high concentrations (500 γ of DDT), no significant interference is found.

Sensitivity

The method is capable of detecting as low as 1.0 γ or approximately 0.07 p.p.m. in a 15-gram sample.

Recovery and Reproducibility

TDE is lost at the various steps in the procedure: extraction, 15%; adsorption, 22%; and dehydrohalogenation and water washes, 8%. The over-all recovery is 62 to 65%, not 55%, as losses cannot be added, but must be applied consecutively. This represents the losses based on the absolute amount of TDE initially present. The relative recovery values range from 95 to 102%, depending on the substrate.

Replicate determinations over a period of time gave:

Added	No. of Runs	Recovery, %	Std. Dev., %
10	5	97.7	±7.0
20	5	98.5	3.2
30	4	99.9	1.8
40	5	100.1	5.7
50	4	101.6	5.1

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INSECTICIDE RESIDUES

Colorimetric Estimation of Malathion Residues in Cottonseed

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A colorimetric method reported previously for the estimation of spray residues of malathion on other plant and animal products has been adapted successfully to the determination of these residues on cottonseed. It is suitable for determining concentrations down to 0.1 p.p.m. in 200 grams of sample.

The basic colorimetric method reported for the determination of malathion [*S*-[1,2-bis(ethoxycarbonyl)-ethyl]*O*,*O*-dimethyl phosphorodithioate] residues in plant and animal tissue (*2, 3*) has been adapted successfully to cottonseed from which edible oil is to be extracted. The residual malathion is removed from the pulverized cottonseed by Soxhlet extraction with hexane. It is extracted from hexane into acetonitrile, which is treated with acid-washed alumina to remove interfering colored components. The malathion is extracted from an aqueous acetonitrile solution into carbon tetrachloride and analyzed by a procedure similar to the above-mentioned method.

The applicability of the method has been tested by analyzing cottonseed samples fortified with known amounts of the insecticide (Table I).

Procedure

Reagents. Acetonitrile, commercial grade. Neutralize with glacial acetic acid, if alkaline.

Alumina, acid-washed powder. Heat 1 pound of aluminum hydroxide powder (Baker and Adamson No. 1233) in a large porcelain dish for 8 hours at 400° C. Cool, then slurry with 750 ml. of 1*N* aqueous hydrochloric acid and stir for 15 minutes. Decant the supernatant liquid and repeat the acid treatment twice. Filter off the solid alumina using suction, wash with water until free from acid, and then wash three times with 150-ml. portions of methanol. Dry in a large, open dish, overnight, in an oven at 100° C. Store in stoppered glass container.

Prepare other reagent as in the colorimetric method (*2*).

Apparatus. Alumina column. Place a cotton plug at the bottom of an ordinary 50-ml. buret cut off at the 30-ml. mark, apply a slight vacuum at the bottom, and pour in dry, acid-washed, alumina powder to give approximately a 7-inch column. Place a cotton plug on top and pass 50 ml. of acetonitrile through the column at the rate of about 5 ml. per minute. Withdraw the acetonitrile until the level reaches about 2 cm. above the alumina and the column is ready for use.

Soxhlet extraction apparatus, large size with 7 × 2⅜ inch paper thimbles.

Calibration Curve. Prepare the calibration curve as described (*2*) for meat, fat, etc., but use aliquots of 0, 0.5, 1, 3, 8, 10, and 15 ml. of the standard solution.

Extraction and Determination of Residues. Pulverize a 200-gram sample of ginned cottonseed in a suitable apparatus. (Blending dry in a Waring Blendor is suitable for pulverizing 20-gram portions at a time.) Extract the finely divided seed for 6 hours, using a Soxhlet apparatus with about 350 ml. of *n*-hexane (commercial) and a solvent distillation rate of about 45 ml. per minute. Filter the hexane extract through a fluted paper; place it on a steam bath, and concentrate to 300 ml. with the aid of a jet of air. Cool and transfer the solution to a 500-ml. separatory funnel with the aid of 50 ml. of *n*-hexane. Add 50 ml. of acetonitrile, shake vigorously for 1 minute, then filter the lower acetonitrile phase through a fluted filter paper into a 400-ml. beaker. Repeat the extraction of the upper layer three more times with 50-ml. portions of acetonitrile and filter each portion into the beaker. Pass the combined aceto-

Table I. Recovery of Malathion from Cottonseed

Sample, Grams	Malathion, P.P.M. Added	Found[a]	Recovery, %
100	0	0.015	
200	0	0.015	
	0	0.025	
	0	0.010	
	0.08	0.05	63
	0.08	0.04	50
	0.15	0.10	67
	0.15	0.12	80
	0.15	0.12	80
	0.30	0.22	73
	0.30	0.21	70
	0.30	0.20	67
	0.73	0.64	88
	0.73	0.60	82
	0.97	0.86	89
	1.14	0.97	85
	2.27	2.03	89
100	4.53	4.23	93

[a] Results on spiked samples are corrected for "apparent" malathion found in untreated control samples.

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nitrile extract at the rate of about 5 ml. per minute, with the aid of slight suction, through a column of acid-washed alumina prepared as described under "Apparatus." The amount of coloring matter varies from sample to sample and it may be that one column can be used for several samples or that more than one column will be required for one sample. Do not allow the level of the acetonitrile to fall below the level of the alumina. After the extract has passed through, wash the column with 50 ml. of acetonitrile. Concentrate the combined acetonitrile filtrate to about 25 ml. over a steam bath with the aid of a jet of air.

Transfer the acetonitrile to a 500-ml. separatory funnel with the aid of two 25-ml. portions of carbon tetrachloride. Add 75 ml. of aqueous 9% sodium sulfate solution, 5 ml. of 12.*N* hydrochloric acid, and 75 ml. of water to the funnel and shake vigorously for 1 minute. Filter the carbon tetrachloride layer through a fluted filter paper into a dry, 250-ml. separatory funnel, but do not allow any of the aqueous layer to run onto the paper and do not wash the paper. Add 50 ml. of carbon tetrachloride to the 500-ml. separatory funnel containing the aqueous layer and shake vigorously for 1 minute. Filter the carbon tetrachloride layer into the 250-ml. separatory funnel and discard the aqueous layer. Add 1 ml. of carbon disulfide solution and 25 ml. of ethyl alcohol to the funnel and mix by swirling. Add 1 ml. of 6.*N* sodium hydroxide and shake for exactly 1 minute. Continue the procedure as for the preparation of the calibration curve beginning with, "Immediately, add 75 ml. of 9% aqueous sodium sulfate solution. . . . etc." Extract the aqueous, acidified layer containing the dimethyldithiophosphoric acid four or more times with 25-ml. portions of carbon tetrachloride until the carbon tetrachloride is proved colorless by measuring the absorbance at 418 mμ. These extractions remove the interfering colored substances originally present in the sample and the xanthates formed by the addition of carbon disulfide in a previous step. Determine from the calibration curve the amount of malathion corresponding to the observed absorbance.

Discussion

Malathion residues will probably be concentrated in the oil; therefore, quantitative extraction of the oil from cottonseed seemed desirable. *n*-Hexane was chosen as the solvent, because the oil and the malathion are both readily soluble in it. Experiments indicated that Soxhlet extraction for 4 hours with *n*-hexane at the rate of about 2.7 liters per hour resulted in essentially complete removal of malathion and oil from 200-gram samples of pulverized seed. To provide a safety factor, a 6-hour extraction has been recommended.

Acetonitrile is used to extract the malathion from the hexane–cottonseed oil solution, because malathion distributes very favorably between the two solvents. Four 50-ml. portions of acetonitrile are sufficient to remove the malathion from 350 ml. of hexane-oil solution.

Acetonitrile extracts from the hexane-oil solution an acidic yellow substance (probably gossypol) which causes high control values on samples containing no malathion and also produces no or low recovery on those containing added amounts of malathion. Acid-washed alumina, used previously for a similar purpose (*1*), proved an excellent adsorbent for these interfering colored materials, while malathion was not adsorbed. A straw-colored pigment is not removed by the alumina, but this does not interfere, because it is removed at a later step.

The calibration curve between 25 and 150 γ is essentially a straight line with a slope of 3.2 (absorbance per milligram of malathion). Below 25 γ, it gradually changes slope and passes through the origin.

Control tests should be run whenever possible on samples known to contain no malathion and suitable corrections should be applied on results obtained for treated samples. Based upon the data presented, the method described appears satisfactory for determining malathion residues in cottonseed in concentrations down to 0.1 p.p.m. and in field-treated samples.

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FUNGICIDES FOR PACKAGED FOODS

Generation of Ammonia for In-Package Fumigation of Foodstuffs

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Ammonia gas is fungicidal to some common mold organisms, but bulk fumigation with this gas may not afford the long-term protection sometimes required under commercial conditions. In-package generators of ammonia have been developed to maintain critical gas concentrations surrounding a fresh commodity over controlled periods, activated by the highly humid environment within a package of actively respiring plant parts. Two types of practical generators are discussed, with applications to citrus fruit handling. The first involves simple hydrolysis of diammonium succinate; the other utilizes the moisture-initiated reaction between dry ammonium sulfate and dry soda ash.

AMMONIA GAS is fungicidal to some common mold organisms, including the blue-green molds, *Penicillium digitatum* Saccardo and *P. italicum* Wehmer, which commonly attack citrus fruits after harvest, during shipment, and in storage (*21*). Decay by these organisms involves germination and vegetative growth of their air-borne spores which have lodged in fresh wounds in the rind of fruits. Methods of mitigating this decay (*14*) have included dipping fruits into fungicidal solutions or suspensions, but existing fungicidal materials afford little residual protection against reinfection by air-borne spores in fresh wounds caused by handling after dipping. However, fumigation with a suitable material after fruits have been packaged protects against this reinfection; biphenyl is currently being used for this purpose.

Citrus fruits packed in fiberboard cartons may be protected with gaseous ammonia (*21, 22*), which according to Klotz