UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
~~WASHINGTON, D.C. 20460~~
Building 306, ARC-East
Beltsville, Maryland 20705

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

October 21, 1982

MEMORANDUM

TO: Alfred Smith
Residue Chemistry Branch
Hazard Evaluation Division

THRU: Ken Kissler KFK
Section Head
Analytical Chemistry Section

THRU: Warren R. Bontoyan WRB
Branch Chief
Chemical and Biological Investigations Branch

SUBJECT: PP#0F2413/FAP#OH5275 Thiodicarb in Soybeans and Cottonseed

Methods trial was requested by Residue Chemistry Branch, Hazard Evaluation Division on the insecticide, Thiodicarb, dimethyl-N,N'-[thiobis [(methylamino) carbonyloxy)] bis [ethanimidothioate] and its metabolite, [methomyl, N- [(methyl-carbomyl) oxy] thioacetimidate in soybeans. The fungicide thiodicarb and its metabolite were analyzed. Thiodicarb and methomyl were hydrolyzed to methomyloxime, S-methyl, N-hydroxythioacetimidate, by heating in aqueous base. The determination of thiodicarb and methomyl was obtained using GLC equipped with a sulphur flame - photometric detector.

Fortification levels for thiodicarb and methomyl requested were 0.1 ppm and 0.2 ppm. Both compounds were analyzed in duplicates. The petitioners method for the "Determination of Thiodicarb Residues in Soybean Seed" dated December 1979 was to be followed. Thiodicarb is a product of Union Carbide Corporation. The method appears satisfactory with the modification explained below.

Modifications:

A. In procedure #3, last sentence,....." to ensure all acetone is gone." A small amount of acetone was allowed to remain to prevent volatilization of compound.

B. In procedure #6,....." add 4 gms of potassium phosphate monobasic....", was omitted due to interferences. A ph of 5-6 is to be maintained by adding only 4 ml conc. HCl. MJB 9/24/85 Step #7

C. "Keeper" solvent, (ethylene glycol) 4-5 drops were added instead of two. No interferences were encountered.

Method summary

Known amounts of Thiodicarb and Methomyl were added to finely ground soybeans. The soy meal was extracted in 90% acetone and 10% methanol for 20 minutes using a magnetic stirrer. The contents were filtered thru a Buchner funnel under vacuum. The filtrate was evaporated to 25 mls and and 30 mls of acetonitrile was added then evaporated again to 5 mls. Acetonitrile was added and partitioned in Hexane. The Hexane was discarded and acetonitrile was evaporated to just dryness. The residue was hydrolyzed in 25 mls of 2.5 N NAOH for 45 minutes at 60° C. Acid was added and a ph of 5-6 was maintained before being transferred to a separatory funnel. The residue was partitioned with methylene chloride, extracted several times, evaporated to just dryness then brought to desired volume for final analysis by GLC-FPD(s).

Comments:

1. The selection of methylene chloride is critical. Several brands of dichloromethane did not pass the acceptability test. MCB's Ommisolv was the only suitable tested solvent.

2. The ph of the solution in step #6 should be kept between 5 and 6.

3. It requires 10 hours to complete a single sample run. It must be emphasized that if step #7 is in progress, it must be finished on the same day.

4. The fortification of soymeal -- vigorous stirring of the meal is required to properly disperse and homogenize the sample.

Elmer H. Hayes

Elmer Hayes, Chemist
Special Project Unit

Results soybean meal

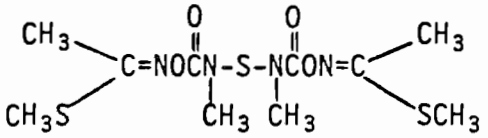
	Thiodicarb				Methomyl			
	Fortified (ppm)	Found	Recovery (%)	Average (%)	Fortified (ppm)	Found	Recovery (%)	Average (%)
Control	0.00	0.00			0.00	0.00		
0.1	0.10	0.09	90	85	0.11	0.10	91	91
	0.10	0.08	80		0.11	0.10	91	
0.2	0.21	0.18	86	74	0.23	0.15	65	76
	0.21	0.13	62		0.23	0.20	87	

1294 C

Acceptable Name: Thiodicarb

Pesticide Reg. Sec. 180.407, 561.386

Structure:



Other names: dimethyl N,N'[thiobis[[(methylimino)carbonyl]oxy]]bis[ethanimidothioate, dicarbasulf, Larvin, UC-51762

Petitioner: Union Carbide Corporation, South Charleston, West Virginia 25303

Method I: THIODICARB-FPD-SOYBEANS "A Method For The Determination of Thiodicarb Residues in Soybean Seed by Gas Chromatography" December 1979

Pesticide Petition: 0F2413/0H5275

Product application: corn, cotton, soybeans

Detection limit: 0.02 ppm

Method trial report: "PP#0F2413/FAP#0H5275 Thiodicarb in Soybeans and Cottonseed" dated October 21, 1982 with modifications to the method. The modifications are an alternate way of running the method, therefore, the method should be published as written with a note that EPA used modifications of certain steps in the procedure.

Note: Methomyl is a metabolite of this chemical and this should be cross-referenced in PAM.

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December 1979

A METHOD FOR THE DETERMINATION OF THIODICARB RESIDUES IN SOYBEAN SEED BY GAS CHROMATOGRAPHY

Method Designation: THIODICARB-FPD-SOYBEANS

UNION CARBIDE CORPORATION
AGRICULTURAL PRODUCTS COMPANY, INC.
Research and Development Department
P. O. Box 8361
South Charleston, West Virginia 25303

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DETERMINATION OF THIODICARB RESIDUES IN SOYBEAN SEED

Thiodicarb = Dimethyl-N,N'-[thiobis[(methylimino)carbonyloxy]] bis[ethanimidothioate]

INTRODUCTION

Thiodicarb residues consist of thiodicarb and its degradation product, methomyl. Methomyl oxime is also quantitated by this procedure, but it is not a significant residue because it is relatively non-toxic and very low in concentration.

Thiodicarb and methomyl are hydrolyzed to methomyl oxime by heating in aqueous base. The total residue in the form of methomyl oxime is then extracted from the acidified aqueous sample with dichloromethane. After concentration and dissolution in acetone, the sample is analyzed by gas chromatography utilizing a flame photometric detector equipped with a filter specific for sulfur and quantitated by comparison with a standard curve.

The validity of the method was tested by fortifying samples of untreated seed with thiodicarb and methomyl and analyzing by the described procedure. Recoveries average 83 percent and sensitivity is 0.02 ppm. Details of recovery experiments for the fortified seed are shown in Table II.

Typical chromatograms for standard solutions, an untreated control, and seed containing residue are shown in Figure 1. Figure 2 is a typical calibration curve.

Laboratory experience has shown that the use of certain brands, grades, or lots of dichloromethane results in a loss of methomyl oxime. At the present time, the reason for the adverse reaction is not known. The available dichloromethane should be tested by the following procedure for any adverse reaction toward the oxime.

Test for acceptability of dichloromethane: Add 100 µg of methomyl oxime and one drop of ethylene glycol to 100 mL of the dichloromethane to be tested. Carefully evaporate the solution just to dryness with dry air and add 25 mL of acetone. Mix well and quantitate the oxime by GC as explained in the following method. A recovery of greater than 90 percent is acceptable and permits the use of the dichloromethane being tested.

Reagents

(a) Solvent Mixture I, 90/10 - acetone/methanol (v/v)

(b) Acetonitrile, analytical grade

(c) Hexane, analytical grade

(d) Ethylene glycol, analytical grade (used as "keeper")

(e) Sodium hydroxide, 2.5 N aqueous (100 g of sodium hydroxide per liter of solution)

(f) Hydrochloric acid, concentrated

(g) Potassium phosphate, monobasic, analytical reagent

(h) Sodium chloride, analytical grade

(i) Dichloromethane (tested for acceptability)

(j) Sodium sulfate, anhydrous, granular

Standard Solutions

(a) Weigh 0.1000 g of methomyl oxime into a 100 mL volumetric flask, dilute to the mark with acetone and shake until solution is complete. Concentration of standard is 1000 µg/mL.

(b) Withdraw a 10 mL aliquot with a pipet and dilute to 100 mL with acetone in a second volumetric flask. Concentration of standard is 100 µg/mL.

(c) Remove 5 mL of stock solution (b) and dilute to 100 mL as above. The standard solution now contains 5 µg/mL of methomyl oxime.

(d) By further dilutions, prepare solutions containing 3, 2, and 1 µg/mL.

(e) Store standards refrigerated when not in use.

NOTE: This mode of preparation of standards is described only as a guide; any other valid procedure is satisfactory.

Apparatus

(a) Hewlett Packard 5840A Gas Chromatograph (Hewlett Packard, Avondale, PA) or the equivalent, equipped with a flame photometric detector incorporating a 394 nm filter selective for sulfur containing compounds. See Table I for chromatographic conditions.

(b) Super Dispax, SD-45N homogenizer with a G302 generator and Tekmar Power Controller, Model TR-10.

(c) Model 186 Precision Water Bath (GCA Corporation, Chicago, IL) or the equivalent.

(d) Magne-4 four-unit magnetic stirrer with hot plate (Cole Parmer Instrument Co., Chicago, IL) or the equivalent.

(e) Centrifuge, Beckman, J-21C (or equivalent).

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Procedure

1. Pregrind the seed to a fine meal in a Waring blender. Weigh 50 g into an 800 mL plastic beaker, add 225 mL of solvent Mixture I and extract at medium speed for 60 seconds with the Tekmar homogenizer. (Adjust the generator for maximum grinding by immersing the head to where all material is agitated from the bottom of the container.)

2. Vacuum filter the contents of the beaker through Whatman No. 1 paper (or equivalent) in a 9 cm Buchner funnel into a 500 mL flask. Return filter cake to the beaker and repeat the extraction and filtration. Rinse the beaker with 50 mL of solvent Mixture I and use as a wash for the filter cake.

3. Transfer the combined filtrate into two 500 mL Erlenmeyer flasks (for quicker evaporation), add two drops of ethylene glycol as keeper to each. Set the flasks in a warm water bath (35-40°C) and evaporate to about 25 mL using a gentle stream of air. Combine the filtrates, add 25 mL of acetonitrile and again evaporate to about 5 mL (to ensure all acetone is gone).

4. Add 45 mL of acetonitrile. Swirl to mix, and transfer to a 250 mL separatory funnel. Add 75 mL of hexane and shake for 30 seconds. Allow layers to separate and discard the top hexane layer. Repeat this clean-up step with an additional 75 mL of hexane, again discarding the hexane. Drain the lower layer into a 250 mL Erlenmeyer flask and add two drops of ethylene glycol. Set the flask in a warm water bath and evaporate <u>just</u> to dryness with a gentle stream of air.

5. To the residue remaining in the flask, add 25 mL of 2.5N aqueous sodium hydroxide and a 2 inch magnetic stirring bar. Set the flask in a water bath positioned on a Magne-4 stirrer-hot plate. Stir and heat for 45 minutes at 60°C.

6. Remove the flask and cool to about 20°C in an ice bath, positioned on a magnetic stirrer. While the solution is being stirred, add 5.0 mL of concentrated hydrochloric acid dropwise, then add 4 g of potassium phosphate monobasic (the pH of the solution should now be buffered between 5 and 6). Saturate the contents of the flask with 10 g of sodium chloride and mix for an additional minute.

7. Transfer the solution to a 250 mL separatory funnel and partition with 50 mL of methylene chloride by shaking gently for 30 seconds, venting to release pressure as required. Drain the bottom organic layer through a bed of anhydrous granular sodium sulfate (supported in a 9 cm funnel by a plug of glass wool). Repeat with an additional 50 mL methylene chloride and then twice with 25 mL quantities. Wash the sodium sulfate with 25 mL of mechylene chloride and add two drops of ethylene glycol.
   NOTE: To avoid any degradation of the pesticide, the complete hydrolysis procedure should be completed on the same day.

8. Set the flask in a warm water bath (35-40°C) and evaporate just to dryness with a gentle stream of air.

9. Dissolve the residue in the flask in a measured volume of acetone. Inject exactly 4 µL into the chromatograph. Quantitate the methomyl oxime by reference to a previously prepared calibration curve.
   NOTE: Store all sample solutions in a refrigerator or freezer when work stops for the day. This storage should be limited to three days.

10. Determine the thiodicarb equivalents in the original sample using the following values and equation:

$$\text{ppm thiodicarb} = (a \times b)/(c \times d)$$

where:
- a = concentration of methomyl oxime in final solution, µg/ml (from calibration curve)
- b = final dilution in mL
- c = sample size in g
- d = 0.59, a molecular weight conversion factor from methomyl oxime to thiodicarb (210 µg MO/354 µg thiodicarb)

TABLE 1

EXPERIMENTAL CHROMATOGRAPHIC CONDITIONS FOR THE HEWLETT PACKARD 5840A GAS CHROMATOGRAPH AND THE COLUMN USED

column temperature, °C	175
injector temperature, °C	185
detector temperature, °C	225
gas flow rates, mL/min	
helium	20
hydrogen	48
oxygen	8
air	50
injection volume, µL	4
attenuation	2
glass column	
length, cm	100
inner diameter, mm	2
packing	
stationary phase	5% SP-1000
support	Supelcoport 100/120
aged	
time, h	48
temperature, °C	250

RECOVERIES OF THIODICARB AND METHOMYL FROM FORTIFIED SOYBEAN SEED

(50 gram samples)

AMOUNT OF COMPONENT ADDED		METHOMYL RECOVERED		THIODICARB RECOVERED	
µg	ppm	µg	%	µg	%
1.0	0.02	-	-	0.92	92
		-	-	0.93	93
		-	-	0.84	84
		-	-	0.93	93
2.0	0.04	1.6	80	1.5	75
		1.6	80	1.7	85
		-	-	1.5	75
5.0	0.10	3.7	74	3.8	76
10	0.20	7.7	77	7.6	76
		9.2	92	8.2	82
		8.5	85	8.5	85
		-	-	7.3	73
		-	-	8.3	83
100	2.0	92	92	85	85
		Average	83%		83%