DETERMINATION OF TOTAL TOXIC TEMIK RESIDUES IN MILK BY GAS CHROMATOGRAPHY

Introduction

This method requires precipitation of milk-solids with phosphoric acid and their removal by filtration. Oxidation of TEMIK Sulfoxide to the sulfone is accomplished by the addition of peracetic acid to the filtrate. The carbamate metabolites are extracted into chloroform. Anhydrous powdered sodium sulfate is used to break the emulsion in the combined chloroform extracts. After appropriate cleanup on a Florisil Column, the sample is analyzed by vapor-phase chromatography and quantitated by comparison with a standard curve.

The chromatographic column should be conditioned overnight at a temperature of 200°C. before being installed since the flame photometric detector is especially susceptible to contamination by excessive column bleed. A properly conditioned column should last for weeks depending on the number of samples injected. Sections of 1/4-inch aluminum tubing, packed with glass wool, are inserted into and connected to the injection and detector ports in the column oven to protect the column packing from the high injection port temperature. This tubing is extended about one inch from the connections into the oven to act as a heat insulator and with reducing fittings it allows convenient attachment of the 1/8-inch aluminum column.

The validity of the method was proven by fortifying samples with TEMIK Sulfoxide and TEMIK Sulfone. The method is specific and the non-toxic oxime and nitrile metabolites of TEMIK do not interfere. The average recovery of TEMIK Sulfoxide and TEMIK Sulfone was about 90 percent. The method will accurately detect residues of 0.0022 ppm.

Reagents

a. Chloroform, "Baker Analyzed" reagent.
b. Acetone, Mallinckrodt AR grade.
c. Ethyl ether, "Baker Aanlyzed" reagent.
d. Hyflo-Supercel
e. Sodium sulfate, anhydrous powder.
f. Florisil, 60/100 mesh PR grade.
g. Oxidizing solution, Becco peracetic acid, 40 percent. (Becco Inorganic Chemicals Division, FMC Corporation, New York, N.Y.).
h. Solvent mixture I: 4 percent acetone - 96 percent ethyl ether.
i. Solvent mixture II: 50 percent acetone - 50 percent ethyl ether.
j. Sodium bicarbonate, Mallinckrodt AR grade.
k. Phosphoric acid (85%), "Baker & Adams" reagent grade.

Contact H. F. LYKINS
UNION CARBIDE TECHNICAL CENTER
SOUTH CHARLESTON, WEST VIRGINIA
Phone: 304-747-4975
Reagents Not Readily Available

Apparatus

a. Micro Tek MT-220 gas chromatograph (Micro Tek Instrument Co., Baton Rouge, La.) equipped with a flame photometric detector incorporating a 394 mu filter specific for sulfur-containing compounds. Column - 1/8" x 12' aluminum, packed with 5 percent Carbowax 20M on 60/80 mesh Gas Chrom Q (Applied Science Lab., State College, Pa.).

NOTE: 1/4" sections of aluminum tubing packed with glass wool are inserted into and attached to both the oven injection port and oven detector port. About one inch of the tubing is allowed to protrude into the oven from each port serving as heat insulation for the column packing, and with reducing fittings affording a convenient way to attach the column.

Conditions -	Oven temperature	160°C.
	Injector temperature	300°C.
	Detector temperature	160°C.
	N_2 carried gas flow	60 cc/min.
	O_2 flow	25 cc/min.
	H_2 flow	200 cc/min.
	Analysis time	10 min.

b. Liquid chromatography column - 200 x 13 mm I.D. glass column equipped with a 2 mm Teflon stopcock and a 250 ml. solvent reservoir.

Standard Solutions

a. Weigh 0.222 g. of TEMIK Sulfone into a 100-ml. volumetric flask, dilute to the mark with acetone and shake until solution is complete.

b. Withdraw a 10-ml. aliquot with a pipet and dilute to 100 ml. with acetone in a second volumetric flask.

c. Remove 2 ml. of stock solution (b) and dilute to 100 ml. as above. The standard solution now contains 4.44 ug./ml. of TEMIK Sulfone.

d. By a similar procedure prepare standard solutions containing 2.2, 1.1 and 0.55 ug./ml. of TEMIK Sulfone.

e. Store solutions at -5°C. and maintain at 0°C. during use.

Procedure

1. Mix 200 grams of milk and 300 ml. of distilled water in a 1-liter beaker.

2. Add 0.5 ml. of phosphoric acid (85%). Mix and allow to stand 15 minutes. Stir occasionally.

3. Add 125 cc. of Hyflo-Supercel and mix thoroughly.

4. Filter under vacuum using a 11.0 cm. diameter filter funnel containing Whatman No. 1 filter paper covered with 1/2 inch of loosely-packed Hyflo.

5. Wash the filter cake twice with 50 ml. of distilled water. The surface of the cake may be scraped with a spatula if filtration is slow.

6. Mix 5 ml. of Becco peracetic acid (40 percent) to the filtrate from (5) and allow the mixture to oxidize for 15 minutes.

7. Carefully add 10 grams of sodium bicarbonate while stirring.

8. Transfer to a 1-liter separatory funnel. Wash the beaker two times with 25-ml. portions of distilled water and add to the separatory funnel.

9. Extract four times with 100-ml. portions of chloroform. Use very mild mixing. Allow each extract to separate at least five minutes. The chloroform layer will be slightly emulsified.

10. Combine the extracts in a 1-liter beaker and add 125 cc. of powdered anhydrous sodium sulfate while stirring. This procedure will break the emulsion.

11. Filter the mixture under vacuum through a 11.0 cm. porcelain filter funnel covered with approximately 25 cc. of powdered anhydrous sodium sulfate. Wash the filter cake with 100 ml. of chloroform.

12. Attach a flask containing the combined extracts to a vacuum manifold with the flask immersed in a 40-50°C. water bath. Start stripping the solvent at 150-200 mm pressure to prevent "bumping" and gradually reduce the pressure to 60-70 mm. Evaporate the extract to a volume of 50-70 ml.

13. Pour the concentrated extracts into a 100-ml. graduate and add chloroform to obtain exactly 100 ml. (Use the added chloroform to rinse the flask.)

14. Place a glass wool plug in the bottom of a glass tube (11 mm I.D.) and add 5 inches of Florisil. Prewet the Florisil with 20 ml. of chloroform.

15. Pour the extracts from (13) on the Florisil column and discard the eluate.

16. First Fraction. - Elute the column with 100 ml. of solvent mixture I and discard.

17. Second Fraction. - Elute the column with 100 ml. of solvent mixture II and collect in a clean 250 ml. Erlenmeyer flask.

18. Immerse the flask in a 40-50°C. water bath and evaporate the solvent with a gentle stream of nitrogen to a volume of about 5 ml.

19. Transfer the concentrated extract from (18) to a tapered test tube. Wash the flask from (18) three times with 2 ml. of acetone and transfer to the test tube.

20. Evaporate the solvent from the test tube to dryness as in (18).

21. Remove the test tube immediately after attaining dryness.

22. Chill the test tube in an ice bath and add 0.5 ml. of 0°C. acetone. Stopper and swirl to dissolve all of the residual pesticide.

23. Inject an 8-ul sample into the chromatograph and quantitate the pesticide by referring the peak height to a standard curve derived by injection of TEMIK Sulfone standards.

24. Calculation

$$\frac{\text{ug. TEMIK Sulfone}}{200} = \text{ppm total toxic TEMIK residues expressed as TEMIK Sulfone}$$

TABLE I. RECOVERY OF TEMIK SULFOXIDE AND TEMIK SULFONE FROM FORTIFIED MILK

Component[a] Added ppm	TEMIK Sulfoxide Recovered ppm	%	TEMIK Sulfone Recovered ppm	%
0			0.0025	
0			0.0025	
0			0.0025	
0.0022	0.0025	115.0	0.0022	100.0
0.0022	0.0026	118.0	0.0021	95.5
0.0022	0.0021	95.5	0.0027	121.0
0.0044	0.0040	91.0	0.0042	95.0
0.0044	0.0042	95.0	0.0044	100.0
0.0055	0.0063	115.0	0.0048	87.0
0.0055	0.0046	85.5	0.0043	78.0
0.011	0.0097	88.0	0.0094	85.5
0.011	0.0097	88.0	0.0099	90.0
0.011	0.0081	73.7	0.0091	82.8
0.011	0.0080	72.8	0.0097	88.2
0.022	0.0146	66.5	0.0168	76.2
0.044	0.0344	78.0	0.0365	83.0
	Average	91.7	Average	90.9
0.0055[b]			0.0051	92.5
0.0055			0.0048	87.4
0.011			0.0093	83.8
0.011			0.0079	72.0

a) Two separate samples, one for each compound, were fortified at this level. All concentrations are expressed as TEMIK Sulfone.

b) An equimolar mixture of TEMIK Sulfoxide and TEMIK Sulfone was added and the Total Toxic residues detected as TEMIK Sulfone.

TABLE II. RESPONSE OF SULFUR-CONTAINING PESTICIDES SUBJECTED TO PROCEDURES OF UC 21149-III-MILK

Pesticide	Fortification[1] ppm	% of Scale at 7.0 minutes retention time
Malathion	0.057	<1.0
Ethion	0.049	<1.0
Carbophenothion	0.061	<1.0
Maneb	0.066	<1.0
Ferbam	0.054	<1.0
TEMIK Sulfone	0.0055	4.0

(1) Fortified 200 grams of milk

(2) 8 ug. injected. Attenuation 10^2 x 16.

TABLE III. PROOF OF EXCLUSION OF NON-CARBAMATE METABOLITES AS INTERFERENCES IN UC 21149-III-MILK

Metabolite	Fortified ppm	Recovered ppm
TEMIK Oxime Sulfone	0.066	<0.0025
TEMIK Nitrile Sulfone	0.076	<0.0025

TABLE IV. RECOVERY OF TEMIK SULFOXIDE AND TEMIK SULFONE FROM THE AQUEOUS FRACTION OF FORTIFIED RAW WHOLE MILK

Component[a] Added	TEMIK Sulfoxide Recovered		TEMIK Sulfone Recovered	
ppm	ppm	%	ppm	%
0.011	0.010	91.0	0.0097	88.2
0.011	0.0093	84.7	0.0094	85.5

a) Two separate samples, one for each compound, were fortified at this level. All concentrations are expressed as TEMIK Sulfone.