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R46 FLUTOLANIL: Validation of an Analytical Method for Residues  
of Flutolanil in Milk, Peanuts and Rice, USA, 1995

DATA REQUIREMENTS

Guideline Reference 171-4(c), OPPTS 860.1340

AUTHOR

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DATE

June 16, 1995

PERFORMING LABORATORY

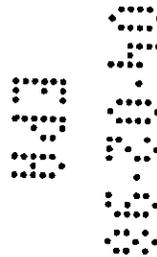
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AU-95R-04





**STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS**

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

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**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

The undersigned, hereby declare that the work to which this report refers was performed according to the procedures herein described and this report provides an accurate record of the results obtained. The study was conducted in accordance with the Good Laboratory Practice Standards as specified in 40 CFR 160 with the exception that one data set was started 2 days prior to the signing of the protocol. This has no effect upon the study.

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**QUALITY ASSURANCE STATEMENT**

This study was inspected and the findings reported to the facility management and to the study director on the listed dates:

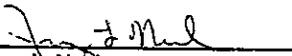
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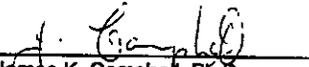
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**SUMMARY****Validation of an Analytical Method for Residues of Flutolanil in Milk, Peanuts and Rice, USA, 1995**

This method is a development of RAM No. AU/01/91 which was independently validated and reported as study number AU-92R-03.<sup>1</sup> The method validated and described in this report remains the same in principle as the original method with modifications being made to the physical method of extraction, changing partitioning solvents, and changing the procedure for methylation. None of the changes and modifications to the original method were considered major since the base method remains intact and the extraction solvents used are not changed. No reduction in analytical recoveries were observed for any of the fortification analytes.

Extractable residues of flutolanil are removed from the crop matrix by solvent blending finely ground samples with a sequence of solvents. Once the residues are solubilized in the extraction solvent, the residues are then cleaved by base hydrolysis which converts parent and all known metabolites to 2-trifluoromethylbenzoic acid the common moiety for all of the residues. The method reported here was validated using the major metabolites identified in each of the following matrices:

Rice	Flutolanil (M-1), and M-4
Peanuts	Flutolanil (M-1), M-3, and M-4
Animal Tissue	Flutolanil (M-1), M-2, M-4, and M-7
Milk	Flutolanil (M-1), M-2, M-4, and M-7
Eggs	Flutolanil (M-1), M-2, M-4, and M-7

Validation results from animal tissue and eggs are included in this report and taken from studies AU-93R-05<sup>7</sup> and AU-93R-06.<sup>8</sup> Results are shown in the tables which follow.

The concentration chosen for the validation corresponds to approximately 50% of the tolerance and 3 to 5 times the tolerance in each matrix.

**Typical Recoveries for Flutolanil Residues in Rice**

Crop Matrix	Fortification Level (ppm)	Percent Recovery of Flutolanil Analytes									
		M-1		M-2		M-3		M-4		M-7	
Rice (Whole)	0.20	78	80	N/A	N/A	N/A	N/A	83	97	N/A	N/A
	30.00	82	79	N/A	N/A	N/A	N/A	85	80	N/A	N/A
Rice Straw	0.20	125	76	N/A	N/A	N/A	N/A	77	84	N/A	N/A
	30.00	78	95	N/A	N/A	N/A	N/A	90	69	N/A	N/A
Rice Hulls	0.20	77	87	N/A	N/A	N/A	N/A	81	71	N/A	N/A
	100.00	78	75	N/A	N/A	N/A	N/A	87	80	N/A	N/A
Rice Bran	0.20	68	83	N/A	N/A	N/A	N/A	84	96	N/A	N/A
	30.00	86	85	N/A	N/A	N/A	N/A	88	98	N/A	N/A
Number		16		0		0		16		0	
Mean		83		N/A		N/A		84		N/A	
Std. Dev.		±13		N/A		N/A		±8		N/A	

**Typical Recoveries for Flutolanil Residues in Peanuts**

Crop Matrix	Fortification Level (ppm)	Percent Recovery of Flutolanil Analytes									
		M-1		M-2		M-3		M-4		M-7	
Peanut Meats	0.20	78	93	N/A	N/A	90	86	85	92	N/A	N/A
	2.00	85	84	N/A	N/A	86	87	93	Lost*	N/A	N/A
Peanut Meal	0.20	83	90	N/A	N/A	93	86	107	92	N/A	N/A
	3.00	101	99	N/A	N/A	100	101	106	104	N/A	N/A
Peanut Hulls	0.20	105	98	N/A	N/A	86	74	81	80	N/A	N/A
	20.00	82	85	N/A	N/A	74	61	81	83	N/A	N/A
Peanut Hay	0.20	90	100	N/A	N/A	101	104	98	98	N/A	N/A
	50.00	99	109	N/A	N/A	109	103	89	101	N/A	N/A
Number		16		0		16		15		0	
Mean		93		N/A		90		93		N/A	
Std. Dev.		±9		N/A		±13		±9		N/A	

\* Sample spilled and went dry at the rotary evaporation step. Actual recovery was 42%.

**Typical Recoveries for Flutolanil Residues in Cattle Tissue\***

Crop Matrix	Fortification Level (ppm)	Percent Recovery of Flutolanil Analytes									
		M-1		M-2		M-3		M-4		M-7	
Muscle	0.05	---	---	79	---	N/A	N/A	---	---	---	---
	1.00	---	---	85	---	N/A	N/A	---	---	---	---
Liver	0.05	---	---	---	---	N/A	N/A	92	---	---	---
	1.00	---	---	---	---	N/A	N/A	75	---	---	---
	10.00	---	---	---	---	N/A	N/A	97	95	---	---
Kidney	1.00	---	---	---	---	N/A	N/A	---	---	94	---
	10.00	---	---	---	---	N/A	N/A	---	---	95	---
Fat	0.05	80	---	---	---	N/A	N/A	---	---	---	---
	1.00	91	---	---	---	N/A	N/A	---	---	---	---
Number		2		2		0		4		2	
Mean		86		82		N/A		90		95	
Std. Dev.		± 8		± 4		N/A		± 10		± 0.7	

\* See Reference 2 (Study AU-93R-05 - Registration Reference Flutolanil/R35)

**Typical Recoveries for Flutolanil Residues in Milk**

Crop Matrix	Fortification Level (ppm)	Percent Recovery of Flutolanil Analytes									
		M-1		M-2		M-3		M-4		M-7	
Milk (Whole)	0.05	73	66	92	88	N/A	N/A	103	89	102	96
	0.20	99	90	109	103	N/A	N/A	103	106	114	116
Number		4		4		0		4		4	
Mean		82		98		N/A		100		107	
Std. Dev.		± 15		± 10		N/A		± 8		± 10	

**Typical Recoveries for Flutolanil Residues in Poultry & Eggs<sup>a</sup>**

Crop Matrix	Fortification Level (ppm)	Percent Recovery of Flutolanil Analytes				
		M-1	M-2	M-3	M-4	M-7
Egg (Whole)	0.05	---	99 83	N/A N/A	75 ---	---
	1.00	---	84 95	N/A N/A	93 ---	---
Egg (White)	0.05	90 95	---	N/A N/A	---	94 ---
	1.00	---	---	N/A N/A	---	122 ---
Muscle (Breast)	0.05	---	---	N/A N/A	---	89 ---
	1.00	---	---	N/A N/A	---	59 ---
Muscle (Thigh)	0.05	86 ---	---	N/A N/A	---	---
	1.00	96 ---	---	N/A N/A	---	---
Liver	0.05	---	99 ---	N/A N/A	---	---
	10.00	---	62 ---	N/A N/A	---	---
	10.00	---	71 ---	N/A N/A	---	---
Fat	0.05	---	---	N/A N/A	---	71 ---
	5.00	---	---	N/A N/A	---	62 ---
	5.00	---	---	N/A N/A	---	72 ---
Skin	0.05	80 ---	---	N/A N/A	---	---
	1.00	95 ---	---	N/A N/A	---	---
Number		6	7	0	2	7
Mean		90	85	N/A	84	81
Std Dev		± 6	± 14	N/A	± 13	± 22

<sup>a</sup> See Reference 3 (Study AU-93R-06 - Registration Reference Flutolanil/R40).

The mean recoveries for flutolanil analytes in the various matrices were all greater than 80%.

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**GENERAL STUDY INFORMATION**

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Experimental Start Date:    June 4, 1995

Experimental Termination Date: June 15, 1995

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### 1. SCOPE

This method is suitable for use as an enforcement method to determine the total extractable residues of flutolanil (see Appendix I) and its metabolites M-2, M-3, M-4, and M-7 in or on rice, peanuts, whole milk and animal tissues. The limit of determination for this procedure has been set at 0.05 ppm for whole milk and animal tissues and at 0.2 ppm for rice and peanut matrices.

The method has been successfully validated by fortifying with the major identified metabolites for the matrix being analyzed. The major analytes used for fortification of rice samples are flutolanil (M-1) and M-4, peanut samples use flutolanil (M-1), M-3, and M-4, and whole milk and animal tissue matrices (other than meats) use flutolanil (M-1), M-2, M-4 and M-7.

### 2. PRINCIPLE

Residues of flutolanil are removed from the rice and peanuts by blending with acetone. Peanut meats are blended with acetone and 80:20 acetonitrile:water. Fat is extracted with a mixture of acetonitrile and hexane. The solvent extracts are concentrated, transferred to Teflon culture tubes and base-hydrolyzed. Whole milk samples and animal matrices other than fat are hydrolyzed directly. The digest containing the hydrolytic product 2-(trifluoromethyl)-benzoic acid (Appendix I), common to flutolanil and all of its metabolites, is diluted with water, acidified, and extracted with dichloromethane. The dichloromethane extracts are concentrated by evaporation then made up to a known volume with acetone. An aliquot of the sample is methylated using an ion-pair technique (Ref. 3) and then diluted with toluene. The methyl ester of 2-(trifluoromethyl) benzoic acid (Appendix I) is quantified by splitless gas chromatography using mass-selective detection (MSD) in the selected ion mode (SIM). The residues are expressed in terms of equivalent flutolanil and/or in terms of its metabolites.

### 3. APPARATUS

Use as a guide: (Equivalent substitution may be made.)

- Whatman glass fiber filter paper (934-AH diameter 9 cm)
- Separatory funnels -125 mL and 250 mL
- Graduated Cylinders, 250 mL stoppered (TC) and 50 mL (TD)
- Boiling flasks --125 mL and 250 mL
- Culture tubes, Pyrex 13 x 100 mm and 25 x 150 mm with screw caps
- Centrifuge IEC, (Model IEC EXD)
- Vortex Mixer

- Water bath, Fisher, Versa Bath, Model 132, with cover
- Class A volumetric flasks, 5 mL, 25 mL, and 100 mL
- Teflon culture tubes (15 mL), Norton Performance Plastics (Part # A1069080)
- Teflon tape, Supelco (Part # 2-0808M)
- Filter Disks LC-13 (0.45  $\mu$ m) Gelman Sciences
- Straight neck vacuum adapter (24/40), Prism Research Glass (Part# PRG-127501)
- Canning (Blending) Jars, purchased locally, (1 pt.)
- Büchner funnels, 9 cm
- Omni Mixer Homogenizer, Omni International (Model #17105)
- Blade Assemblies, Omni International (Part #17080)
- Heating block, Pierce, Reacti-Therm III with Reacti-Vap Evaporator (Parts # 18830 and 18780)
- DB-17 fused silica capillary column 30 m x 0.25 mm i.d., 0.25  $\mu$ m film thickness, J & W Scientific (Cat. # 122-1732)
- Hewlett Packard 5890A Gas Chromatograph with capillary split/splitless inlet with 5970 B mass selective detector equipped with Model 7673A autosampler

#### 4. REAGENTS

Use as a guide: (Equivalent substitution may be made.)

- Toluene, pesticide grade
- Methanol, pesticide grade
- Sulfuric acid ( $H_2SO_4$ ), concentrated and 30% (v/v), ACS reagent
- Acetone, pesticide grade
- Dichloromethane (DCM), pesticide grade
- Acetonitrile (MeCN), pesticide grade
- Acetonitrile:H<sub>2</sub>O (80:20 v/v)
- Hexane, pesticide grade
- $Na_2SO_4$ , anhydrous
- $Na_2SO_4$ , anhydrous, acid treated (see Note)
- Sodium chloride (NaCl),
- Sodium hydroxide (NaOH), 50% (w/w)
- Filtration columns (6 mL), J.T. Baker, Inc. (Cat. No. 7121-06)
- Olive oil, Aldrich (Cat. # 24, 816-9) 50% (v/v) in acetone
- Tetrabutyl ammonium hydroxide (TBAH), 1 M in methanol, Aldrich (Cat. # 23,018-9)
- Methyl iodide (MI), Aldrich (Cat. # 1-850-7)
- Analytical standards of known purity for compounds M-1, M-2, M-3, M-4, M-7, 2-TFBA, and 2-TFBA Me ester (see Appendix I)

NOTE: To acid treat the  $\text{Na}_2\text{SO}_4$ , weigh approximately 500 g of anhydrous  $\text{Na}_2\text{SO}_4$  into a 1000 mL boiling flask and cover with anhydrous ethyl ether. Slowly add 4 mL of concentrated  $\text{H}_2\text{SO}_4$  with swirling. Rotary evaporate the  $\text{Na}_2\text{SO}_4$  to remove the ethyl ether. Transfer the  $\text{Na}_2\text{SO}_4$  to a large beaker or suitable open glass container. Heat the  $\text{Na}_2\text{SO}_4$  for two hours at 150 °C with occasional stirring to break clumps. Allow the  $\text{Na}_2\text{SO}_4$  to cool and transfer to a stoppered flask or bottle. Store at room temperature until needed.

## 5. PROCEDURE

### 5.1 Extraction (Rice & Peanuts excluding Peanut Meats)

1. Weigh a finely ground representative crop sample (20.0 grams) into a blending jar.
2. Fortify the recovery samples with flutolanil (M-1) or metabolites as required. Add 100 mL of acetone and blend for 1 minute
3. Decant the solvent (leave the crop residue in the blending jar) into a Büchner funnel containing glass fiber filter paper and filter the extracts under vacuum into a 250 mL graduated cylinder.
4. Add an additional 100 mL of acetone and blend the crop residue again for 1 minute. Repeat Step 3 above.
5. Rinse the filter cake with acetone and dilute the contents of the graduated cylinder to 250 mL with acetone. Stopper and mix the contents of the cylinder.
6. Take a 50 mL aliquot of the extracts from Section 5.1, Step 5 and transfer to a 125 mL boiling flask. Rotary evaporate the extracts to dryness at approximately 40 °C and re-dissolve in 2 mL of acetone. Proceed to Section 5.4, Base Hydrolysis.

### 5.2 Extraction (Peanut Meats & Meal/Presscake)

1. Weigh a finely ground representative crop sample (20.0 grams) into a blending jar.
2. Fortify the recovery samples with flutolanil (M-1) or metabolites as required. Add 100 mL of acetone and blend for 1 minute.

3. Decant the solvent (leave the crop residue in the blending jar) into a Büchner funnel containing glass fiber filter paper and filter the extracts under vacuum into a 250 mL graduated cylinder.
4. Add 100 mL of 80:20 acetonitrile:water and blend the crop residue again for 1 minute. Repeat Section 5.2, Step 3 above except collect the filtrate in a 250 mL separatory funnel.
5. Add 3 grams of granular NaCl to the filtrate contained in the separatory funnel from Step 4, stopper and shake well. Allow the salt water to separate. Drain the lower aqueous layer and any residual salt and discard. Combine the upper organic layers into the graduated cylinder from Section 5.2, Step 3. Dilute the contents of the graduated cylinder to 250 mL with acetone. Stopper and mix the contents of the cylinder. (Note: The contents will be cloudy.)
6. Take a 50 mL aliquot of the extracts from Section 5.2, Step 5 and transfer to a 125 mL boiling flask. Rotary evaporate the extracts to dryness at approximately 40 °C and re-dissolve in 2 mL of acetone. Proceed to Section 5.4, Base Hydrolysis.

### 5.3 Extraction (Fat)

1. Weigh a 10.0 gram sample into a blending jar. Fortify the recovery samples with flutolanil (M-1) or metabolites as required. Add 50 mL acetonitrile and 50 mL of hexane and blend for 2-3 minutes. Decant the solvent into a Büchner funnel containing glass fiber filter paper and filter the extracts under vacuum into a 250 mL separatory funnel. Drain the acetonitrile into a 250 mL boiling flask. Retain the hexane.
2. Transfer the filter cake (including filter paper) back to the blending jar and repeat Step 1 above. Combine the filtrate and the retained hexane in the 250 mL separatory funnel. Shake the separatory funnel for approximately 1 minute and allow the layers to separate. Drain the acetonitrile into the 250 mL boiling flask, combining the extracts with those from Section 5.3, Step 1. Discard the hexane.
3. Rotary evaporate the extracts to dryness at approximately 40 °C under vacuum and re-dissolve the residue in 2 mL of acetone.

#### 5.4 Base Hydrolysis

**CAUTION.** It is essential that the Teflon tubes fit the heating block correctly. If they do not fit tightly, they will not achieve the correct hydrolysis temperature and as a result the hydrolysis may not go to completion. Defective Teflon culture tubes may fail or the caps may be blown off during hydrolysis. This is especially true of the hydrolysis of eggs and tissues. Old tubes which show signs of stretching or stripped threads should not be used. Tubes may be reused; however, the caps should fit snugly to avoid leakage during hydrolysis. It is highly recommended that the hydrolysis take place behind a protective screen or in a fume hood with the sash pulled down.

**Note:** It has been found that it is necessary to add a small amount of olive oil (or an oil of similar composition such as peanut oil) to serve as a "keeper" during base hydrolysis. The addition of excessive amounts of oil may hinder the base hydrolysis or the methylation. Samples which are oily in nature such as peanut meats, peanut hulls or rice bran normally do not require the addition of 250  $\mu$ L of 1:1 acetone olive oil (v/v). If small amounts of residual oils are left after Step 6 then the addition of the olive oil:acetone prior to base hydrolysis is not required.

1. Prepare Teflon culture tubes for base hydrolysis by wrapping the threads of the culture tube with at least four turns of Teflon tape.
2. Transfer the acetone fractions from Section 5.1, 5.2, or 5.3, Step 6 into the Teflon culture tubes containing 250  $\mu$ L of 1:1 acetone olive oil (v/v).
3. Rinse the flask 3 times with 2 mL portions of acetone and transfer each rinse to the Teflon culture tube.
4. Evaporate the acetone mixture on a dry block at 40 °C under a stream of air between 0.75 and 1.25 hr. Make sure all acetone and acetone vapor are removed from the culture tubes before adding NaOH.
5. Add 2 mL of 50% (w/w) NaOH cap tightly and mix. Place the tubes on a heating block at approximately 200 °C for 3-4 hr. Allow the samples to cool to room temperature. Samples may be hydrolyzed after working hours by controlling the heating block with a timer.

**Note:** Successful hydrolysis is dependent on temperature, NaOH concentration and time. The recommended parameters have

proven to be adequate, but may be adjusted if necessary to achieve satisfactory results. The parameters listed should be considered as minimum hydrolysis conditions.

#### 5.5 Direct Base Hydrolysis (Milk, Tissue & Eggs)

There is no "extraction" for milk, tissue or egg samples. Tissues must be well trimmed and relatively fat free. Eggs and tissues should be homogenized and/or ground well. Milk, tissue and egg samples are base hydrolyzed directly in Teflon culture tubes. Slightly different base hydrolysis conditions are outlined in the following steps:

**CAUTION:** Solutions used for fortifying milk, tissues & eggs (Direct Base Hydrolysis) should be prepared in methanol rather than acetone. The solvent volume should be kept to a minimum (0.5 mL or less) for milk tissues and eggs. Larger amounts of solvent can soften tubes and cause rapid pressure build-up during hydrolysis such that the tubes may be stretched or may fail prematurely.

- 1 Weigh a 5.0 gram sample directly in a Teflon culture tube (Prepared as in Section 5.4, Step 1) Fortify the recovery samples with flutolanil (M-1) or metabolites as required with fortification solutions prepared in methanol. No olive oil is required.
- 2 Mix the sample well, and allow the sample to stand at room temperature for approximately 15 minutes with the cap off.
- 3 Add 3 mL of 50% NaOH to the tissue samples, or 3.0 grams of solid NaOH to the egg or milk samples cap tightly and mix well.
- 4 Place the tubes on a heating block at approximately 200 °C for 3-4 hr. Allow the samples to cool to room temperature. Samples may be hydrolyzed after working hours by controlling the heating block with a timer.

#### 5.6 Acid Partitioning

1. Quantitatively transfer the hydrolysis products from Section 5.4, Step 5 or Section 5.5, Step 4 to a 125 mL separatory funnel by rinsing the Teflon culture tube 3 times with 5 mL portions of deionized water, followed by a 5 mL rinse of 30% conc. sulfuric acid to water (v/v) (8 mL for milk, tissues, and eggs). Finally rinse the tube with an additional 5 mL portion of deionized water, followed by a 2 mL rinse of acetone. Combine all rinses and shake to mix well.

(Note: Tubes should be tapped and shaken vigorously to dislodge adhering solid material especially in the initial rinses. A spatula or probe may be needed to break-up tissue and egg samples.)

2. Partition the sample ( $\text{pH} \leq 1$ ) three times with 10 mL of dichloromethane (vent the separatory funnel) Shake each partition for approximately one minute and allow the layers to separate. Drain the lower partition through  $\approx 5$  grams of acid treated sodium sulfate contained in a 6 mL disposable filtration column into a 125 mL boiling flask. Rinse the sulfate with 10 mL of dichloromethane.
3. Add 1 mL of toluene to the sample and rotary evaporate to approximately 1 mL at 40 °C with reduced pressure. **DO NOT ALLOW THE SAMPLE EXTRACTS TO GO DRY!**
4. Transfer the sample to a 5 mL volumetric flask. Rinse the boiling flask with small portions (2 x 2 mL) of acetone and add to the volumetric flask. Bring the sample to volume with acetone and mix well.

#### 5.7 Methylation

Option. The efficiency of the methylation may be monitored by fortifying a 1 mL aliquot of a control sample from Section 5.6, Step 4 and/or plain acetone with 0.2  $\mu\text{g}$  of trifluoromethyl benzoic acid in acetone. The sample should then be methylated as in Section 5.7, Steps 1 through 3 below

1. Transfer a 1.0 mL aliquot of the sample to a 13 x 100 mm culture tube. Add 100  $\mu\text{L}$  of methyl iodide (MI) and 50  $\mu\text{L}$  tetrabutyl ammonium hydroxide solution (TBAH) to the sample. Cap the tube, mix well, and incubate the sample for 2 hr. at approximately 40 °C.
2. Allow the sample to cool. Transfer the sample to a 25 mL volumetric flask. Rinse the tube with several portions of toluene and transfer to the flask. Bring the sample to volume with toluene and mix well.
3. Transfer an aliquot to a GC vial for analysis by GC/MSD. If a precipitate forms, the sample may be filtered through a 0.45  $\mu\text{m}$  filter disk prior to analysis.

Option: To serve as an additional cleanup, especially for milk samples the following solid phase extraction (SPE) cleanup may be used. This procedure does not require column conditioning or column profiling. The (SPE) cartridge is used as if a filter. This step is occasionally required for dirty samples. If interference occurs in the chromatography, an aliquot of the methylated samples should be cleaned up using this procedure and injected on the GC

#### 5.8 Amino SPE Cleanup

1. Place approximately 0.75 grams of acid treated anhydrous sodium sulfate into an unconditioned 3 mL aminopropyl bonded solid phase extraction cartridge.
2. Transfer an aliquot (2 mL) from Section 5.7, Step 3 to the top of the column. Pressurize the solid phase extraction cartridge with a pipette bulb and collect the eluate in a GC vial for analysis by GC/MSD.

### 6. GC/MSD ANALYSIS

#### 6.1 Preparation of Analytical Standards

1. Prepare a stock solution of 2-(trifluoromethyl) benzoic acid methyl ester in ~~toluene~~ ~~Make serial dilutions of the stock 2-TFBA Me-ester to give working solutions in the range of 0.05 to 0.001 µg/mL in toluene~~
2. Make repeated 2 µL injections of 2-TFBA-Me-ester standard solution(s), using the GC conditions in Appendix II, until a constant response is obtained.
3. Standardize the GC/MSD under the conditions in Appendix II by making 2 µL injections of the 2-TFBA Me-ester working solutions interspersed with the samples.
4. Construct a standard curve by plotting the natural logarithm of the peak areas vs. the natural logarithm of the standard concentrations to obtain a least squares regression line (see Appendix III). Alternatively, the natural logarithm of the peak heights may be used.

#### 6.2 Analysis of Samples

1. Inject a 2 µL aliquot of the sample from Section 5.7, Step 3 or Section 5.8, Step 2 into the GC/MSD under the conditions in

Appendix II. An injection volume of 2  $\mu$ L was found to give satisfactory results. Variations in equipment or sample characteristics may require different injection volumes. Make dilutions of the samples as necessary to maintain peak areas (heights) within the range of the standard curve.

2. Compare the peak area of the unknown samples with the standard curve. Determine the concentration of the 2-TFBA Me-ester in the injected aliquot as follows:

$$T = \exp \frac{\ln(A) - Y}{S}$$

Where:  $T$  = Concentration in  $\mu$ g/mL of 2-TFBA-Me-ester  
 $A$  = Peak Area of unknown  
 $Y$  = Y intercept of standard calibration curve  
 $S$  = Slope of standard calibration curve  
 $\exp$  = Base of natural logarithm, 2.71828

3. Calculate the total residues in terms of flutolanil as follows.

$$PPM \text{ Flutolanil} = (T / C) \times F \times D$$

Where:  $T$  = Concentration of 2-TFBA-Me-ester  
 $C$  = Crop/solvent ratio (gm/mL)  
 $F$  = Flutolanil molecular weight factor (1.584)  
 $D$  = Dilution factor, as needed

### 6.3 Fortification Experiments

1. This method may be validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified prior to the addition of the extraction solvent or prior to the direct hydrolysis of the whole milk with M-1, M-2, M-3, M-4, or M-7 in acetone. Standards used for the fortification of milk, tissues and eggs should be prepared in methanol. Desired concentrations of fortification standards are left up to the discretion of the analyst. Standards used for fortifying milk, tissues and eggs should be prepared so that the delivery solvent volume does not exceed 0.5 mL. Analyze the control and fortified samples by the procedure outlined in the method. Typical chromatograms from representative matrices are shown in Appendix IV.

2. Calculate the final PPM for the control and fortified samples as in Section 6.2. Note The molecular weight correction factors for flutolanil (M-1), M-2, M-3, M-4, and M-7 are found in Appendix I

3. Calculate the percent recovery as follows:

$$\% \text{ Recovery} = \frac{D - C}{TF} \times 100$$

Where: *D* = PPM of compound recovered.  
*C* = PPM of compound found in control.  
*TF* = PPM of compound fortified.

4. Typical recoveries for various matrices are shown in the tables below.

**Table 1** Typical Recoveries for Flutolanil Residues in Rice

Crop Matrix	Fortification Level (ppm)	Percent Recovery of Flutolanil Analytes									
		M-1		M-2		M-3		M-4		M-7	
Rice (Whole)	0.20	78	80	N/A	N/A	N/A	N/A	83	97	N/A	N/A
	30.00	82	79	N/A	N/A	N/A	N/A	85	80	N/A	N/A
Rice Straw	0.20	125	78	N/A	N/A	N/A	N/A	77	84	N/A	N/A
	30.00	78	95	N/A	N/A	N/A	N/A	90	69	N/A	N/A
Rice Hulls	0.20	77	87	N/A	N/A	N/A	N/A	81	71	N/A	N/A
	100.00	78	75	N/A	N/A	N/A	N/A	87	80	N/A	N/A
Rice Bran	0.20	68	83	N/A	N/A	N/A	N/A	84	96	N/A	N/A
	30.00	86	85	N/A	N/A	N/A	N/A	88	98	N/A	N/A
Number		16		0		0		16		0	
Mean		83		N/A		N/A		84		N/A	
Std. Dev.		±13		N/A		N/A		±8		N/A	

**Table 2** Typical Recoveries for Flutolanil Residues in Peanuts

Crop Matrix	Fortification Level (ppm)	Percent Recovery of Flutolanil Analytes									
		M-1		M-2		M-3		M-4		M-7	
Peanut Meats	0.20	78	93	N/A	N/A	90	86	85	92	N/A	N/A
	2.00	85	84	N/A	N/A	86	87	93	Lost <sup>a</sup>	N/A	N/A
Peanut Meal	0.20	83	90	N/A	N/A	93	86	107	92	N/A	N/A
	3.00	101	99	N/A	N/A	100	101	106	104	N/A	N/A
Peanut Hulls	0.20	105	98	N/A	N/A	86	74	81	80	N/A	N/A
	20.00	82	85	N/A	N/A	74	61	81	83	N/A	N/A
Peanut Hay	0.20	90	100	N/A	N/A	101	104	98	98	N/A	N/A
	50.00	99	109	N/A	N/A	109	103	89	101	N/A	N/A
Number		16		0		16		15		0	
Mean		93		N/A		90		93		N/A	
Std. Dev.		±9		N/A		±13		±9		N/A	

<sup>a</sup> Sample spilled and went dry at the rotary evaporation step. Actual recovery was 42%.

**Table 3** Typical Recoveries for Flutolanil Residues in Cattle Tissue<sup>a</sup>

Crop Matrix	Fortification Level (ppm)	Percent Recovery of Flutolanil Analytes									
		M-1		M-2		M-3		M-4		M-7	
Muscle	0.05	---	---	79	---	N/A	N/A	---	---	---	---
	1.00	---	---	85	---	N/A	N/A	---	---	---	---
Liver	0.05	---	---	---	---	N/A	N/A	92	---	---	---
	1.00	---	---	---	---	N/A	N/A	75	---	---	---
Kidney	1.00	---	---	---	---	N/A	N/A	---	---	94	---
	10.00	---	---	---	---	N/A	N/A	---	---	95	---
Fat	0.05	80	---	---	---	N/A	N/A	---	---	---	---
	1.00	91	---	---	---	N/A	N/A	---	---	---	---
Number		2		2		0		4		2	
Mean		86		82		N/A		90		95	
Std. Dev.		±8		±4		N/A		±10		±0.7	

<sup>a</sup> See Reference 2 (Study Au-93R-05 - Registration Reference Flutolanil/R35).

**Table 4** Typical Recoveries for Flutolanil Residues in Milk

Crop Matrix	Fortification Level (ppm)	Percent Recovery of Flutolanil Analytes									
		M-1		M-2		M-3		M-4		M-7	
Milk (Whole)	0.05	73	66	92	88	N/A	N/A	103	89	102	96
	0.20	99	90	109	103	N/A	N/A	103	106	114	116
Number		4		4		0		4		4	
Mean		82		98		N/A		100		107	
Std. Dev.		± 15		± 10		N/A		± 8		± 10	

**Table 5** Typical Recoveries for Flutolanil Residues in Poultry & Eggs<sup>a</sup>

Crop Matrix	Fortification Level (ppm)	Percent Recovery of Flutolanil Analytes									
		M-1		M-2		M-3		M-4		M-7	
Egg (Whole)	0.05	---	---	99	83	N/A	N/A	75	---	---	---
	1.00	---	---	84	95	N/A	N/A	93	---	---	---
Egg (White)	0.05	90	95	---	---	N/A	N/A	---	---	94	---
	1.00	---	---	---	---	N/A	N/A	---	---	122	---
Muscle (Breast)	0.05	---	---	---	---	N/A	N/A	---	---	89	---
	1.00	---	---	---	---	N/A	N/A	---	---	59	---
Muscle (Thigh)	0.05	86	---	---	---	N/A	N/A	---	---	---	---
	1.00	96	---	---	---	N/A	N/A	---	---	---	---
Liver	0.05	---	---	99	---	N/A	N/A	---	---	---	---
	10.00	---	---	62	---	N/A	N/A	---	---	---	---
	10.00	---	---	71	---	N/A	N/A	---	---	---	---
Fat	0.05	---	---	---	---	N/A	N/A	---	---	71	---
	5.00	---	---	---	---	N/A	N/A	---	---	62	---
	5.00	---	---	---	---	N/A	N/A	---	---	72	---
Skin	0.05	80	---	---	---	N/A	N/A	---	---	---	---
	1.00	95	---	---	---	N/A	N/A	---	---	---	---
Number		6		7		0		2		7	
Mean		90		85		N/A		84		81	
Std. Dev.		± 6		± 14		N/A		± 13		± 22	

<sup>a</sup> See Reference 3 (Study AU-93R-06 - Registration Reference Flutolanil/R40)

## 7. DISCUSSION

A set of samples (8 to 12) can be analyzed by one person in approximately two working days (16 hours). On the first day it has been found that the base hydrolysis step is a convenient stopping place. The samples may be left to hydrolyze at the end of the day and turned off by means of a timer. The samples should be well cooled and ready for the acid partition the following morning. Samples are usually ready for GC/MSD analysis by early afternoon the second day.

## 8. TEST SUBSTANCES

The following test substance were used in fortification experiments and for calibration standards (Structural Information may be found in Appendix I)

<u>Material</u>	<u>Repository Log #</u>	<u>Purity</u>
Flutolanil (M-1)	RL 244/91	100%
Flutolanil Metabolite (M-2)	RL 89/90	98.2%
Flutolanil Metabolite (M-3)	RL 90/90	99.7%
Flutolanil Metabolite (M-4)	RL 141/91	99.9%
Flutolanil Metabolite (M-7)	RL 236/91	96.9%
2-TFBA Methyl Ester	RL 404/93	99.5%

## 9. ARCHIVING

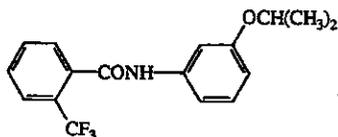
A copy of the protocol, final report, raw data, computer generated listings of raw data and supporting documentation are maintained in the Archives of the AgrEvo Research Center, 703 NOR-AM Road, Pikeville, NC 27863

## 10. REFERENCES

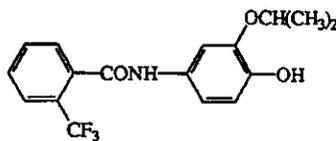
1. Bowman, M. C. "Method for The Determination of Total Residues of Flutolanil in Rice and Rice Bran" (Mar. 1992). MRID: 42606609  
Registration Reference: Flutolanil/R19.
2. Campbell, J. K., "Residues of Flutolanil in the Meat and Milk of Cattle, Dosed with Flutolanil at 1, 3, and 10 Times the Estimated Maximum Daily Intake for 28 Days, USA, 1993 NOR-AM Report AU-93R-05, (Jan. 1994). MRID: 43175808  
Registration Reference: Flutolanil/R35

3. Campbell, J. K., "Residues of Flutolanil in the Meat and Eggs of Hens, Dosed with Flutolanil at 1, 3, and 10 Times the Estimated Maximum Daily Intake for 28 Days, USA, 1993 NOR-AM Report AU-93R-06, (Jan. 1994). MRID 43175805  
Registration Reference Flutolanil/R40

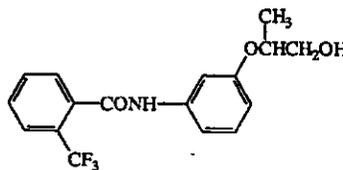
**Appendix I Structural Data**



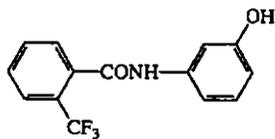
Flutolanil (M-1)



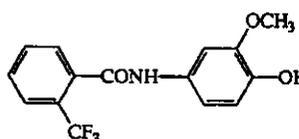
M-2 Metabolite



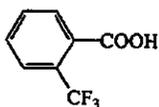
M-3 Metabolite



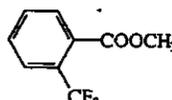
M-4 Metabolite



M-7 Metabolite



2-TFBA



2-TFBA Me-ester

Compound Identification	Chemical Name	Molecular Weight	Conversion Factor
Flutolanil (M-1)	3'-Isopropoxy-2-(trifluoromethyl)benzanilide	323.3	1.584
M-2	4'-hydroxy-3'-isopropoxy-2-(trifluoromethyl)benzanilide	339.3	1.662
M-3	3'-(1-hydroxyethyl)ethoxy-2-(trifluoromethyl)benzanilide	339.3	1.662
M-4	3'-hydroxy-2-(trifluoromethyl)benzanilide	281.2	1.378
M-7	4'-hydroxy-3'-methoxy-2-(trifluoromethyl)benzanilide	311.3	1.525
2-TFBA acid	2-(trifluoromethyl)benzoic acid	190.1	0.9314
2-TFBA-Me	2-(trifluoromethyl)benzoic acid methyl ester	204.1	1.0000

Appendix II GC/MSD Operating Conditions

These should be used as a guide. Adjustments may be made to the oven temperature program as necessary to provide satisfactory chromatography or separation of the analytical targets from any co-eluting material.

Gas Chromatograph

**Instrument:** Hewlett-Packard 5890A gas chromatograph with capillary split/splitless inlet operated in the splitless mode, containing a 4 mm i.d. Cyclosplitter liner (Restek Corporation Cat. No. 20706)

**Column:** Fused silica capillary DB-17 bonded phase 30 m x 0.25 mm i.d., 0.25  $\mu$ m film thickness (J&W 122-1732)

**Carrier Gas:** Helium (Ultra pure 99.999%).  
Head pressure set to 10 psi.

**Split Flow:** 60 mL/min.

**Septum Purge:**  $\approx$  3.5 mL/min.

**Temperatures:** Injection Port - 250 °C  
Detector - 250 °C

Oven - 90 °C hold for one minute, then program at 20 °C/min. to 150 °C, then 20 °C/mm to 250 °C for 4.0 minutes.

**Retention Times:** 2-TFBA Me-ester = 4.4 minutes

Appendix II (continued)Injection

**Autosampler:** Hewlett-Packard 7673A  
2- $\mu$ L injection volume, residence time less than one second  
(fast injection)

**Detector:** Hewlett-Packard 5970B mass selective detector (MSD)  
operated in the selected ion mode (SIM)

**Purge Valve:** Initial Value - OFF  
On Time - 0.75 minutes  
Off Time - 0.00 minutes  
Manual tune with ions 131, 169, 219  
Solvent delay - 3.5 minutes  
Ions - 145 m/e and 173 m/e  
Dwell - 100 milliseconds  
4 1 cycles/second

**Electron Multiplier**  
Voltage 200 mV above manual tune  
Low mass resolution

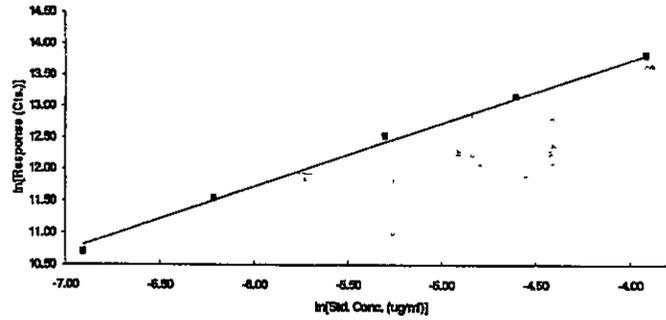
**Integration Parameters:**

Init. Width	- 0.05
Init. Thresh	- 11
Init. Reject.	- 100.00
Shoulders	- off
Baseline Valley	- on, 0.001 minutes
Peak Width	- 0.040, 2.4 minutes
Integrator	- off, 0.001 minutes - on, 4.300 minutes - off, 4.600 minutes

**Appendix III Typical Calibration Data**

Standard Calibration Curve:		2-TFBA Me-ester		
Retention Time (min.)	Standard Solution Reference Number	Standard Conc. (µg/mL)	Peak Area (Cts.)	Statistical Data
4.45	AU/59/04	0.001	44834	Slope = 1.0051 Y-int. = 17.7548 Corr = 0.9987
4.45	AU/59/05	0.002	102954	
4.45	AU/59/06	0.005	275644	
4.45	AU/59/07	0.010	518971	
4.45	AU/59/08	0.020	1014702	
4.45	AU/59/09	0.050	2354973	

STANDARD CALIBRATION CURVE (2-TFBA Me)



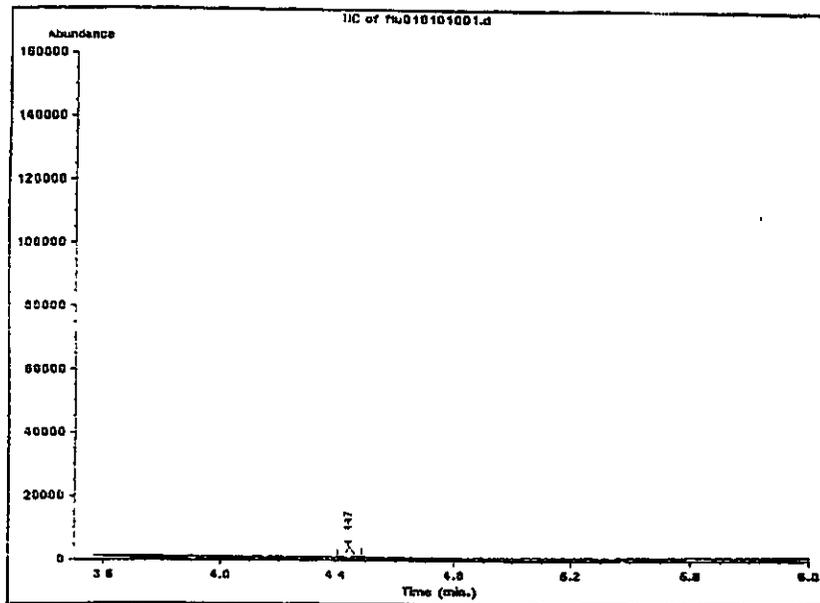
**Appendix IV Representative Chromatograms**

Figure 1	0.001 µg/mL 2-TFBA Me-ester Standard.....	32
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Appendix IV (continued)

Figure 1 0.001 µg/mL 2-TFBA Me-ester Standard

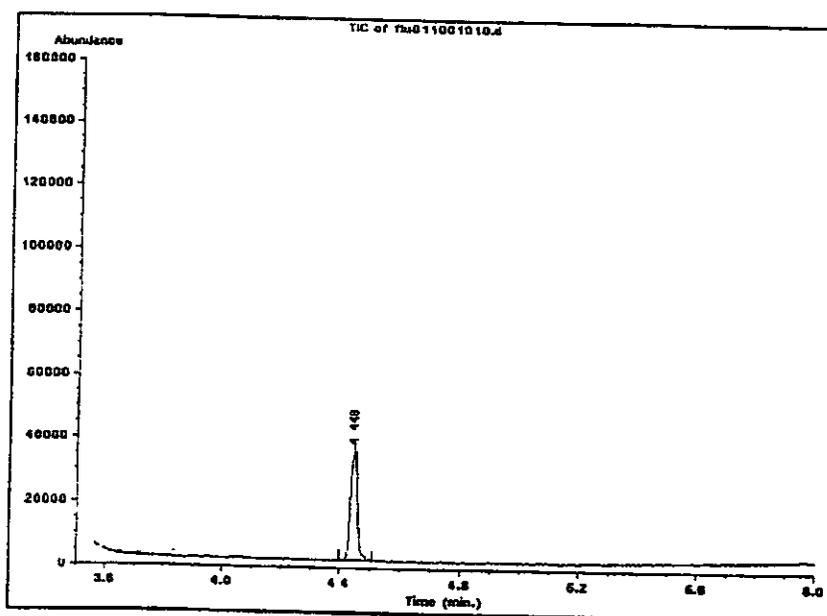
Sample Description: AU/59/04 0.001 µg/mL 2-TFBA Me-ester Standard				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.45	2-TFBA Me-ester	44834	N/A	N/A



Appendix IV (continued)

Figure 2 0.010 µg/mL 2-TFBA Me-ester Standard

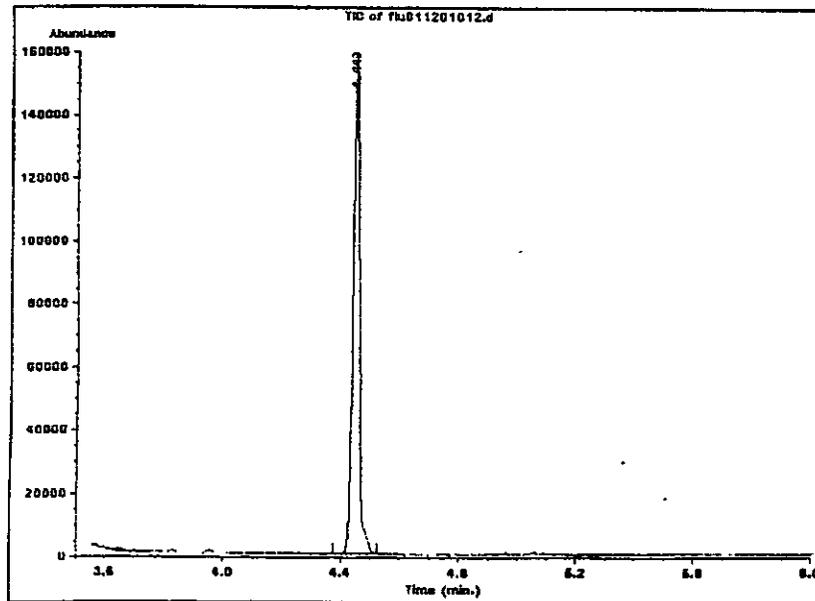
Sample Description: AU/59/07 0.010 µg/mL 2-TFBA Me-ester Standard				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.45	2-TFBA Me-ester	518971	N/A	N/A



Appendix IV (continued)

**Figure 3** 0.050 µg/mL 2-TFBA Me-ester Standard

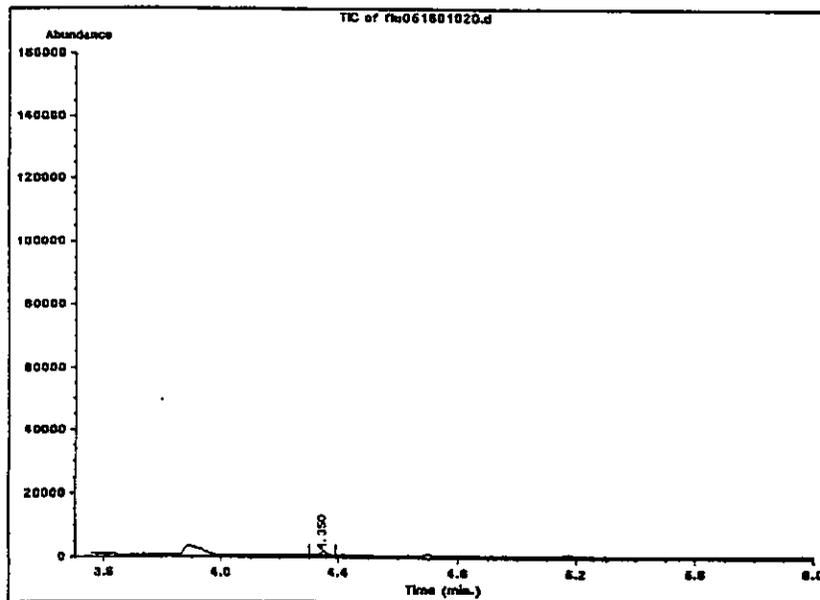
Sample Description: AU/59/09 0.050 µg/mL 2-TFBA Me-ester Standard				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.45	2-TFBA Me-ester	2354973	N/A	N/A



Appendix IV (continued)

Figure 4 Rice Grain Control

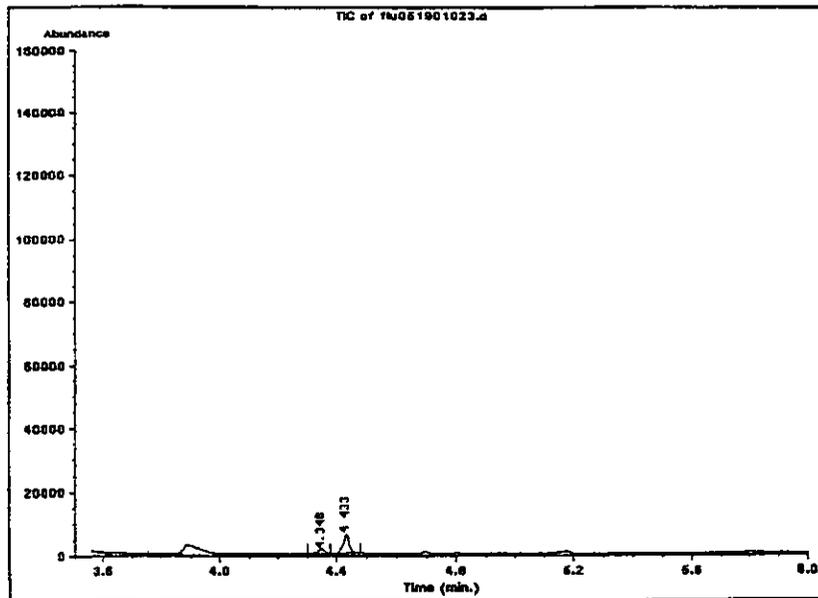
Sample Description: 109-078 01 Rice Grain Control				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
---	2-TFBA Me-ester	0	N D.	N/A



Appendix IV (continued)

**Figure 5** Rice Grain Fortified with M-1 at 0.20 ppm

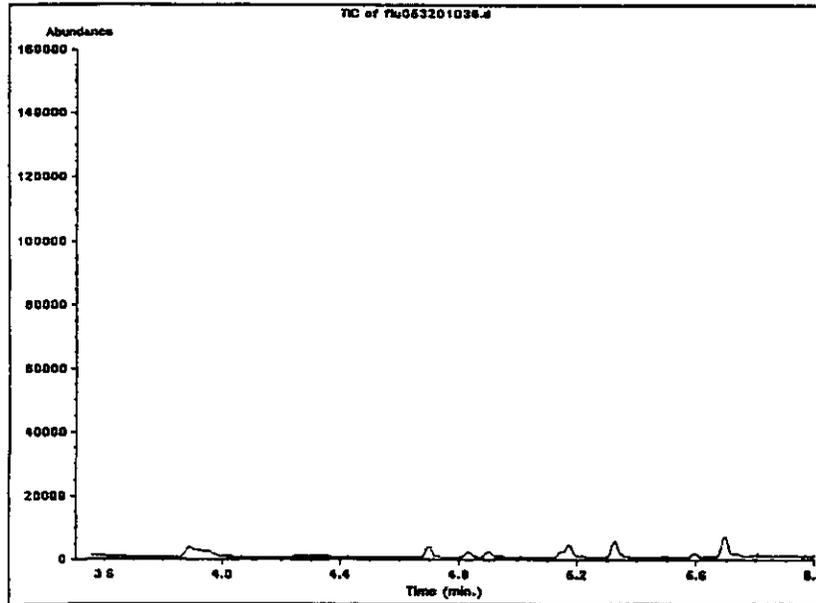
Sample Description:		109-078 01 Rice Grain Fortified with M-1 at 0.20 ppm		
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.43	2-TFBA Me-ester	90406	0.16	80



Appendix IV (continued)

Figure 6 Rice Bran Control

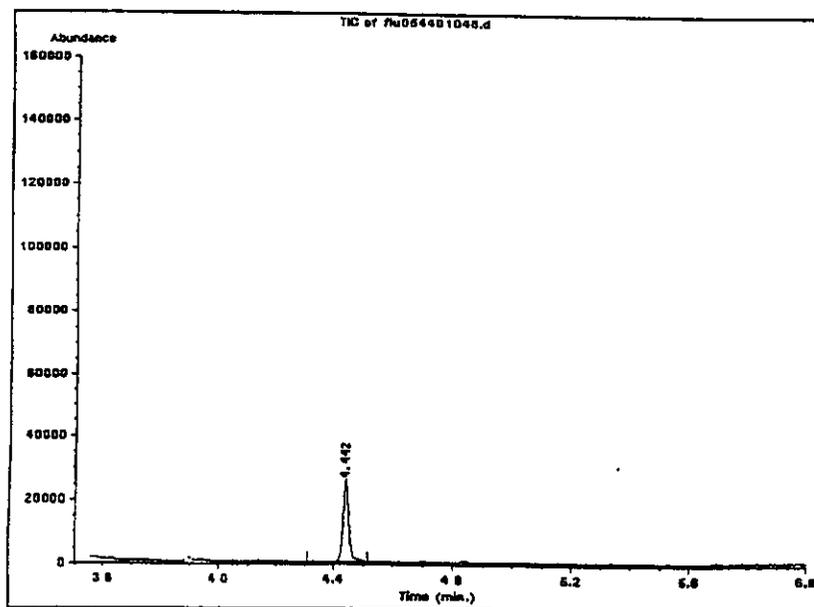
Sample Description: 135-122.01 Rice Bran Control				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
---	2-TFBA Me-ester	0	N.D.	N/A



Appendix IV (continued)

Figure 7 Rice Bran Control Fortified with M-4 at 30.00 ppm

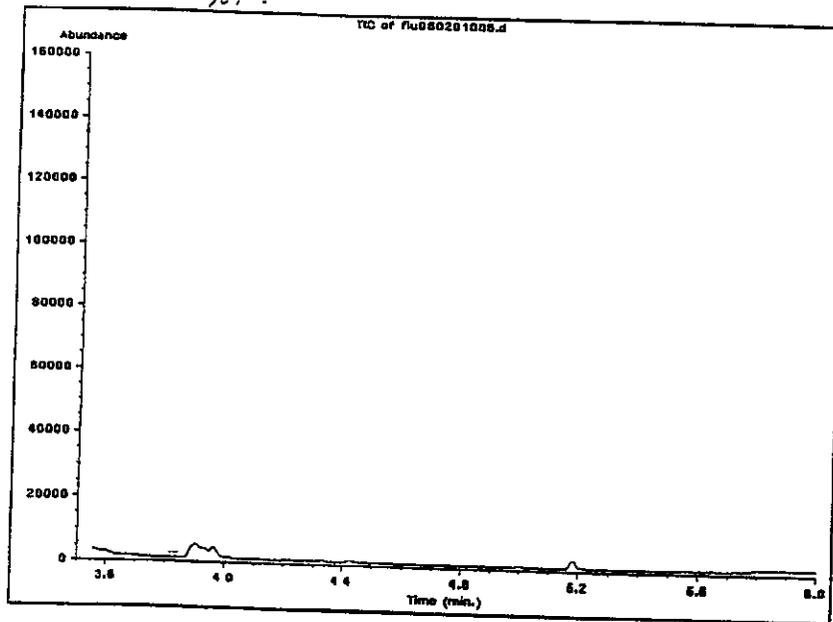
Sample Description:		135-122.01 Rice Bran Fortified with M-4 at 30.00 ppm - Diluted 1-50 mL		
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.44	2-TFBA Me-ester	365973	29.52	98



Appendix IV (continued)

**Figure 8** Peanut Hulls Control

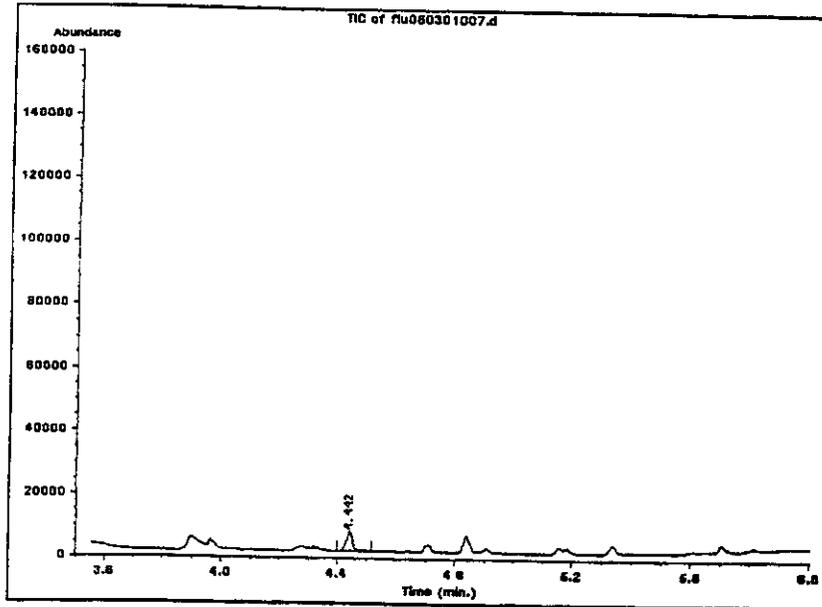
Sample Description: 109-075 03 Peanut Hulls Control				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
---	2-TFBA Me-ester	0	N D.	N/A



Appendix IV (continued)

**Figure 9** Peanut Hulls Control Fortified with M-3 at 0.20 ppm

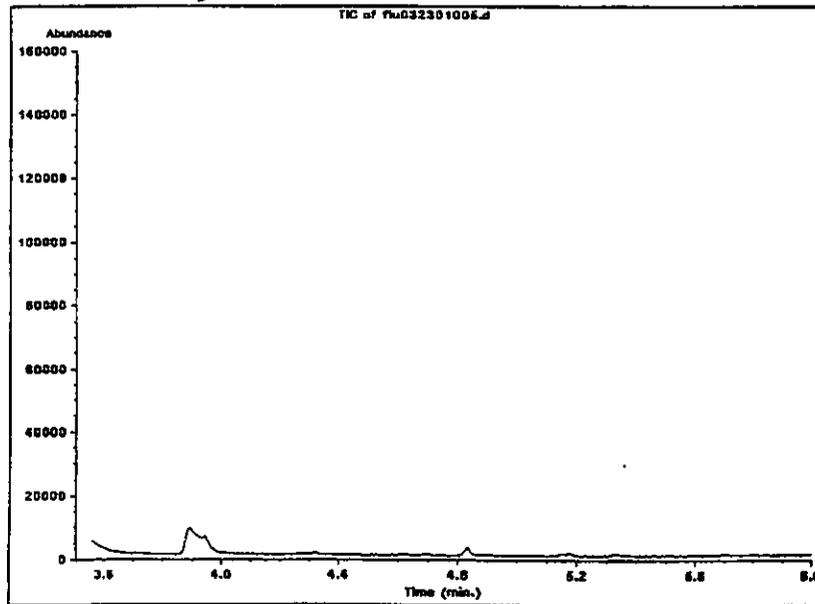
<b>Sample Description:</b>		109-075.03 Peanut Hulls Control Fortified with M-3 at 0.20 ppm		
Retention Time (min)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.44	2-TFBA Me-ester	100948	0.17	86%



Appendix IV (continued)

Figure 10 Peanut Meats Control

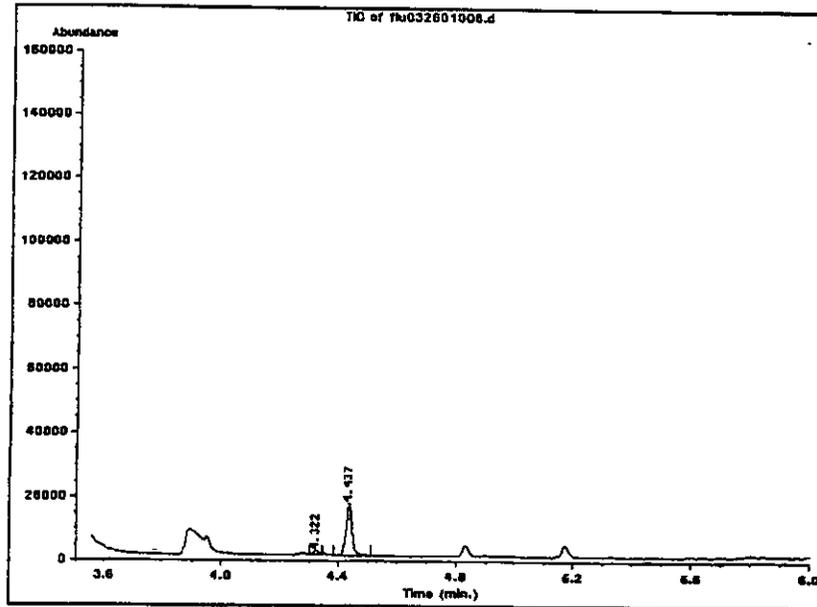
Sample Description: 134-186 01A Peanut Meats Control				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
---	2-TFBA Me-ester	0	N D.	N/A



Appendix IV (continued)

Figure 11 Peanut Meats Control Fortified with M-1 at 0.20 ppm.

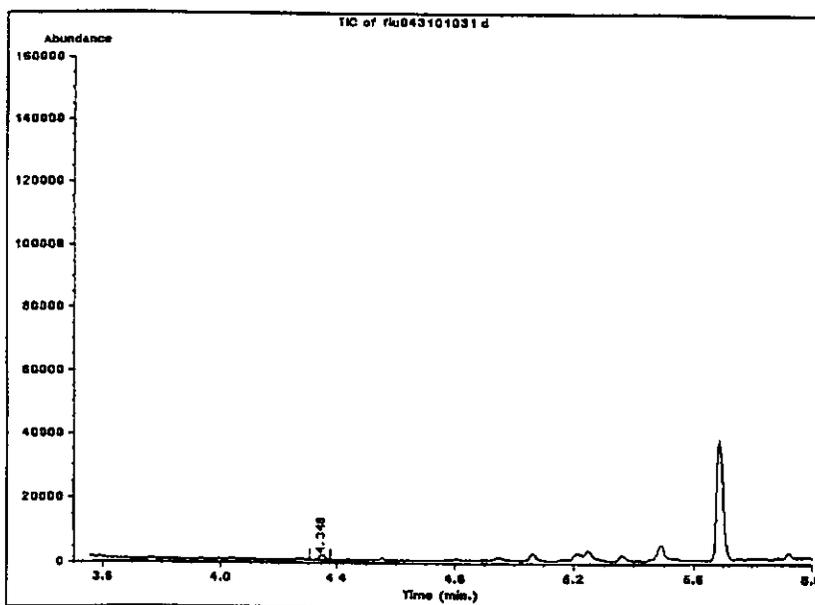
Sample Description:		134-186 01A Peanut Meats Control Fortified with M-1 at 0.20 ppm		
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.44	2-TFBA Me-ester	240649	0.19	93



Appendix IV (continued)

Figure 12 Whole Milk Control

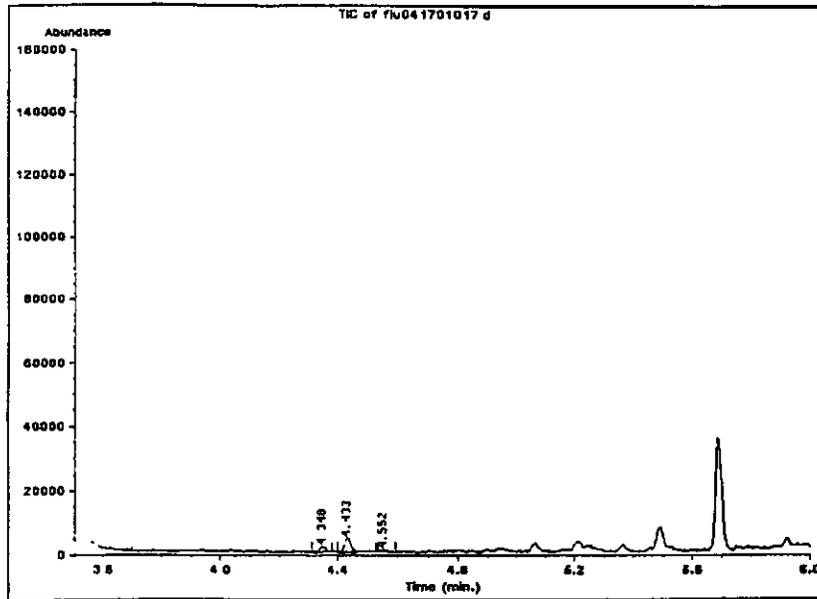
Sample Description: 127-042.06 Whole Milk Control with Amino SPE Cleanup				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
----	2-TFBA Me-ester	0	N.D.	N/A



Appendix IV (continued)

Figure 13 Whole Milk Control Fortified with M-2 at 0.05 ppm

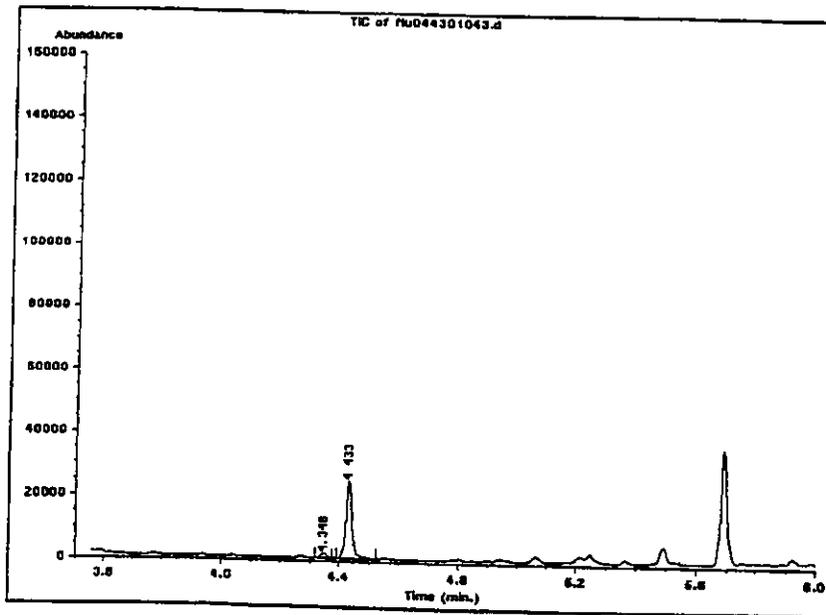
<b>Sample Description:</b>		127-042.06 Whole Milk Fortified with M-2 at 0.05 ppm with Amino SPE Cleanup		
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.43	2-TFBA Me-ester	73085	0.046	92



Appendix IV (continued)

Figure 14 Whole Milk Control Fortified with M-7 at 0.20 ppm

Sample Description: 127-042.06 Whole Milk Fortified with M-7 at 0.20 ppm				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.43	2-TFBA Me-ester	360996	0.23	116%

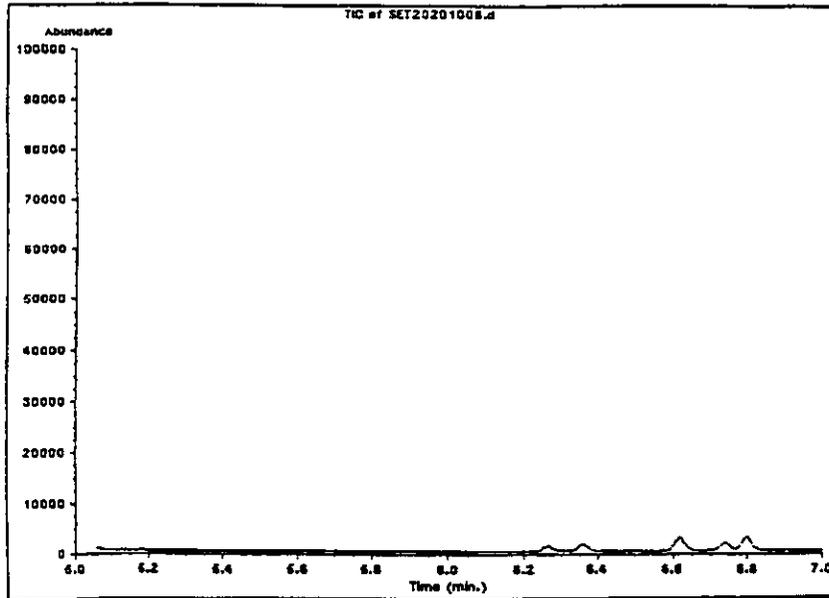


Appendix IV (continued)

Figure 15 Liver Control<sup>a</sup>

Sample Description: 109-166 95 Liver Control				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
----	2-TFBA Me-ester	0	N D	N/A

<sup>a</sup> See Reference 2

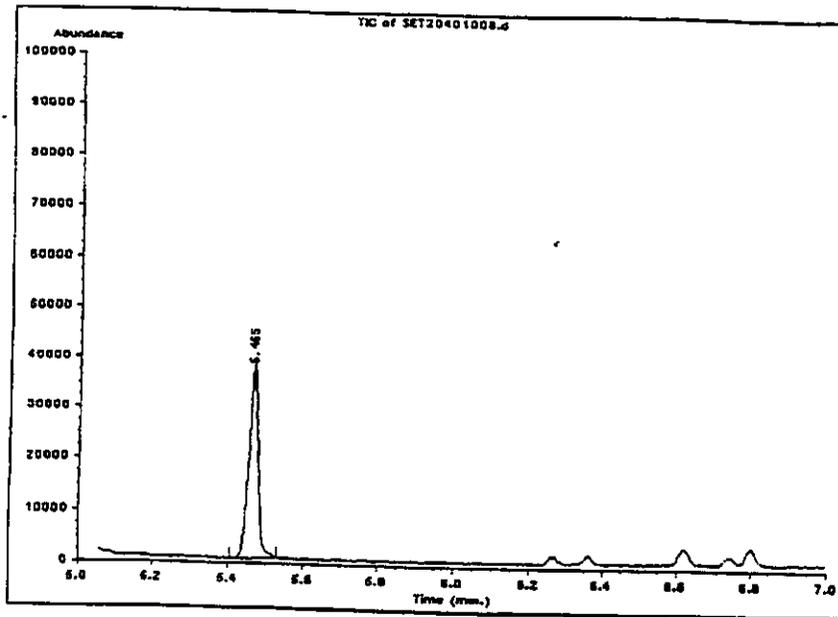


Appendix IV (continued)

Figure 16 Liver Control Fortified with M-4 at 1.00 ppm<sup>a</sup>

Sample Description: 109-186 95 Control Liver Fortified with M-4 at 1.00 ppm				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
5.47	2-TFBA Me-ester	654916	0.75	75%

<sup>a</sup> See Reference 2

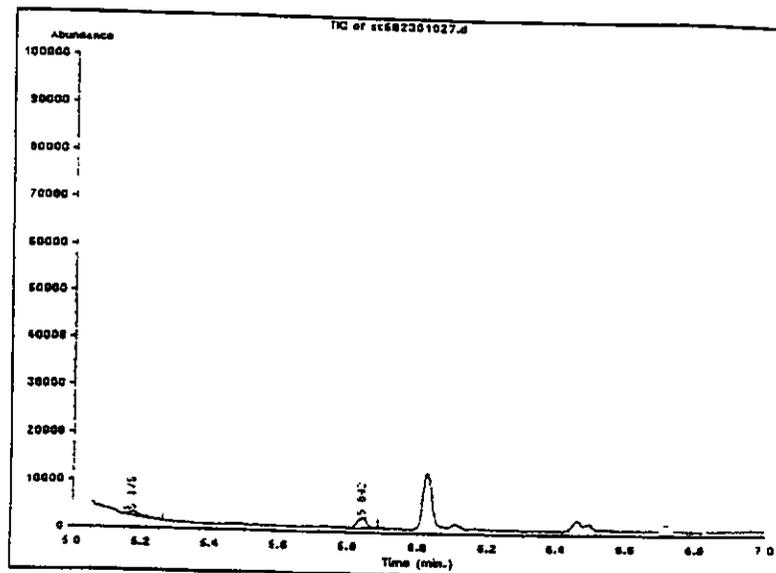


Appendix IV (continued)

Figure 17 Egg Yolk Control

Sample Description: 109-181.50 Egg yolk Control				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
---	2-TFBA Me-ester	0	N D	N/A

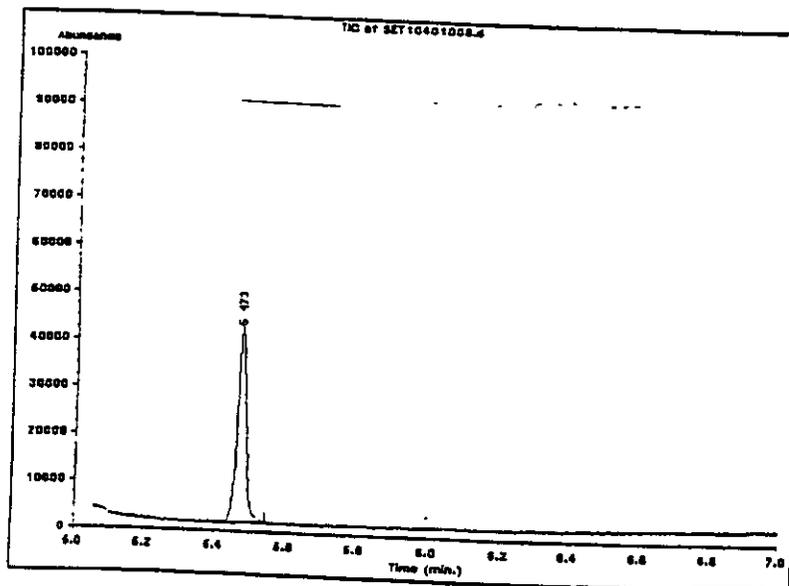
<sup>a</sup> See Reference 3.



Appendix IV (continued)

**Figure 18** Control Egg Sample Fortified with M-2 at 1.00 ppm

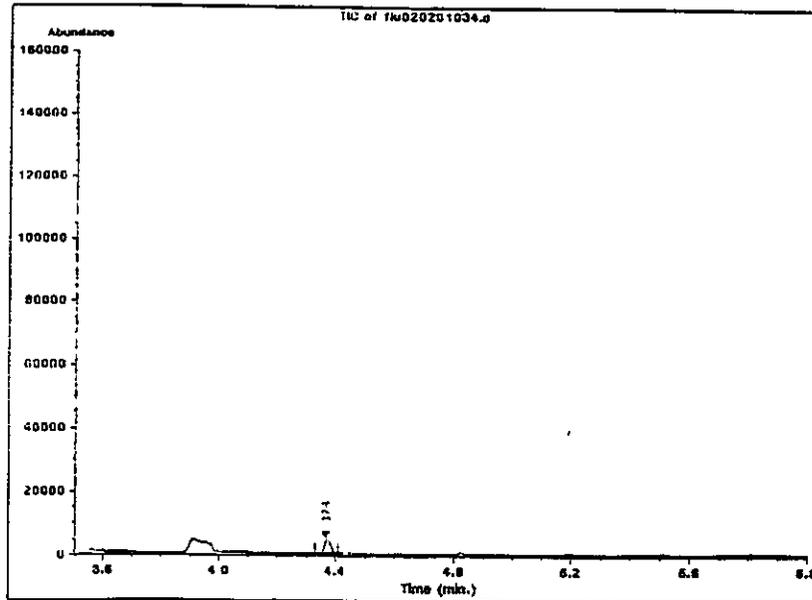
<b>Sample Description:</b>		109-151.54 Control Egg Sample Fortified with M-2 at 1.00 ppm		
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
5.47	2-TFBA Me-ester	737545	0.84	84%



Appendix IV (continued)

Figure 19 Reagent Blank

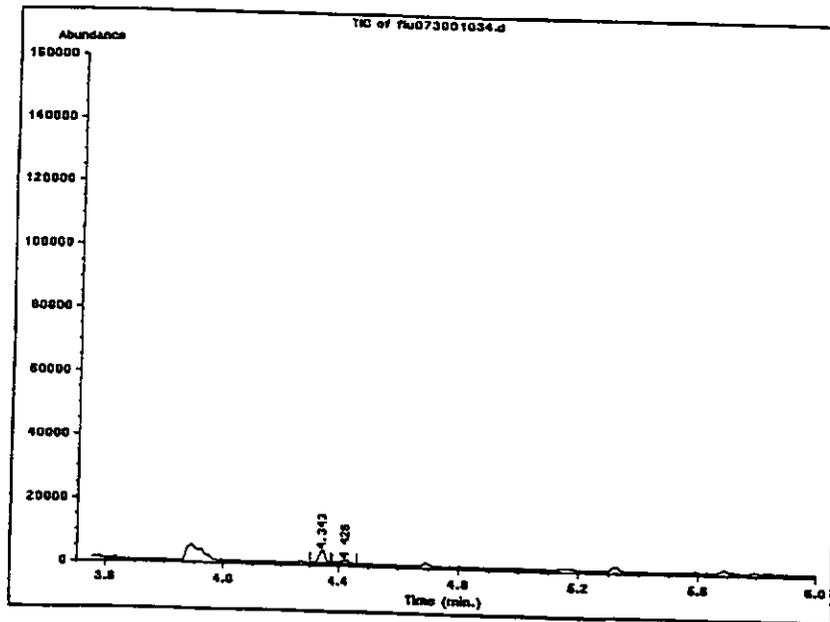
Sample Description:		Reagent Blank		
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
---	2-TFBA Me-ester	0	N D	N/A



Appendix IV (continued)

Figure 20 Rice Straw Control

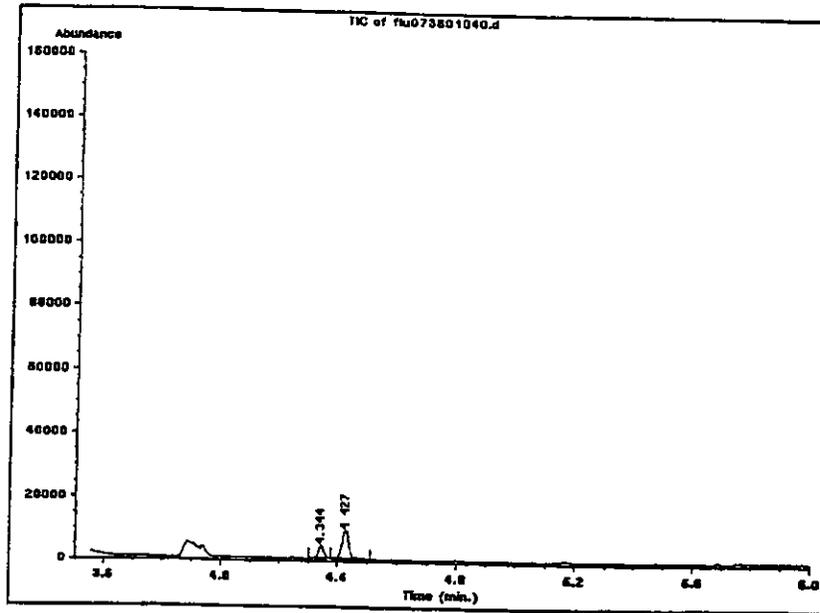
Sample Description: 109-168.02 Rice Straw Control				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.43	2-TFBA Me-ester	24165	0.02	N/A



Appendix IV (continued)

Figure 21 Rice Straw Control Fortified with M-4 at 0.20 ppm

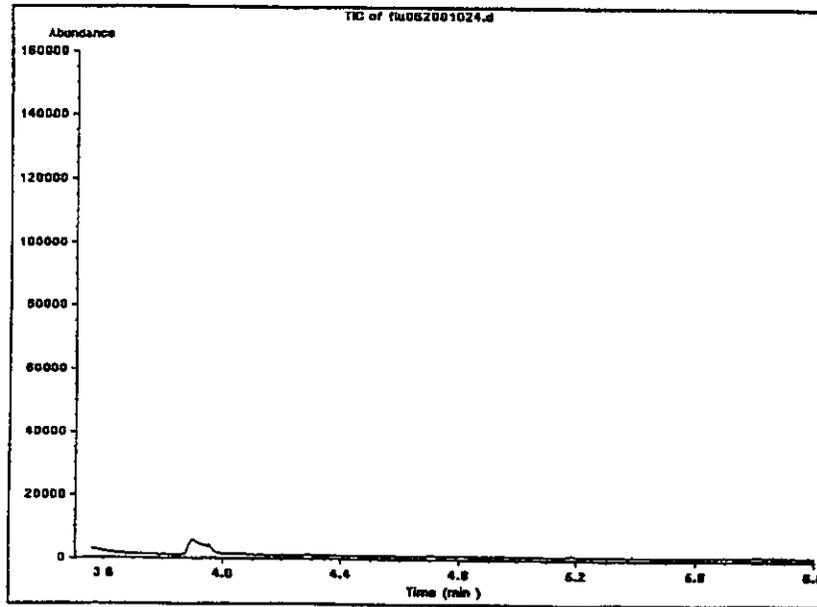
<b>Sample Description:</b>		109-168 02 Rice Straw Control Fortified with M-4 at 0.20 ppm		
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.43	2-TFBA Me-ester	149192	0.17	84%



Appendix IV (continued)

Figure 22 Rice Hull Control

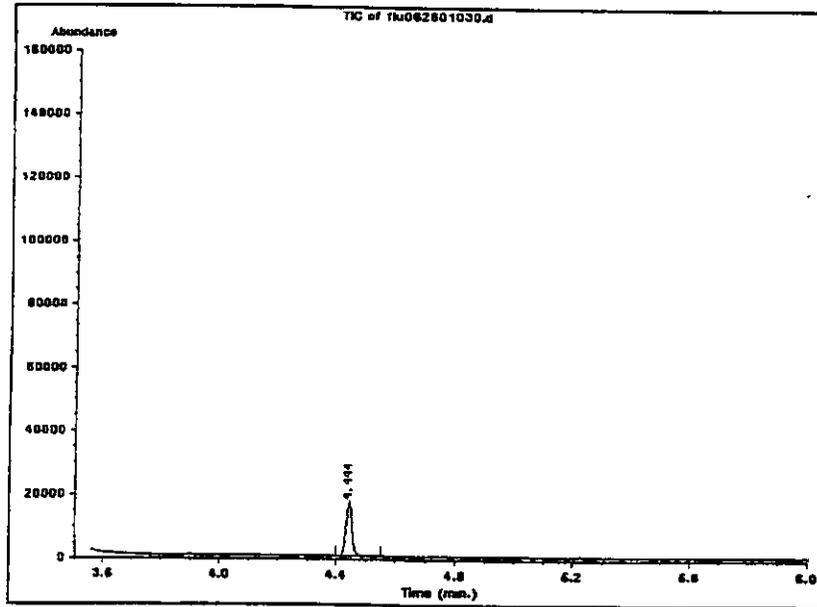
Sample Description: 108-071.02 Rice Hull Control				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
---	2-TFBA Me-ester	0	N.D	N/A



Appendix IV (continued)

Figure 23 Rice Hull Control Fortified with M-1 at 100 ppm

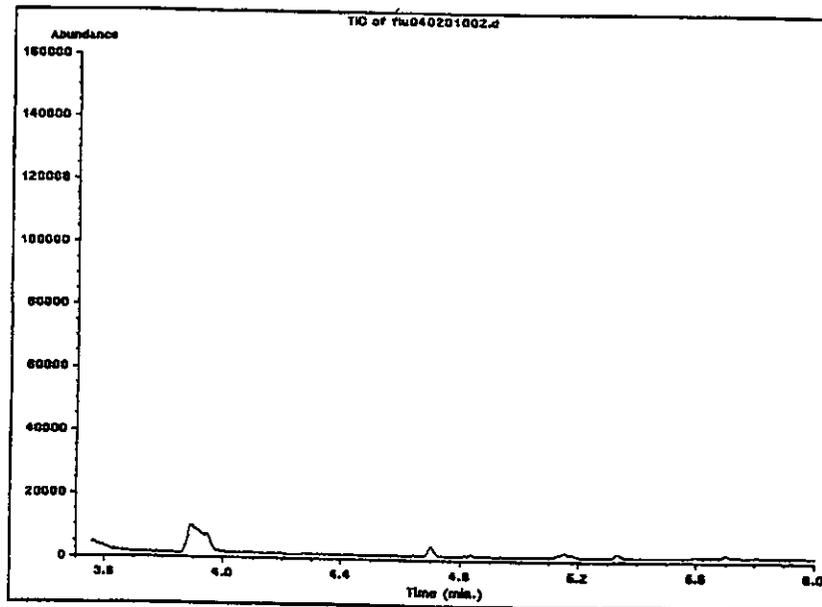
<b>Sample Description:</b>		108-071.02 Rice Hull Control Fortified with M-4 at 100 ppm - Diluted 1-200 ml.		
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.44	2-TFBA Me-ester	246449	78.24	78%



Appendix IV (continued)

Figure 24 Peanut Meal/Presscake Control

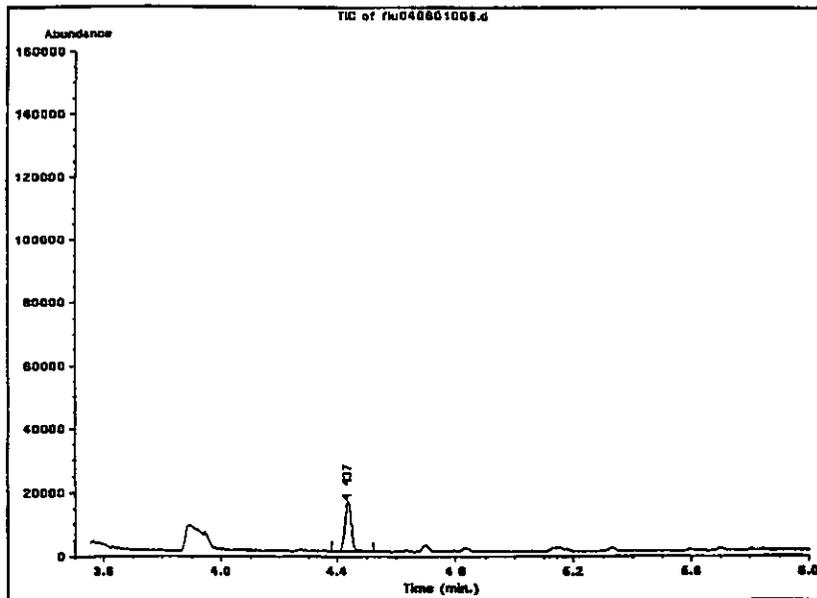
Sample Description: 135-001.02 Peanut Meal/Presscake Control				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
---	2-TFBA Me-ester	0	N D.	N/A



Appendix IV (continued)

**Figure 25** Peanut Meal/Presscake Fortified with M-3 at 0.20 ppm

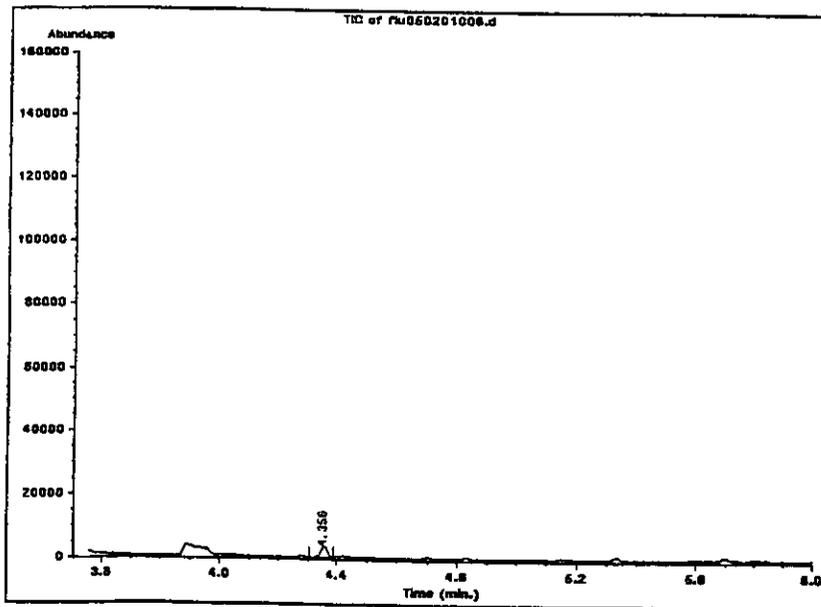
<b>Sample Description:</b>		135-001.02 Peanut Meal/Presscake Control Fortified with M-3 at 0.20 ppm		
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.44	2-TFBA Me-ester	232694	0.19	93%



Appendix IV (continued)

Figure 26 Peanut Hay Control

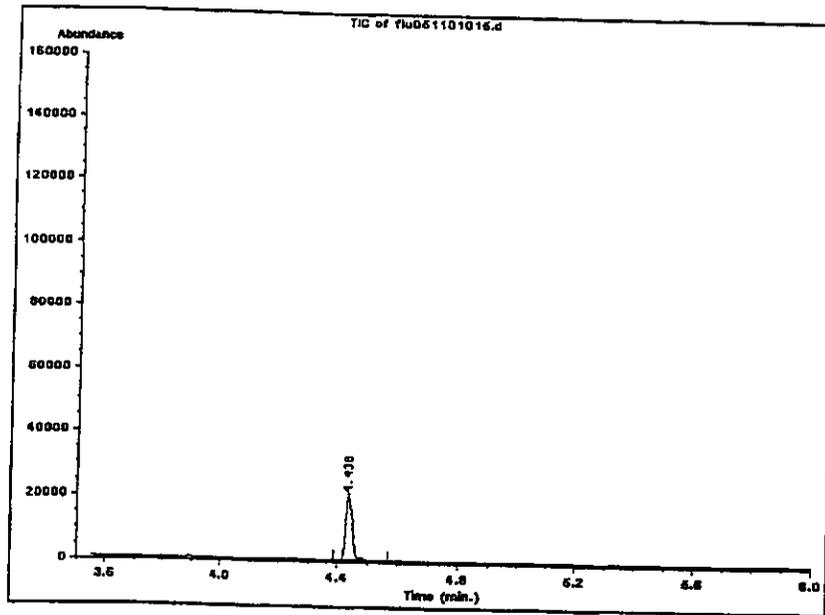
Sample Description: 109-075 02 Peanut Hay Control				
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
---	2-TFBA Me-ester	0	N D.	N/A



Appendix IV (continued)

Figure 27 Peanut Hay Control Fortified with M-4 at 50.0 ppm

Sample Description:		109-075.02 Peanut Hay Control Fortified at 50 ppm with M-4 Diluted 1-100 mL		
Retention Time (min.)	Analyte of Interest	Peak Area Cts.	Residue Conc. (ppm)	Analytical Recovery (%)
4.44	2-TFBA Me-ester	302245	50.66	101%



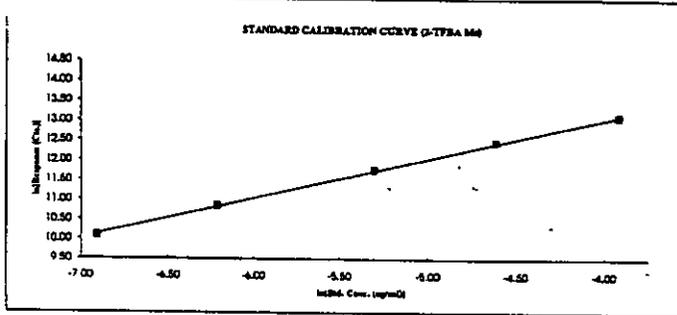
Appendix IV (continued)

Figure 28 Typical Calculation Spreadsheet for an Analytical Run

Agrive Chemical Company Residue Chemistry Calculation Spreadsheet

Study No. AU952M

Standard Solu. Ref. #	Standard Conc. (ug/ml)	Response (Counts)	In Std. Conc.	In Counts	Calculated Line	Standard Calibration Curve		
						Slope	Y Intercept	Corr. Coeff.
AL/5904	0.0010	23898	-4.91	10.08	10.13	1.0029	17.0231	0.9998
AL/5905	0.0020	31056	-4.21	10.84	10.82			
AL/5906	0.0050	128740	-5.20	11.77	11.74			
AL/5907	0.0100	267777	-4.64	12.47	12.45			
AL/5908	0.0200	497848	1.91	13.12	13.13			
AL/5909	0.0500	1237280	1.00	14.02	14.05			



Agrive Sample #	Sample Weight (g)	Amount Analyte Added (ug)	Recovery Level (ppm)	Response (Counts)	In(Counts)	Det. Conc. of Analyte (ppm)	Crop / Soil Rate (g/ml)	Determined Res. (ppm)	Percent Recovery
109-875.02 CK	20.0	0.000	0.00	0	NA	N.D.	0.0220	N.D.	NA
109-875.02 (M-1)	20.0	4.000	0.20	90809	11.42	0.00363	0.0220	0.16	90%
109-875.02 (M-1)	20.0	4.000	0.20	101542	11.33	0.00404	0.0220	0.20	100%
109-875.02 (M-2)	20.0	4.000	0.20	97423	11.49	0.00379	0.0220	0.20	101%
109-875.02 (M-2)	20.0	4.000	0.20	100109	11.31	0.00398	0.0220	0.21	104%
109-875.02 (M-4)	20.0	4.000	0.20	114150	11.60	0.00456	0.0220	0.20	98%
109-875.02 (M-4)	20.0	4.000	0.20	113718	11.64	0.00453	0.0220	0.20	98%
109-875.02 Test	1.0	0.300	0.20	223452	12.32	0.00891	0.0400	0.21	104%

Percent Analyte	Conversion Factor
Phenol (M 1)	1.5840
M-2 Metabolite	1.6620
M-3 Metabolite	1.6620
M-4 Metabolite	1.3780
M 7 Metabolite	1.3250
2-TPBA	0.9214

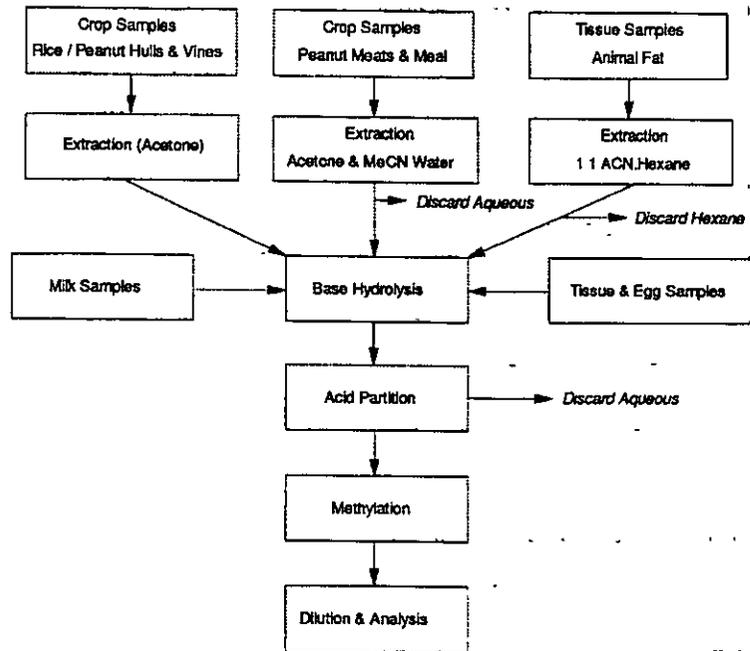
Note: Test includes a surrogates check by fortifying with 2-TPBA. Data not reported as a recovery.

Analyte Compliance Date: 4-Nov-83

Inspector: GC #6

Analyte Signature: *jsd me*

Appendix V Flow Diagram of Method AU/02/95



**EPA ADDENDUM For Residue Analytical Method  
PP# 6F4693/6H5749**

- 1)     ACB made slight modifications to the GC/MSD instrument parameters  
       The parameters for the GC/MSD were  
       HP Model 5973 GC/MSD

       Column - J&W DB-17 MS, 30 m x 0.25 mm ID, 0.25 um film thickness  
       He Flow - 1.0 mL/min  
       Transfer Line Temperature - 280°C  
       Injection Temperature - 250°C

       Temperature Program - 90°C for 1.0 min., rate 20°C/min to 150°C, then 20°C/min to 250°C  
       for 4.0 min

       Injection volume - 2 uL

       Ions monitored - m/z 173 for quantitation and m/z 145 for confirmation, dwell time set for 200  
       milliseconds.

- 2)     In the methylation part of the procedure (Section 5.7 of procedure, step 1), the method  
       called for adding 100 uL of methyl iodide and 50 uL tetrabutyl ammonium hydroxide solution  
       to the sample and incubating the sample for 2 hr. at 40° C. With this procedure, as written,  
       ACB experienced low recoveries: 12-71 % for M-4 in peanut hay and 24-56 % flutolanil in  
       peanut nut meat.

       Because of the above, ACB made the following modifications to the procedure listed in  
       Section 5.7, step 1. Instead of adding 100 uL methyl iodide, **250 uL** methyl iodide were  
       added. Also the samples were incubated for 2 hr. at **80° C**, instead of 40° C.