Residue Analysis of Endosulfan in Pigeon Peas[1]

James A. Singmaster III[2]

ABSTRACT

Samples of pigeon peas treated with two applications, 8 days apart, with 0.56 or 1.12 kg/ha of active Endosulfan were analyzed by gas chromatography for residues of the two isomers of Endosulfan and a metabolite, Endosulfan sulfate. The 0.56 kg treatment had total residues among six machine-shelled samples picked 6 h after last application that varied from 0.61 to 1.10 p/m, all of which are below the 2.0 p/m tolerance assigned to Endosulfan in peas by the Food and Drug Administration. Analysis of six machine-shelled samples harvested 6 h after the last application of 1.12 kg revealed total residues from 1.4 to 2.3 p/m. Five days after the last application, six hand-shelled samples from the 0.56 kg treatment had 0.07 to 0.11 p/m total residues, while the 1.12 kg treatment had 0.13 to 0.33 p/m. The disappearance of the two isomers of Endosulfan and the appearance of Endosulfan sulfate between the two harvest dates agree fairly well with previous reports on other crops.

Preparation of samples for analysis followed the standard chlorinated hydrocarbon residue methodology, except for a slight change in the partition step, and elimination of the column cleanup. Recoveries varied from 71–117% in samples fortified from 0.1 to 3.0 p/m levels, and little interference from co-extractives was found at even the lowest level.

INTRODUCTION

Experiments conducted by the former Entomology Department of the Station revealed that low dosages of Endosulfan[3] give excellent control of pod boring larvae, primarily *Heliothis zea* and *Heliothis virescens*, in pigeon peas, *Cajanus cajan* (8). Before this use of Endosulfan can be recommended to Puerto Rican farmers, it has to be registered with the Pesticide Registration Division of the Environmental Protection Agency, and a tolerance for residues has to be assigned by the Food and Drug Administration. Otherwise, pigeon peas that are shipped to the States, usually as canned products, could be confiscated if the Food and Drug Administration should analyze samples and find residues of Endosulfan. Normally, the company manufacturing the pesticide obtains the registration and tolerance for its pesticide. But if the use is small, the company may not wish to expend the money for the data required, as the return may not compensate its expenditure. In such a case, the parties interested in a pesticide's use have to arrange for much of the data required for the registration and tolerance of the pesticide. Such is the case for Puerto Rico's use of Endosulfan in pigeon peas.

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[2] Assistant Chemist, Agricultural Experiment Station, Mayagüez Campus, University of Puerto Rico, Río Piedras, P.R.

[3] 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide.

An experiment was established in 1969–70 to analyze field treated pigeon peas for residues from various Endosulfan treatments in order to obtain some of the data required for the registration and tolerance. At first the samples were analyzed by a colorimetric method (7), but the results showed no detectable residues (2) after prolonged storage in the freezer. Some trial samples from this experiment were analyzed by gas chromatography, which revealed an excessive amount of a metabolite of Endosulfan known as Endosulfan sulfate. Since some samples in this experiment were picked right after spraying and yet showed considerable Endosulfan sulfate, the tentative conclusion was that delays in shelling and freezing the samples or the prolonged frozen storage may have allowed conversion of Endosulfan to the metabolite to occur. A further complication in these samples was detection of residues in untreated control plots ascribed to drift among the randomized plots.

Consequently, a second field experiment was arranged in 1971 and close coordination with picking, shelling and analyzing was planned in order to eliminate any delays that might increase the amount of the metabolite. This time the analysis was done by gas chromatography so that the low levels of residues suggested by the previous analyses would be detected as well as the metabolite. Analysis of Endosulfan by gas chromatography has revealed that it consists of two isomers referred to as Endosulfan I and II,[4] both of which are toxic and have to be accounted for in the residue (6). Fortunately, both the isomers and Endosulfan sulfate can be separated easily on any of several gas chromatography columns commonly used for pesticides (9). However, cleanup procedures in the literature were not described for samples containing Endosulfan sulfate, and some work done on cleanup procedures is included in this report on the analysis of the second field experiment for Endosulfan residues in pigeon peas.

MATERIALS AND METHODS

The pigeon peas for the analysis came from a planting at the Isabela Substation. The rates of application were 0.56 and 1.12 kg active Endosulfan per hectare in 935 l of water using the 50% wettable powder formulation. Two sprays were made, the first on March 16, 1971, and the second, 8 days later. The two treated lots and an upwind check plot were divided into six subplots to provide six samples for each treatment. The first picking was finished within 6 h of the last spray, and a second small picking was made 5 days later.[5]

[4] Endosulfan I is also referred to as α-Endosulfan; and Endosulfan II, as β-Endosulfan.

[5] The author wishes to thank Mr. Frank Juliá, of the Isabela Substation, for the spraying and picking arrangements and Dr. Mario E. Pérez-Escolar, of the Entomology Department, for the preparation of the Endosulfan applications.

The first set of samples after overnight storage at room temperature was shelled by machine to give samples of 1–1.5 kg each. These were kept frozen until extracted the following week. The second picking also was stored overnight at room temperature. Most of the samples in the second picking were too small for machine-shelling, and all were hand-shelled. The check samples from the second picking had to be combined to make a composite because of the small quantity in several of the samples. These samples were frozen for 2 days and then extracted. None of the samples waited more than a week for analysis, or spent more than 24 h at room temperature.

The following extraction step based on the FDA (3) method for chlorinated insecticides was used on these samples. Pigeon peas (100 g) were blended with purified acetonitrile (4) (200 ml) for 3 min. The resulting puree was filtered through glass wool into a graduate cylinder (250 ml), and the volume of extract was recorded. The extract was transferred to a separatory funnel (1 L) with a Teflon stopcock, and then distilled water (ca. 600 ml), saturated sodium chloride solution (20 ml), and distilled hexane (100 ml) were added in that order. The funnel was shaken gently for at least 1 min before being allowed to stand for separation of layers. After separtion of the layers, the lower layer was drawn off and discarded and the upper layer was collected in a graduated cylinder. This partition step is a slight variation of the FDA method developed for Metazole (10).

Columns of Florisil[6] were tested for cleanup of the partitioned extracts following the reported elution procedures for Endosulfan (5). When Endosulfan sulfate was not recovered by those procedures, more polar solvents including methanol (100 ml) were then passed through the columns on which only standards had been added. The presence of Endosulfan compounds was assayed by the GLC method to be described.

The adsorbent mixture cleanup used for colorimetric analysis of Endosulfan was tested as described (7). When no Endosulfan sulfate was recovered, further washings with other solvents including methanol were passed through the collected adsorbent. The presence of Endosulfan compounds was assayed by the GLC method described below.

As cleanup procedures were unsuccessful, the hexane extract was dried with anhydrous sodium sulfate and analyzed directly by gas chromatography. Frequently, dilution was required to bring response down to the scale being used for the standards.

[6] Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

The conditions for the gas chromatographic analysis were as follows:

Instruments: MicroTek Model 2500-R equipped with a nickel 63 electron capture detector operated in pulsed mode with a collecting voltage of 52 volts.

Column: 1.2 m by 6 mm dia. glass, filled with 5% QF-1 on 80/100 mesh Gas Chrom Q, conditioned at 225° C overnight.

Carrier gas: Nitrogen, ultrapure, with a molecular sieve filter in the line, 60 ml/min flow with pressure at 2.0×10^5 pascal,[7] and no scavanger flow.

Temperatures: Inlet 235°C. Column 200°C. Outlet 238°C. Detector 355°C.

Attenuators: Input 10^3. Output 4×10^{-13}.

The reponse was recorded on a 1 millvolt Minneapolis Honeywell recorder and was quantitated by measurement of peak height. All samples were injected from a Hamilton 25 μl syringe using 5 μl of extract.

The standard used was a mixture of equal amounts by weight of Endosulfan I and II and Endosulfan sulfate[8], whose reported purities were 99.3, 97.5 and 100%, respectively. Two sets of these compounds were individually weighed out in 100 to 200 mg samples and placed in separate volumetric flasks (100 ml). One set was dissolved in hexane to make standards for the gas chromatographic analysis, and the other in acetonitrile to make standards for recovery studies. Since Endosulfan sulfate would not dissolve readily in hexane, a few drops of acetonitrile were added first to the flask to dissolve the compound, and then the hexane was added. Appropriate aliquots were combined to make a stock solution containing 10 μg/ml of each of the three Endosulfan compounds. The stock solution of the compounds in acetonitrile was used as was to fortify some check pigeon pea samples to 0.1, 0.3, 0.6, 1.0, 2.0 or 3.0 p/m levels for recovery studies. The stock solution of compounds in hexane was diluted to make standards containing 25, 50, 100, 150, 200, 250 and 300 picograms (pg) of each compound per five microliters for analysis on the gas chromatograph. The peak height response of the compounds was used to make standard curves. The separation of the three compounds in the gas chromatograph is shown on the chromatogram A in figure 1, and table 1 gives the retention times and peak heights. One of the standards of Endosulfan compound was chromatographed after every other sample was analyzed on the chromatograph to check instrument performance throughout the analysis.

[7] Pascal is the metric unit for pressure and is equivalent to $Ca.10^{-5}$ atmospheres or 15×10^5 lb/in^2.

[8] The author expresses his appreciation to the Niagara Chemical Division of the FMC Corporation, Middleport, N.Y., for providing these standard compounds.

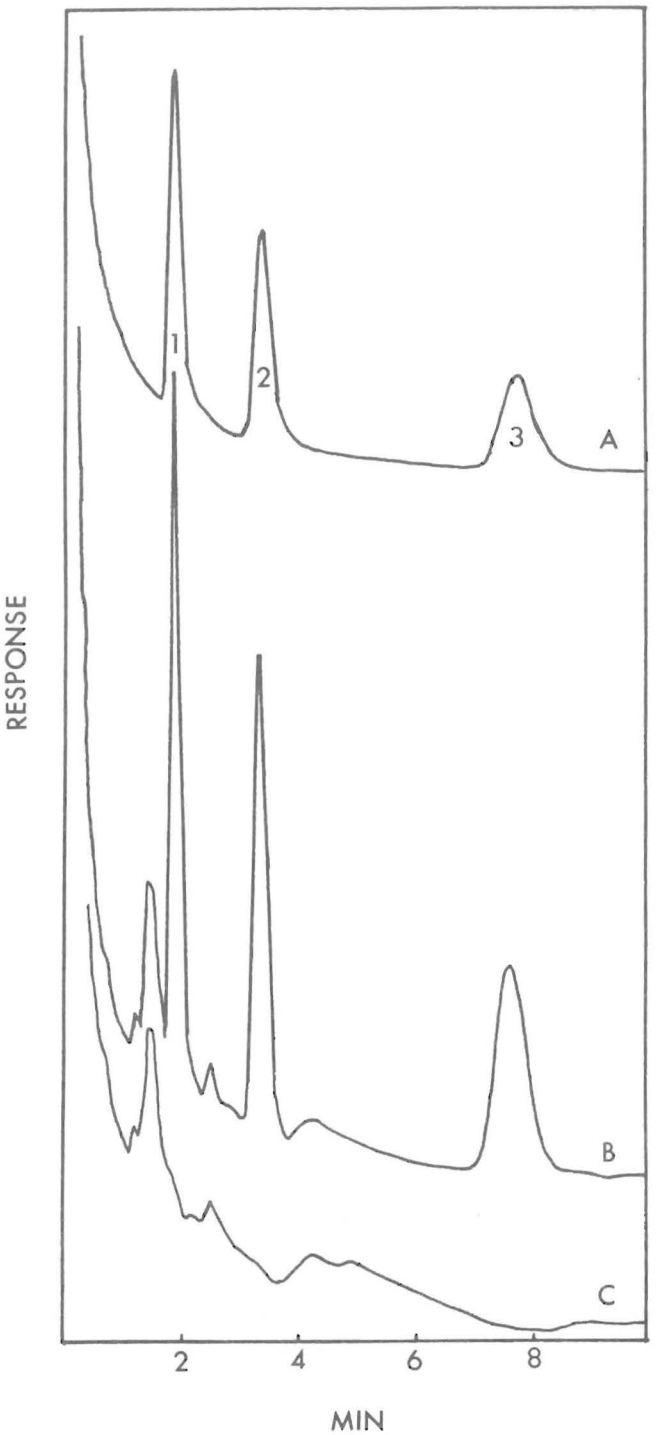

FIG. 1.—Chromatograms of Endosulfan compounds and pigeon pea samples (see text for conditions of gas chromatograph). A. Standards of 100 pg each: 1, Endosulfan I; 2, Endosulfan II; 3, Endosulfan Sulfate. B. Extract of untreated pigeon peas fortified at 0.1 p/m level with each of the three Endosulfan compounds (Recoveries in table 2). C. Extract of same sample used in B but unfortified.

Calculations were based on a volume of 273 ml for total extractable liquid in the acetonitrile extraction, as the percentage of water in pigeon peas found in several tests varied from 72 to 75. The following formula modified from the F.D.A. procedure (3) was used to calculate the parts per million from the picograms measured in the sample injected into the chromatograph:

$$\text{p/m} = \frac{0.0576\, A \times B}{C}$$

A represents the picograms detected; B, the dilution factor if the hexane extract had to be diluted to keep recorder response on scale; and C, the volume of the acetonitrile extract used in the hexane partition step, which usually was the total volume collected in the graduated cylinder.

TABLE 1.—*Retention time and peak heights of Endosulfan compounds injected in 5 microliters of hexane under conditions stated in text*

Compound	Picograms	Retention time	Peak height 100 full scale
Endosulfan I	200	2 min. 4 sec.	53
Endosulfan II	200	3 min. 30 sec.	34½
Endosulfan sulfate	200	8 min.	17½

TABLE 2.—*Recoveries of Endosulfan compounds from untreated pigeon pea samples fortified at various p/m levels*

Compound		Levels of each compound in p/m					
		0.1	0.3	0.6	1.0	2.0	3.0
Endosulfan I	p/m recovered	0.096	0.32	0.70	.78	2.0	3.4
	percent recovered	96	107	117	78	100	113
Endosulfan II	p/m recovered	0.096	0.32	0.69	.78	2.2	3.4
	percent recovered	96	107	115	78	110	113
Endosulfan sulfate	p/m recovered	0.092	0.29	0.54	.71	1.8	2.7
	percent recovered	92	97	90	71	90	90

RESULTS AND DISCUSSION

The recovery studies presented in table 2 indicated that recoveries (71 to 117%) fluctuated somewhat. Perhaps some loss of Endosulfan sulfate compared to that of the other two Endosulfan compounds occurred even though very simple sample preparation was used. These recoveries are more consistent than some done with the trial samples from the first field experiment, where recoveries fluctuated greatly. Part of the fluctuation in those recoveries has since been attributed to using the F.D.A. partition procedure (3), which has been shown to lose from 5 to 20% of the petroleum ether through vaporization during the solvent partition and

washing steps[9]. The simple partition procedure used in this report loses no more than 5% and usually less than 2% of the hexane through vaporization.

The attempt to use the cleanup step of a colorimetric method (7) was not successful because of the loss of Endosulfan sulfate. In addition, some Endosulfan II appeared to be lost. In the reported colorimetric method, the 70 to 30% mixture of Endosulfan I and II was used, and a loss of 50% of Endosulfan II did not appear too significant because there was only a 15% loss in total residue in the standard. But in view of the results of the gas chromatographic analysis at 5 days (tables 3 and 4), which reveals two to three times as much Endosulfan II as I, the colorimetric analysis using this cleanup procedure might well give inaccurate results as only 50% recovery may be occurring for the major residue component of the isomers at that time. Therefore, the colorimetric method (7) that was

TABLE 3.—*Residues of Endosulfan compounds in p/m on pigeon pea samples 6 h and 5 days after last application of 50% wettable powder formulation of Endosulfan applied at rate of 0.56 kg active ingredient per hectare in 935 L of water*

Time	Compound	Sample No.					
		1	2	3	4	5	6
6 h	Endosulfan I	0.22	0.29	0.24	0.40	0.46	0.46
	Endosulfan II	0.36	0.38	0.39	0.59	0.59	0.55
	Endosulfan sulfate	0.03	0.03	0.03	0.07	0.06	0.05
	Total	0.61	0.70	0.66	1.06	1.11	1.03
5 days	Endosulfan I	0.006	0.008	0.007	0.009	0.008	0.010
	Endosulfan II	0.023	0.032	0.022	0.042	0.022	0.022
	Endosulfan sulfate	0.041	0.068	0.040	0.045	0.057	0.075
	Total	0.070	0.108	0.069	0.096	0.087	0.107

worked out using the 70 to 30% technical mixture of Endosulfan I and II ought to be tested for recoveries of each isomer individually.

In the calculations used for the analysis, the amount of water in thawed pigeon pea samples was found to be 73%, quite above the reported value of 62% (1). A major factor responsible for the higher value may be the freezing of samples, which allows several hours for respiration to increase the percentage of water that is expired from the peas in quantities causing considerable ice formation inside the plastic storage bags. This water has to be included in the sample for analysis because the residue may volatilize with it. Another factor is the age of the pigeon peas, since the fresh pigeon peas sold commercially tend to be light green to white

[9] Singmaster, J. A., 1970. Unpublished.

indicating that they are several days more mature than the bright green samples collected in these experiments. The commercially sold peas, when shelled, often appear dried out probably because they are shelled by hand, which leaves them exposed to the atmosphere for a considerable time before packaging. If "fresh" shelled pigeon peas from the market are measured for water content, the percentage of water might be considerably lower than that of pigeon peas taken fresh from the field.

A chromatogram of an untreated sample fortified at the level of 0.1 p/m of each of the three Endosulfan compounds is shown in figure 1B. On comparison with the chromatogram C in the same figure of the same sample, but unfortified, one sees no interference of any consequence in the regions of the peaks for the three Endosulfan compounds being

TABLE 4.—*Residues of Endosulfan compounds in p/m on pigeon pea samples 6 h and 5 days after last application of 50% wettable powder formulation of Endosulfan applied at rate of 1.12 kg active ingredient per hectare in 935 L of water*

Time	Compound	Sample No.					
		1	2	3	4	5	6
6 h	Endosulfan I	0.50	0.65	1.12	0.96	1.02	0.94
	Endosulfan II	0.76	0.84	1.07	1.09	0.95	1.05
	Endosulfan sulfate	0.12	0.07	0.11	0.09	0.11	0.17
	Total	1.38	1.58	2.30	2.14	2.08	2.18
5 days	Endosulfan I	0.031	0.016	0.013	0.026	0.031	0.030
	Endosulfan II	0.050	0.041	0.038	0.068	0.070	0.073
	Endosulfan sulfate	0.102	0.077	0.082	0.104	0.165	0.230
	Total	0.183	0.134	0.133	0.198	0.266	0.333

analyzed; therefore, no cleanup was needed. If levels below 0.01 p/m had to be analyzed, cleanup probably would be necessary because the hexane extracts would have to be concentrated. Since the tolerance allowed in peas is 2.0 p/m, response was sufficient to obliterate slight interferences; and residues could still be measured at 1/10 of the tolerance, the 0.2 p/m level. Fortunately, the field-treated samples had residue levels that fitted very nicely between the 2.0 and 0.01 p/m levels, and no concentration was required.

Table 3 presents the residues for each of the three compounds and the total residue for each sample harvested 6 h and 5 days after the last treatment of Endosulfan at the rate of 0.56 kg active ingredient per hectare. Residues 6 h after the last spraying were from 0.61 to 1.11 p/m in total, all of which are lower than the 2.0 p/m tolerance for peas. Since this 0.56 kg active ingredient per hectare rate is the one that the Station

is planning to recommend pending these results, the Station can now request registration because the results show the residues should be below 2.0 p/m. In actual practice the residues should be even lower than those encountered after 5 days, which were less than 0.2 p/m, because the recommendation will be mainly for the one harvest crop where applications probably will be finished 2 weeks before harvest. Even in the regular crop subject to several harvests, residues from this treatment should present no problem as the recommendation would be to harvest 5 days after the last spraying.

Table 4 presents the residues for each of the three compounds and the total residue for each sample harvested 6 h and 5 days after the lst treatment of Endosulfan at the rate of 1.12 kg active per hectare. The samples, 6 h after the last spraying contained total residues from 1.38 to 2.30 p/m, which are just double the residues found in the samples treated at half the rate. The reason for analyzing a rate above what is being planned for registration is to have data to show what kind of residues might occur if poor mixing or spraying techniques occur. These data indicate that the residue from a rate double that planned for registration would barely exceed the tolerance 6 h after spraying. In a 5-day waiting period after the last spraying, the residue should be reduced over 80% to levels 0.35 p/m or less according to the data, which would be well within the tolerance.

This reduction in residue between the 6-h and 5-day samples may be somewhat larger than it actually is due to the difference in the shelling procedures. In some previous studies with DDT residues in pigeon peas,[10] several samples had to be hand-shelled while the rest were machine-shelled, and residues on the machine-shelled samples were 2 to 3 times higher than those on the hand-shelled samples. The higher level in machine-shelled peas probably is due to the rubbing of the shelled peas on the outside of the shells as the peas work from the beaters to the sieve holes in the shelling machine. In this experiment, the samples for the 5-day harvest were on the order of a few hundred grams and had to be shelled by hand.

Since all samples were analyzed within a week of harvest, and the results of the 6-h samples show very little Endosulfan sulfate, the conclusion appears justified that the large amount of this metabolite found in the test samples of the first field experiment represented changes occurring from delays in shelling the peas or during storage. Correspondence with the manufacturer's chemists[11] revealed that the conversion to the sulfate had not been found during frozen storage, and this was confirmed

[10] Singmaster, J. A., 1967. Unpublished.

[11] Cook, R. C. 1971. Personal Correspondence, FMC Corp., Middleport, N.Y.

in some analyses of the 6-h samples in this experiment stored for 2 mo in the freezer. Consequently, delays in getting the samples frozen or possibly a prolonged thawing period appears responsible for the high levels of Endosulfan sulfate that spoiled the trial analyses for the first field experiment. Therefore, rapid sample preparation and analysis are required for Endosulfan.

Another difference between the former trial and this field experiment was noticed, which presents something of a problem in analyzing check samples from randomized treatments in this crop. In the trial experiment mentioned in the Introduction, treatments were replicated and randomized, and the check samples were found to have low levels of residues ascribed to drift. In the second field experiment, randomized plots were not used, and the check plot was placed upwind from the treated plots. No residues were detectable in the check samples confirming that drift into check plots in the first field experiment spoiled the samples and contributed to the fluctuations in recoveries. The drift arises because some varieties of pigeon pea often are over 5 ft high at the time for these sprays requiring upward spraying for good coverage, thereby, allowing drift to occur over a considerable range. Consequently, replication and randomization of treatments for residue analysis in this and other tall crops may result in detectable residues in checks if sprays are made near harvest.

The behavior of the three compounds as presented in tables 3 and 4 agrees with previous data. Endosulfan in the formulation consists of 70% Endosulfan I, and 30% Endosulfan II, so a large loss of Endosulfan I occurs in 6 h as it is slightly below the level of Endosulfan II at that time in most samples. At the 6-h sampling, Endosulfan sulfate is barely detectable, but at 5 days it is the main component present as seen in figure 2 also. Maier-Bode (6) in his review summarized several analyses on other crops which showed essentially the same pattern. The one point of difference between his summary and the results presented here is either a much faster disappearance of Endosulfan I or a much slower disappearance of Endosulfan II in this work. Maier-Bode, reporting on some of his own work in his review, had shown in fodder grass that after 7 days, the Endosulfan I residue was present at a slightly higher level than the Endosulfan II residue, while at 14 days the former was slightly lower. In this study, Endosulfan I already is slightly less than Endosulfan I at 6 h and decidedly less at 5 days. Several explanations for the difference in ratios of I and II between pigeon peas and fodder grass appear plausible. A difference in adsorption of the two isomers on the pods may cause a smaller percentage of Endosufan I to be transferred to the peas in shelling resulting in a lower I:II ratio on the peas compared to the pods that might have a ratio similar to that on fodder grass.

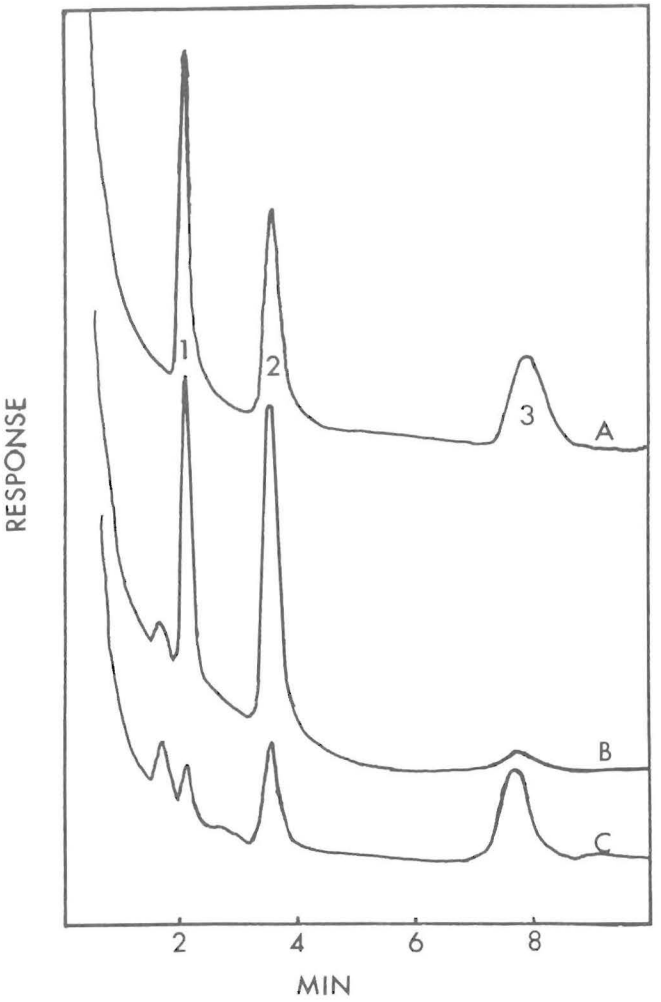

FIG. 2.—Chromatograms showing change in Endosulfan compounds on treated pigeon peas with respect to time (see text for conditions of gas chromatograph). A. Standards of 100 pg each: 1, Endosulfan I, 2; Endosulfan II; 3, Endosulfan Sulfate. B. Extract of pigeon peas harvested 6 hr after treatment at 0.56 kg/hectare active ingredient using 50% wettable Endosulfan formulation (sample 1 in table 3). C. Extract of pigeon peas harvested 5 days from same plot and treatment as in B above, but concentrated five-fold.

Another explanation would be acceleration of the already well known preferential loss of I over II on plant surfaces (6) due to a warmer, moister and perhaps windier[12] climate and/or to a higher exposure to solar radiation.

[12] The location of the field plots in Isabela, P.R., is subject to trade winds which make applications, except in the early morning or late afternoon, very difficult.

RESUMEN

Muestras de gandules tomadas de parcelas tratadas con 0.56 y 1.12 kg in.a./ha de Endosulfan, en dos aplicaciones con un intervalo de 8 días entre sí, fueron analizadas por cromatografía de vapor, para determinar los residuos de los dos isómeros de Endosulfan y de uno de sus metabolitos, el sulfato de Endosulfan. En las muestras tratadas con 0.56 kg i.a./ha se encontraron residuos con valores entre 0.61 y 1.10 ppm, siendo éstos menores que la tolerancia de 2.0 ppm establecida por la Administración de Alimentos y Drogas (FDA) para Endosulfan en guisantes verdes. Los residuos encontrados en muestras de parcelas que recibieron 1.12 kg in.a./ha fueron de 1.4 a 2.3 ppm. Los análisis de gandules cosechados en parcelas tratadas con ambas dosis se llevaron a cabo en seis muestras de gandules cosechados 6 horas después de la última aplicación de Endosulfan y descascarados a máquina. Cinco días después de la última aplicación de Endosulfan, los análisis de seis muestras de gandules descascarados a mano mostraron residuos con valores de entre 0.07 y 0.11 ppm para el tratamiento de 0.56 kg y de 0.13 a 0.33 ppm para el de 1.12 kg. La desaparición de los dos isómeros de Endosulfan y la aparición del sulfato de Endosulfan, en el intervalo entre las dos cosechas, concuerda relativamente bien con los resultados informados para otros cultivos.

En la preparación de las muestras para ser analizadas se siguió el método estándar de residuos de hidrocarburos clorinados. Sólo se introdujo un pequeño cambio en la etapa de la extracción líquido-líquido y se eliminó la limpieza por cromatografía de columna. Los porcentajes de recuperación en las muestras enriquecidas con 0.1 a 3.0 ppm variaron entre 71 y 117. Ni siquiera en el nivel más bajo de enriquecimiento se encontró una interferencia apreciable de parte de los coextractos. En este trabajo se tomaron precauciones especiales para evitar que, al asperjar, el plaguicida fuera llevado por el viento a las parcelas testigo, y el que ocurriese manipulación prolongada de las muestras antes de congelarlas. Con esto se eliminaron ciertas anormalidades en los reultados que hicieron inservible un experimento anterior para el análisis de residuos de Endosulfan.

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