samples, the hexane layer contains the pigments with the aqueous layer being virtually colorless.

Cleanup and Recovery: The effectiveness of florisil as a chlorophyll adsorbent is apparent when added to a leaf extract and mixed. The green pigments are readily adsorbed leaving the carotenoids in solution. The possibility that lindane, like the yellow pigments, may remain sufficiently in solution by such a treatment so that analysis of the supernatant may be adequate was explored. This procedure avoids subjecting an extract containing a relatively volatile pesticide such as lindane to evaporation. A 2 ml volume of rice-leaf extract in hexane, as obtained above, was shaken vigorously with 0.4 g of florisil. Complete removal of

chlorophyll was apparent by this treatment as indicated by the clear yellow supernatant.

Recovery of added lindane is presented in Table 1. The results compare well with published data on acceptable recovery of microquantities of pesticides (2).

TABLE 1

Recovery of lindane added to rice samples

Sample	Added	Recovered	Recovery
	ppm		%
Plant	0.20	0.18	90
	0.40	0.36	90
	0.40	0.39	98
	0.60	0.54	90
Soil	0.20	0.20	100
	0.20	0.19	95
	0.40	0.35	88
Water	0.10	0.09	90
	0.33	0.33	100

Comments: Besides rapidity, simplicity and avoidance of evaporative losses, another advantage of the method is the use of small amounts of solvents thus minimizing the introduction of solvent-soluble electron-capturing contaminants.

The few manipulations required and the clean solvent separation obtained doubtless add much to the precision of the method.

The lindane insecticide employed in entomological experiments at the International Rice Research Institute is a material called "Dol Granule", a product of Japan. Because this

is essentially a diluted form of benzene hexachloride, the alpha and delta isomers which usually appeared in the chromatograms were similarly estimated.

It is the opinion of the author that other 6% ether-petroleum ether elutable pesticides designated by Mills (6) may be similarly determined after cleanup by the above method, particularly if recoveries in the 85% range are considered acceptable.

Acknowledgment

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