CARBARYL AND MONOCROTOPHOS RESIDUES IN COTTONSEEDS, OIL, AND CAKE[1]

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Residues of carbaryl and monocrotophos have been determined in cottonseed and oil by spectrophotometry. Carbaryl residues in the seed and oil were 1.0 and 0.8 mg/kg, respectively; while monocrotophos residues were found to be 0.9 and 1.4 mg/kg, respectively.

Parallel experiment with ^{14}C-carbaryl and ^{14}C-monocrotophos was also conducted and residues were determined in a liquid scintillation counter. ^{14}C-carbaryl residues in the seed and oil were found to be 1.5 and 0.4 mg/kg, respectively, a residue level matching residues determined spectrophotometrically. ^{14}C-monocrotophos residues in the seed and oil were found to be 0.06 and 0.12 mg/kg; respectively, a level not detectable by the spectrophotometric method.

One of the increasing concerns around the world is the danger to health spawned by endless varieties of pesticides and chemical fertilizers used to boost farm production. Pest-control chemicals and food additives are essential to adequate food production, manufacture, marketing, and storage. However, without continuing surveillance and intelligent control, some of those that persist in our foodstuffs could at times conceivably endanger the public's health.

The use of cottonseed protein as a protein supplement represents the most important role which cottonseed can and will play in feeding the malnourished and protein-deficient people of developing or underdeveloped countries. Seventy-five percent of the world's supply of cottonseed is processed to obtain oil and meal, the latter being one of the commonest protein supplements for dairy animals, swine and poultry.

Carbaryl and monocrotophos are two of the many pesticides used in big cotton farms in the Philippines. In the country, pesticide residue tolerances have not been established. Since there is a general paucity of residue data, and the cotton industry is still in the developmental stage, the nature and magnitude of residue(s) in cotton by-products is not known. Hassan et. al (1976) reported that in cotton, the residues of Phosvel, an organophosphate insecticide, obtained by radiometric method of analysis were: 0.34 mg/kg in cottonseed; 0.82 mg/kg in the oil; and 0.24 mg/kg in the cake.

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It is the purpose of this study to determine the residues of carbaryl and monocrotophos in cottonseed, oil, and cake by chemical and radiometric techniques.

MATERIALS AND METHODS

Field Experiment

A. *Non-Nuclear.* Deltapine variety of cotton were planted in an experimental garden divided into two blocks. Each block was further subdivided into three equal plots. The following treatment were given to each plot:

Plot B — Monocrotophos [Azodrin 202R — a formulation containing 20 percent dimethyl-1-methyl-3-(methylamino)-3-oxo-1-propenylphosphate, (E) isomer] sprayed at the rate of six tablespoons per 16 L of water to give an approxiate concentration of 0.003 g ai/plant.

Plot C — Carbaryl [Sevin 85S — a formulation containing 85 percent 1-naphthelenyl methylcarbamate] sprayed at the rate of two tablespoons per gallon of water to give an approximate concentration of 0.014 g ai/plant.

Due to heavy insect infestations, the plants were sprayed six times. The bolls were harvested upon maturity.

Ammonium sulfate was applied before planting and before bolling periods. There was a total of 18 mm rainfall for the growing season.

B. *Radiometric.* ^{14}C-Carbaryl (6.7 mg/plant) was dissolved in a small amount of acetone and the solution was made to 10 ml with water. The chemical suspension was sprayed on the leaves of cotton plants. Spraying was done three times at two-week intervals. The bolls were harvested upon maturity.

N-methyl-^{14}C-Monocrotophos (0.5 mg/plant) was dissolved in a small amount of acetone and the solution was made to 10 ml with water. The chemical solution was sprayed on the leaves of cotton plants. Spraying was done three times at two-week intervals. The bolls were harvested upon maturity.

Carbaryl Residues in Cottonseeds.

Extraction. Twenty five g of ground delinted cottonseeds were macerated with 150 ml of methylene chloride and 100 g of powdered anhydrous sodium sulfate, and blended for two min at low speed in an Osterizer. The liquid was decanted and filtered with suction into a 500-ml flask through a Buchner funnel with rapid filter paper covered with a thin layer of Hyflo Super-Cel. The blender and filter pad were rinsed with 50 ml of methylene chloride. Most of the residue was returned from the filter paper to the blender and reextracted with 50 ml of methylene chloride.

One ml of diethylene glycol solution was added to the flask and the Buchner funnel was replaced with its original filter. The flask with the Buchner funnel was placed on a steam bath and suction was applied. When the volume was reduced to about five ml, the flask was removed from the steam bath and swirled until almost dry. The suction and funnel were removed and the flask was allowed to cool.

The sides of the flask were rinsed down with three ml of acetone and swirled to dissolve the residue. While gently swirling, 15 ml of the coagulating solution was added and the solution was made to stand for 10 min with occassional swirling. It was filtered with suction through a small filter funnel containing Whatman No. 42 filter paper coated with two mm thick Hyflo Super-Cel. The precipitate was washed three times with 2-ml portions of 10 per cent aqueous solution, allowing each washing to remain in contact with the precipitate for about 15 sec before suction was applied. The filtrate and washings were transferred to a 25-ml volumetric flask and made to volume with 10 percent aqueous acetone solution.

Determination. Five ml of sample solution was pipetted into a 50-ml beaker, two ml of alcoholic potassium hydroxide solution was added and mixed. After three min, one ml of acetic acid was added and, with swirling, one ml of cold chromogenic reagent [saturated p-nitrobenzenediazonium fluoborate]. The solution was made to stand for two min then its absorbance (A_1) was determined in a 1-cm cell at 477 nm against water.

Blank absorbance (A_2) was obtained by carrying another five ml aliqout of sample solution through the above procedure, but two ml of ethanol was substituted for the alcoholic potassium hydroxide. The amount of carbaryl, in ug, corresponding to the corrected sample absorbance ($A^{corr} = A_1 - A_2$) was determined from the standard curve.

Preparation of Standard Curve. A stock solution of carbaryl was prepared by dissolving 80 mg of the insecticide in a 100-ml volumetric flask with acetone. 80 and 8 ug/ml solutions were prepared by serial dilution: these are referred to as Solution A and B, respectively. One and three ml portions of Solution *B* and one, two, and three ml portions of Solution A were pipetted into separate 25-ml volumetric flasks and made to three ml with acetone. Coagulating solution (15 ml) was added and the solution was diluted to volume with 10 percent aqueous acetone solution. The procedure for determination above was then followed and absorbances were determined for these standard solutions. Absorbance (corrected for blank) was plotted against ug carbaryl to obtain the calibration curve.

Carbaryl Residues in Cottonseed Oil.

Extraction. Oil was extracted from 250 g of ground delinted cottonseeds with petroleum ether in a Soxhlet extractor. The oil extract was concentrated by evaporation of the solvent after about five hours of extraction.

The oil was transferred into a 100-ml separatory funnel containing 20 ml of n-hexane. Twenty ml of acetonitrile saturated with n-hexane was added and the mixture was shaken. The lower acetonitrile layer was separated and further extracted twice with 10-ml portions of n-hexane. The hexane extracts were combined and further extracted with 10 ml of acetonitrile. The acetonitrile extracts were combined and diluted with 200 ml of water and the diluted solution was extracted with about 70 ml of ethylacetate. The ethylacetate extract was passed through a 10 x 1 cm column of anhydrous sodium sulfate. Ten ml aliquots were taken from 100 ml eluent and carbaryl concentration was determined as for cottonseed. ^{14}C-Carbaryl residue in the cake was determined as the difference of residue values in cottonseed and oil.

Monocrotophos Residues in Cottonseeds.

Special Reagents:

Light Petroleum, boiling range 40° to 60° C

Activated carbon — 14 to 22 mesh carbon was heated at 600° C in closed crucibles for two hours to remove organic impurities, boiled twice with concentrated hydrochloric acid for 30 min, washed free from acid with water and dried in an oven at 100° to 110° C.

Ammonium molybdate solution — 50 g of ammonium molybdate was dissolved in 400 ml of 10 N sulfuric acid, and diluted to one L with distilled water.

Stannous chloride solution, concentrated — 10 g of stannous chloride dihydrate was dissolved in 25 ml of concentrated hydrochloric acid, sp. gr. 1.180

Stannous chloride solution, dilute — the concentrated solution was diluted 200-fold with 1N sulfuric acid. A fresh solution was prepared daily.

Ethanolic sulfuric acid — concentrated sulfuric acid, 5 ml, was mixed with 245 ml of absolute ethanol

Extraction. Twenty five g of ground delinted cottonseeds was macerated with 100 ml of methylene chloride for fifteen min. The mixture was filtered and the solids on the pad was washed with 60 ml of methylene chloride. The combined filtrate and washings were transferred to a separatory funnel and the lower organic layer was run into a 259-ml conical flask. The aqueous layer was washed with two 15-ml portions of methylene chloride and the washings were added to the main extract. The aqueous layer was discarded and the methylene chloride solution was heated on a hot-plate in a current of air until all the solvent has evaporated, taking care not to over-

heat the flask in removing the last traces. While still warm, five ml of methanol was added and the solution was poured into a 100-ml separatory funnel.

The flask was washed with 30 ml of light petroleum and 25 ml of water, and the washings were added to the contents of the separatory funnel. The funnel was shaken vigorously, the layer allowed to separate, and the lower aqueous layer was run back into the conical flask. The light petroleum layer was reextracted with five to 10 ml of water and the aqueous layer was decanted into a 100-ml flask.

The aqueous layer was reextracted with 30 ml of light petroleum and 10 ml of water which was used previously to rinse the flask. The mixture was shaken vigorously and separated as before. The funnel was rinsed with a little light petroleum and the rinsings were added to the petroleum extract. Again the aqueous portion was returned to the funnel and extracted with four 20-ml portions of chloroform. The chloroform extracts were combined.

Chromatography. The chloroform extract was evaporated to about 10 ml by heating on a hot-plate; a current of air was blown across the surface of the solvent to cool the liquid and to minimize loss of the volatile insecticide. With chloroform as the liquid phase, a column was prepared from four g of activated carbon in a glass tube 1.5 cm in diameter. The extract was transferred to the column and eluted with 100 ml of chloroform at the rate of 1.5 ml per min.

Treament of Eluates. The chloroform eluate was partially evaporated off with a stream of air, the last traces of chloroform being boiled away from the mixed acids and water in the final stages of the determination. To the eluate, 10 ml of water, four ml of 1N perchloric acid, five ml of nitric acid, and one ml of hydrochloric acid were added and the solution was heated until brown fumes began to appear. The solution was then evaporated until fumes of perchloric acid were evolved. Again it was heated to fumes with five ml of water, two ml of nitric acid, and again with five ml of water before four ml of ammonia solution was added. The excess ammonia was removed by boiling.

Determination. The test solution containing the phosphorous was placed in a 100-ml separatory funnel and the volume was brought to 19 ml. To the solution was added 3.5 ml of ammonium molybdate and the solution was mixed after adjusting the volume to 14 ml.

A 10 ml portion of 1:1 (v/v) isobutyl alcohol-benzene mixture was added to the solution and the funnel was shaken vigorously. The layers were allowed to separate and the lower layer was discarded. The remaining organic layer was washed with five to ten ml of 1N sulfuric acid and the lower layer was discarded. Fifteen ml of the dilute stannous chloride solution was added,

the mixture was shaken for five to 10 sec, and the layers were allowed to separate. The lower aqueous layer was discarded, and the organic layer was allowed to fill the bore of the separatory funnel's tap, not allowing any of this layer to run to waste. Any of the aqueous layer left in the stem of the separatory funnel was removed with a piece of cotton-wool, and the organic layer was run, which was blue if phosphorous was present, into a 10-ml volumetric flask. The volume at this stage was about eight ml.

The separatory funnel was washed with ethanolic sulfuric acid, and the washings were added to the contents of the flask. The volume was adjusted to the mark with the ethanolic sulfuric acid, and the mixture was shaken thoroughly. The absorbance was measured in a spectrophotometer at 690 nm. A mixture of four parts of isobutyl-alcohol-benzene mixture and one part of ethanolic sulfuric acid was used as the blank. The amount of phosphorous (ug/ml) corresponding to the corrected sample absorbance was determined from the standard curve. The amount of monocrotophos (ug) was calculated using the following equation:

$$\text{Wt. monocrotophos (ug)} = \frac{\text{Total P content (ug)}}{0.14}$$

where 0.14 = ug P/ ug monocrotophos obtained from absorbance readings of standard monocrotophos solution.

Calibration Curve. The reference standard used was potassium dihydrogen phosphate. A stock solution was prepared from which test solutions containing one ug/ml and above were derived. From each test solution, ten ml aliquots were pipetted and readied for spectrophotometric measurement of phosphorous. Absorbance (corrected for blank) was plotted against ug phosphorous per 10 ml to obtain the calibration curve.

Monocrotophos Residues In Cottonseed Oil.

Oil was extracted from ground delinted cottonseeds and the residues of monocrotophos was separated and determined as for cottonseeds.

Monocrotophos residue in the cake was calculated as the difference between residues in cottonseed and oil.

^{14}C-Carbaryl Residues in Cottonseeds

Extraction. About 10 g of ground delinted cottonseeds were macerated with 50 ml of acetonitrile and 20 g of anhydrous sodium sulfate in a blender for two min. and then allowed to settle for one min. The acetonitrile extract was filtered through a sintered glass funnel. The extraction was repeated twice with 20-ml portions of acetonitrile and the filtered acetonitrile extracts were combined in a 100-ml volumetric flask and made to volume. One ml aliqouts of the acetonitrile extract were taken for ^{14}C counting in a Packard Liquid Scintillation Counter.

Standardization and Liquid Scintillation Counting. Standard solutions of ^{14}C-carbaryl were prepared from a standard stock solution at the following concentrations and activities:

Soln. 1 = 0.06799 mg/ml = 2.1145 x 10^{-4} mCi/ml
Soln. 2 = 0.00068 mg/ml = 0.0212 x 10^{-4} mCi/ml
Soln. 3 = 0.00340 mg/ml = 0.1057 x 10^{-4} mCi/ml
Soln. 4 = 0.00680 mg/ml = 0.2115 x 10^{-4} mCi/ml
Soln. 5 = 0.01360 mg/ml = 0.4230 x 10^{-4} mCi/ml

One ml aliquots of each standard solution were taken for standard counting in the liquid scintillation counter.

Scintillation counting solutions were prepared by adding nine ml of scintillation solvent solution to every one ml aliquot of sample solution in a Packard scintillation vial. The scintillation solvent solution consisted of 3.5 g of PPO, 0.15 g of dimethyl POPOP and 50 g of naphthalene in 500 ml dioxane. Blank solutions of the scintillation solvent, acetonitrile and n-hexane solvents were also prepared and the radioactivity was determined. The efficiency of the counter was calculated from standard counts using the equation.

Efficiency = cpm/dpm
where: cpm = counts per minute
dpm = disintegration per minute
2.22×10^9 dpm = - mCi

The residues of ^{14}C-carbaryl were determined by using the equations:

$$\text{mci } ^{14}C = \frac{\text{cpm}}{\text{Eff}} \times \frac{\text{1mci}}{2.22 \times 10^9 \text{ dpm}} \times \frac{\text{Vol. of sample}}{\text{Vol. of aliquot}}$$

$$\text{mg/kg } ^{14}C\text{-carbaryl} = \frac{\text{mci } ^{14}C/0.00311 \text{ mci/mg}}{\text{wt. of sample (kg.)}}$$

^{14}C-Carbaryl Residues in Cottonseed Oil

Extraction. Cottonseed oil was extracted from 10 g of ground delinted cottonseeds with n-hexane by Soxhlet extraction. The oil extract was then poured into a separatory funnel containing 20 ml of n-hexane. Twenty ml of acetonitrile saturated with n-hexane was added and the mixture was shaken. The lower acetonitrile layer was separated and extracted twice with 10-ml portions of n-hexane. The hexane extracts were also combined and extracted once with 10 ml of acetonitrile. The acetonitrile extracts were collected in a 50-ml volumetric flask and the solution was made to volume with acetonitrile. The hexane extracts were also combined in another 50-ml volumetric

flask and made to volume with n-hexane. One ml aliquots were taken from both extract solutions for ^{14}C counting in the liquid scintillation counter following the procedure described earlier.

^{14}C-Monocrotophos Residues in Cottonseed, Oil, and Cake.

The procedure was basically the same as that used for the analysis of ^{14}C-Carbaryl.

RESULTS AND DISCUSSION

Separate plots of Deltapine variety of cotton were treated six times with non-labeled monocrotophos (3 mg per plant) and carbaryl (14 mg per plant) On maturity, about two months after the last spraying, the bolls were harvested and residues from the seed and oil were extracted following standard procedures (Benson and Finocchiaro, 1965; Laws and Webley, 1961). Carbaryl was determined spectrophotometrically by coupling 1-naphthol, released from carbaryl by alkaline hydrolysis, with p-nitrobenzenediazonium flouborate, to give a colored product with maximum absorbance at 477 nm. The spectrophotometric method for monocrotophos was based on determination of phosphorous content of the residue.

In the parallel experiments for radiometric determination of residues, ^{14}C-carbaryl (6.7 mg per plant) and ^{14}C-monocrotophos (0.5 mg per plant) were sprayed three times on experimental plants and the bolls were similarly harvested upon maturity. The active residues were extracted from seed and oil by standard procedures and determined by liquid scintillation counting. ^{14}C-carbaryl residues were of the same magnitude as the non-active carbaryl residues while ^{14}C-monocrotophos seems to vary proportionately with the amount of insecticide applied when compared with the non-active monocrotophos residues (see Tables 1 and 2). Radiometric determination allows us to detect residues of monocrotophos to as low as 0.3 mg/kg which was not detectable by the spectrophotometric method.

Carbaryl residues in the cake varies from 0.02 to 1.1 mg/kg, and monocrotophos residues from 2.3 to 0.18 mg/kg.

TABLE 1. **Residues; in mg/kg; of carbaryl and monocrotophos in cottonseed and cottonseed oil by spectrophotometry.**

	1	2	3	4	5	Average
A. Carbaryl						
Cottonseed	2.3	0.1	0.8	1.2	0.6	1.0
Cottonseed Oil	1.0	0.9	0.7	0.8	0.8	0.8
B. Monocrotophos						
Cottonseed	ND	ND	3.2	0.4	—	0.9
Cottonseed Oil	ND	ND	ND	2.1	4.7	1.4

TABLE 2. **Residues; in mg/kg; of carbaryl and monocrophos in cottonseed and cottonseed oil by radiometry.**

	TRIALS 1	2	3	4	5	Average
A. Carbaryl						
Cottonseed	2.0	2.1	1.9	0.8	0.6	1.5
Cottonseed Oil	0.3	0.8	0.5	0.1	0.4	0.4
B. Monocrotophos						
Cottonseed	0.04	0.03	0.1	0.05	—	0.06
Cottonseed Oil	0.1	0.08	0.2	0.1	—	0.12

CONCLUSIONS

The use of both nuclear and conventional methods constitutes a suitable approach for studying agrochemical residue problems. This allows comparison of both techniques, if experimental conditions are kept constant. The nuclear technique also facilitates the identification of the chemical species of the residue.

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