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Analytical Method

Department: Residue Chemistry

Date: August 11, 1997

RAM Number: BF/11/97

Title: The Determination of Residues of Buprofezin in Beef Tissues and Milk via Solid-Phase Extraction and Gas Chromatography with MS and Nitrogen Phosphorus Detection

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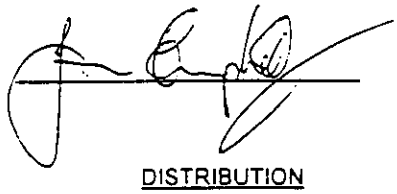
Signed:  Date: 12-Aug-97

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Residue Methods Book

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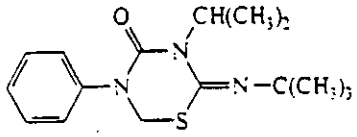
1. SCOPE

This method is suitable for the determination of extractable residues of buprofezin (BF01) in beef tissues (liver, kidney, muscle and/or fat) and milk. The limit of quantitation for this procedure has been set at 0.05 ppm for beef tissues and 0.01 ppm for milk.



Common Name:	Buprofezin (BF01)
C.A. Name:	2-[(1,1-dimethylethyl)imino]tetrahydro-3-(1-methylethyl)-5-phenyl-4H-1,3,5-thiadiazin-4-one
IUPAC Name:	2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one
CAS Reg. No.:	[69327-76-0]

2. PRINCIPLE

Extractable residues of buprofezin are removed from beef tissues (liver, kidney, muscle, and/or fat) and milk by blending with acetonitrile. The resulting extract is filtered, concentrated, diluted with 1M hydrochloric acid, and extracted with dichloromethane. Beef muscle and fat samples are washed with hexane prior to the dichloromethane extraction. The dichloromethane extract is dried through a sodium sulfate pad and rotary evaporated to dryness. Buprofezin residues are reconstituted in toluene, cleaned up by solid-phase extraction (spe) on aminopropyl columns. Tissue and milk samples are quantified by GC/MSD and GC/NPD, respectively.

3. APPARATUS

Use as a guide; equivalents may be substituted.

- Autosampler vials, Class A glass, crimp top, Hewlett Packard
- Blender, Sorvall Omni-Mixer, Model 17105, Omni International
- Blender Blade Assembly, Omni International
- Blending jars (any pint-size Mason jars)
- Boiling flasks, 125- and 500-mL
- Büchi Rotovapor RE-124
- Büchi Waterbath B-481
- Büchner funnels (9 cm) and filter adapters (24/40)

- Bulb, The Safety, Bel Art Products F-37888
- Class A volumetric pipets (0.5-, 1.0-, 2.0-, 4.0-, 5.0-, 6.0-, and 10.0-mL)
- Disposable glass pipets, 5-mL
- Fiber glass sliver (glass wool), 8 micron, Pyrex
- Fused silica megabore column, DB-1, 15 m x 0.53 mm i.d., 1.5-µm film thickness, J & W Scientific
- Fused silica megabore column, DB-5MS, 30 m x 0.25 mm i.d., 0.25-µm film thickness, J & W Scientific
- Glass fiber filter paper, Whatman 934-AH
- Glass powder funnels, long-stem, 75-mm
- Glass stoppers, 24/40, Pyrex
- Graduated cylinders, 50- and 1000-mL, TD
- Hewlett-Packard 5890A gas chromatograph with capillary split/splitless inlet and nitrogen phosphorus detector equipped with a Model 7673A autosampler
- Finnigan GCQ gas chromatograph with capillary split/splitless inlet and a 4.0 mm i.d. split sleeve with wool (Restek Corp. Cat. No. 20782)
- Lab spoons
- Micropipettes, 250-µL
- Separatory funnels, 250-mL, with stoppers
- Solid-phase extraction columns, Bakerbond spe™ aminopropyl (500 mg, 3-mL), J. T. Baker
- Transfer Pasteur pipets, flint glass, 5.75" and 9"
- Volumetric flasks, 50- and 100-mL

4. REAGENTS

All solvents should be pesticide grade or better. Equivalents may be substituted.

- Acetone, HPLC grade
- Acetonitrile, pesticide grade
- Analytical standards of buprofezin (BF01) of known purity
- Dichloromethane, HPLC grade
- Hexane, pesticide grade
- Hydrochloric Acid, 1.0 M solution in deionized water
- Sodium Sulfate, granular anhydrous, ACS reagent
- Toluene, HPLC grade
- Water, deionized

5. PROCEDURE

5.1 Extraction

1. Weigh 25 grams of a representative beef tissue (liver, kidney, muscle, and/or fat) and milk (50 grams) sample into a blending jar. Fortify the recovery samples at this point with buprofezin (see Step 2 of Section 6.3).

2. Add 150 mL of acetonitrile to the sample, and blend at a medium speed for 3 - 5 minutes.

3. Vacuum filter the extract through a Büchner funnel (lined with Whatman 934-AH filter paper) into a 500-mL boiling flask.

4. Rinse the blending jar with 50 mL of acetonitrile. Vacuum filter the rinse into the 500-mL boiling flask.

5. Rotary evaporate the combined tissue extract to a volume of approximately 40 - 50 mL , and evaporate the milk extract to near dryness under reduced pressure at 40 °C (± 5 °C). (Note: Solvent bubbling will occur, necessitating close monitoring during evaporation.)

6. Add 50 mL of 1.0 M hydrochloric acid to the boiling flask. Transfer the resulting acidified extract to a 250-mL separatory funnel. Proceed to Section 5.2 for beef muscle and fat extracts. Proceed to Section 5.3 for milk and all other beef tissue extracts.

5.2 Hexane Wash (for beef muscle and fat only)

Wash the acidified extract from Step 6 of Section 5.1 with hexane (2 x 50 mL). (Note: The requisite hexane volumes should be added to the original boiling flask as a rinse prior to addition to the separatory funnel.) For each wash, shake for approximately 30 seconds. Allow the layers to separate. Drain the aqueous (lower) layer into the original 500-mL boiling flask. Discard the hexane wash.

5.3 Dichloromethane Extraction

1. Extract the acidified aqueous solution from Section 5.2 and/or Step 6 of Section 5.1 with dichloromethane (1 x 50 mL followed by 1 x 25 mL for beef tissue) and (3 x 25 mL for milk). (Note: The requisite dichloromethane volumes should be used to rinse the boiling flask prior to addition to the separatory funnel.)

For each extraction, shake for approximately 30 seconds. Allow the layers to separate. Drain the dichloromethane (lower) layer through a sodium sulfate pad (approximately 90 g of sodium sulfate in a glass wool-plugged powder funnel) into a 500-mL boiling flask. Rinse the sulfate pad with 25 mL of dichloromethane after the final extraction. Discard the aqueous layer.

2. Rotary evaporate the combined dichloromethane extracts from Step 1 of Section 5.3 to dryness under reduced pressure at 40 °C (± 5 °C). Reconstitute the buprofezin residue from tissue and milk samples in 4.0 mL and 5.0 mL of toluene, respectively.

5.4 Bakerbond SPE™ Aminopropyl Column Cleanup

NOTE on **column characterization:** A column profile should be performed on each batch of aminopropyl columns prior to use. The elution pattern can vary depending on the manufacturer and/or batch. The column profile should be performed on a 2.0-mL aliquot of a buprofezin standard solution containing 0.60 µg/mL of the buprofezin analytes in toluene. Load the aliquot onto a conditioned (see Step 1 of Section 5.4) aminopropyl column. Completely drain the toluene from the column into a 125-mL boiling flask. (Note: Column breakthrough of buprofezin occurs; this necessitates collection of the load.) Elute the column with 2 x 2 mL of a solution of 70:30 toluene:acetone, collecting each elution fraction in a separate 125-mL boiling flask. Rotary evaporate the load and the two fractions to dryness under reduced pressure at 40 °C (± 5 °C). Reconstitute each fraction in 2 to 5 mL of toluene, and analyze by GC/MSD or GC/NPD. Compare the responses with those of known standards. The total recovery should be greater than 90%. If necessary, adjust the volume of the solution of 70:30 toluene:acetone used for elution to obtain such recovery. Typically, this elution volume is 2 mL of 70:30 toluene:acetone. The load is retained and combined with the eluate.

1. Condition each column with one column volume of toluene. Do not allow the column to go dry prior to sample application. (Note: In this and subsequent steps, manually expel the solvent by pressurizing the column through use of a safety bulb, with pipet fitting removed.)
2. Transfer a 2-mL aliquot of the extract from Step 2 of Section 5.3 onto a conditioned aminopropyl column. Completely drain the load into a 125-mL boiling flask.
3. Elute the remaining buprofezin with 2 mL of a solution of 70:30 toluene:acetone, combining the eluate with the load. Then, rinse

the inner neck of the boiling flask with a small amount of acetone to dissolve any eluate that may have splashed onto this surface.

4 Rotary evaporate the combined load and eluate to dryness under reduced pressure at 40 °C (± 5 °C). Reconstitute the residue in an appropriate volume of toluene (5 to 10 mL for tissue samples and 2 to 4 mL for milk samples). Transfer an aliquot to a GC vial for analysis.

6. GC/MSD AND GD/NPD ANALYSIS

6.1 Preparation of Analytical Standard Solutions

1. Prepare individual stock solutions of buprofezin in acetone. The solution should be at a known concentration of approximately 1000 µg/mL.

2. Prepare fortification standard solutions of buprofezin (typically at 1.0, 10, and 100 µg/mL for each) in acetone. These standard solutions are made from serial dilutions of the respective stock solution with acetone.

3. Prepare a standard solution containing a nominal concentration of 10 µg/mL of buprofezin in toluene. From this standard solution, make serial dilutions with toluene to yield calibration standards of typically 0.60, 0.40, 0.20, 0.10, 0.04, and 0.02 µg/mL of buprofezin in toluene.

6.2 Detection of Sample Residues

1. Equilibrate the GC system under the conditions listed in Appendix I for tissue samples and Appendix II for milk samples by making 2-µL injections of calibration standard solutions until a uniform response (of less than 10% variation for equivalent standards) is obtained. An injection volume of 2 µL was found to produce satisfactory results. Variations in equipment or sample characteristics may require different injection volumes. If so, adjust the injection volume of the standard solution as well as the test sample or fortified sample matrix (see Step 3 of Section 6.2) accordingly.

2. Determine peak areas and/or peak heights for the injected calibration standards. Typical calibration data are presented in Appendix III. Representative standard chromatograms are presented in Figures 1, 2, 15, and 16 of Appendix IV. Calculate a least squares regression line of peak area and/or peak height (counts) versus standard concentration (µg/mL) (Appendix III).

3. Inject a 2-µL aliquot of the test sample (or fortified sample matrix) from Step 4 of Section 5.4 into the GC system under the conditions stated in Appendix I and/or Appendix II. Make dilutions as necessary to maintain the response within the range of the standard curve.

4. Compare the peak area and/or heights of the analyzed sample with the standard curve. Calculate the total residue concentration of the buprofezin analyte by Equation 1.

$$ppm\ analyte = \frac{[(y - b) / M]}{C} \qquad \text{(Equation 1)}$$

where: y = Peak area and/or heights for analyte (counts)
b = y-Intercept of the standard calibration curve (counts)
M = Slope of the standard calibration curve (counts mL/µg)
C = Sample solvent ratio expressed as grams of sample per mL at injection (g/mL). This incorporates all dilutions made to the sample.

6.3 Fortification Experiments

1. Untreated (control) samples may be analyzed using the procedure described to verify that any endogenous substances present in the samples do not interfere with the final determination of buprofezin. See Figures 3, 6, 9, 12, and 17 of Appendix IV for representative chromatograms of untreated (control) beef tissue and milk samples.

2. Recoveries are determined by analyzing fortified control samples in conjunction with each sample set according to the described procedure. Tissue samples are fortified, prior to extraction, with 1.25 µg or greater of buprofezin. For example, add 125 µL of a 10 µg/mL standard solution of buprofezin in acetone to 25 g of untreated beef tissue for a 0.05 ppm matrix spike. Therefore, milk samples are fortified, prior to extraction, with 0.5 µg or greater of buprofezin. For example, add 500 µL of a 1.0 µg/mL standard solution of buprofezin in acetone to 50 g of untreated milk for a 0.01 ppm matrix spike. See Figures 4, 7, 10, 13, 18 and 19 of Appendix IV for representative chromatograms of fortified beef tissue and milk samples.

3. Calculate the final buprofezin concentration values for the control and recovery samples according to Equation 1 provided in Step 4 of Section 6.2.

4. Calculate recoveries of buprofezin by Equation 2.

$$Recovery\ (\%) = \frac{R - S}{T} \times 100 \qquad \text{(Equation 2)}$$

where: R = ppm of buprofezin analyte found in fortified sample
S = ppm of buprofezin analyte found in control sample
T = theoretical ppm of buprofezin analyte in fortified sample

5. Calculate the corrected analyte concentration (ppm) in each treated sample by Equation 3.

$$Corrected\ Analyte\ Concentration\ (ppm) = \frac{Final\ Analyte\ Concentration\ in\ Treated\ Sample}{Average\ Percent\ Recovery\ of\ Analyte} \times 100$$

(Equation 3)

7. DISCUSSION

A set of 8-12 samples can be analyzed by one analyst in approximately 1.5 working days, assuming that all materials and equipment are available. A recommended stopping point in the analytical procedure, if necessary, is Step 2 of Section 5.3. Samples should be reconstituted in toluene and refrigerated overnight. Column cleanup should be performed on the next working day.

The limit of quantitation for this method in beef tissues and whole milk is 0.05 and 0.01 ppm, respectively. This is based on the recoveries achieved at those concentrations. Typically, experimental recoveries were within the range of 70 - 120% with standard deviations of less than 20%. Results are presented in Tables 1 and 2.

Table 1 Recovery Data for Buprofezin in Beef Tissues

Matrix	Fortification Level (ppm)	% Recovery of Buprofezin
Beef Liver	0.05	85
	0.10	91
Beef Kidney	0.05	105
	0.20	98
Beef Muscle	0.05	98
	0.20	83
Beef Fat	0.05	89
	0.20	85
	Number of Analyses:	8
	Mean (%):	91.8
	Std. Deviation (%):	7.8

Table 2 Recovery Data for Buprofezin in Milk

Target Analyte	Fortification Level (ppm)	Analytical Recoveries (%)
Buprofezin	0.01	75, 98, 88
	0.05	78, 101, 93
Number =		6
Mean (%) =		88.8
Std. Dev. (%) =		10.6

Appendix I Instrument Conditions (GC/MSD) for Beef tissues

Gas Chromatography

Instrument:	Finnigan GCQ gas chromatograph with capillary split/splitless inlet operated in the splitless injection mode and containing a 4.0 mm i.d. split sleeve with wool (Restek Corp. Cat. No. 20782)		
Column:	Fused silica megabore DB-5MS bonded phase, 30 m x 0.25 mm i.d., 0.25-µm film thickness (J & W Scientific)		
Carrier Gas:	Helium (Ultrapure 99.999%) Head pressure set to 11 psi @ 90 °C Constant velocity 40 cm/sec		
Split Valve:	Closed before injection @ 90 °C Opened 1.0 min after injection		
Septum Purge:	Approximately 3.0 mL/min		
Temperatures:	Injection Port:	250 °C	
	Oven:	Programmed	
		Initial:	90 °C for 1.0 min
		Ramp 1:	20 °C/min to 320 °C; then hold for 2.5 min
	MS Interface:	0.00 °C	
	Transfer Line:	250 °C	
	Ion Source:	200 °C	
Retention Times:	ca. 9.8 min for buprofezin		

Injection

Autosampler:	CTC A200S 2-µL injection volume + 0.5 µL of air, residence time of 0.5 sec.
Detector:	GCQ MS Detector (MSD)
Acquisition Time:	12.00 minutes

Appendix I (continued)

Seconds Per Scan: 0.50 seconds

Mass Defect: 0.0 mmu/amu

Seg	Start	Scan Mode	Ion Mode	µS	Parameters
1	5.00	Full Scan	(+)	6	Mass 65 to 190
2	8.00	Full Scan	(+)	5	Mass 100 to 325

Target Ions:

Buprofezin 249, 190, 175 ions

Integration Parameters: Data was collected on a GCQ Software Data Processing system.

Appendix II Instrument Conditions (GC/NPD) for Whole milk

Gas Chromatography

Instrument:	Hewlett-Packard 5890A gas chromatograph with capillary split/splitless inlet operated in the direct injection mode and containing a 6.3 mm o.d. x 78.5 mm cyclo uniliner (Restek Corp. Cat. No. 20337)
Column:	Fused silica megabore DB-1 bonded phase, 15 m x 0.53 mm i.d., 1.5-µm film thickness (J & W Scientific)
Carrier Gas:	Helium (Ultrapure 99.999%) Head pressure set to 8.5 psi @ 165 °C Column flow rate set to 31.2 mL/min @ 165 °C
Split Flow:	0.0 mL/min @ 165 °C
Septum Purge:	Approximately 2.25 mL/min
Temperatures:	Injection Port: 275 °C Detector: 300 °C
	Oven: Programmed Initial: 165 °C for 2.0 min Ramp: 20 °C/min to 185 °C; then hold for 2.0 min Ramp A: 20 °C/min to 235 °C; then hold for 2.5 min Ramp B: 25 °C/min to 310 °C; then hold for 2.0 min
Retention Times:	Buprofezin: approximately 7.8 min

Injection

Autosampler:	Hewlett-Packard 7673A 2-µL injection volume, residence time of less than one second (fast injection)
Detector:	Hewlett-Packard nitrogen phosphorus detector (NPD)

Appendix II (continued)

Fuel Gases:	Hydrogen:	3.0 - 3 5 mL/min
	Air:	90 - 100 mL/min
Make-Up Gas:	Helium:	31 mL/min
Purge Value:	Initial value:	Off
Integration Parameters:	Data was collected on a PE Nelson Data system using Access*Chrom software Revision 1.9	

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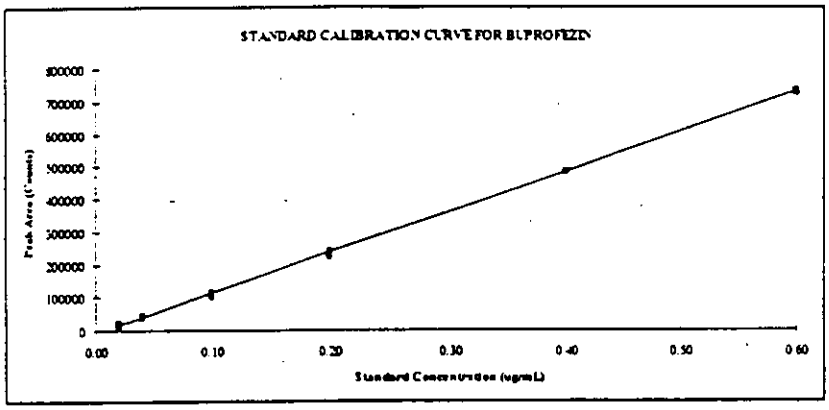
Appendix III Standard Calibration Data for Beef tissue

Standard Calibration Data for Buprofezin

Standard Calibration Curve:		Buprofezin		
Retention Time (min)	Standard Solution Reference Number	Standard Conc. (µg/mL)	Peak Area (Counts)	Statistical Data
9.80	BF/62/06	0.02	19892	Slope 1234500
9.78	BF/62/05	0.04	43654	Y-Int. -9032.8
9.78	BF/62/04	0.10	106129	
9.78	BF/62/03	0.20	226921	Coeff. 0.999
9.78	BF/62/02	0.40	505851	
9.82	BF/62/01	0.60	722282	



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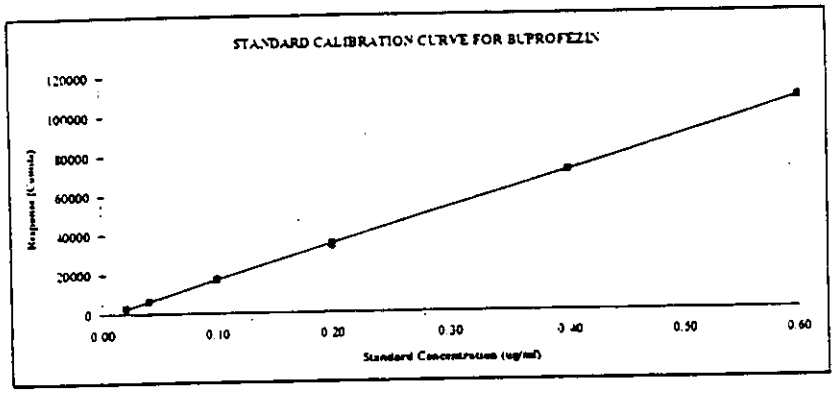
Appendix III (continued) Standard Calibration Data for Whole milk

Standard Calibration Data for Buprofezin

Standard Calibration Curve:		Buprofezin		
Retention Time (min)	Standard Solution Reference Number	Standard Conc. (µg/mL)	Peak Height (Counts)	Statistical Data
7.76	BF/53/12	0.02	3025	Slope 180186
7.76	BF/53/11	0.04	6336	Y-Int. -998.6
7.76	BF/53/10	0.10	17314	
7.76	BF/53/09	0.20	33819	Coeff. 1.000
7.76	BF/53/08	0.40	71206	
7.75	BF/53/07	0.60	107361	



Appendix IV Representative Chromatograms

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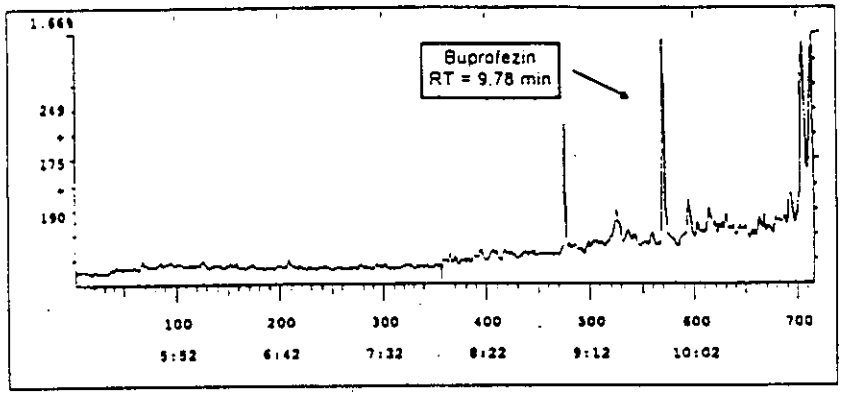
Appendix IV (continued)

Figure 1 0.04 µg/mL Standard of Buprofezin

Sample Description:	BF/62/05	0.04 µg/mL Standard		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
9.78	Buprofezin (BF01)	43654	N/A	N/A



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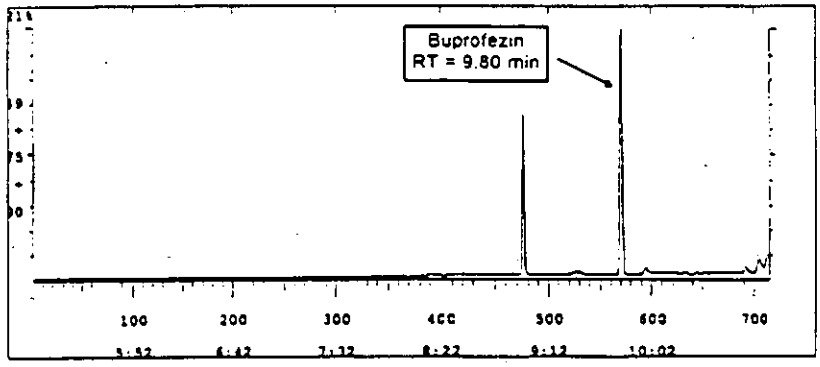
Appendix IV (continued)

Figure 2 0.60 µg/mL Standard of Buprofezin

Sample Description:	BF/62/01		0.60 µg/mL Standard	
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
9.80	Buprofezin (BF01)	722282	N/A	N/A



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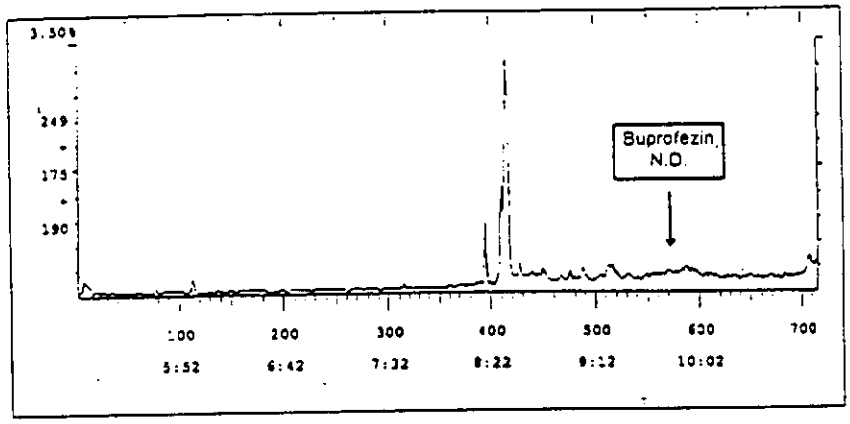
Appendix IV (continued)

Figure 3 Beef Liver Control

Sample Description:	151-030.39	Beef liver control		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
N/A	Buprofezin (BF01)	N.D.	N/A	N/A



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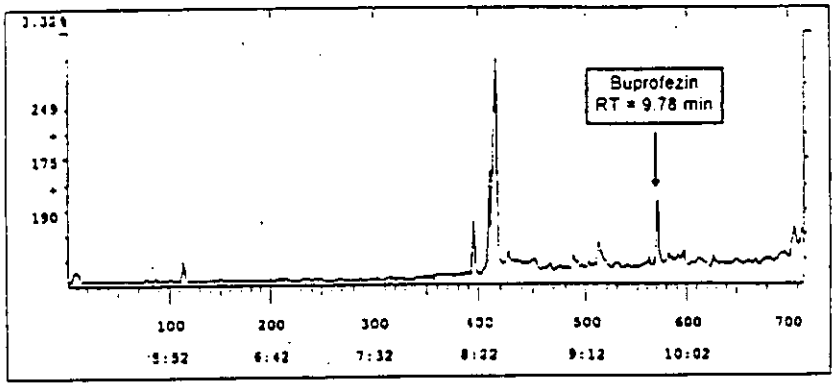
Appendix IV (continued)

Figure 4 Beef Liver Control Fortified at 0 05 ppm

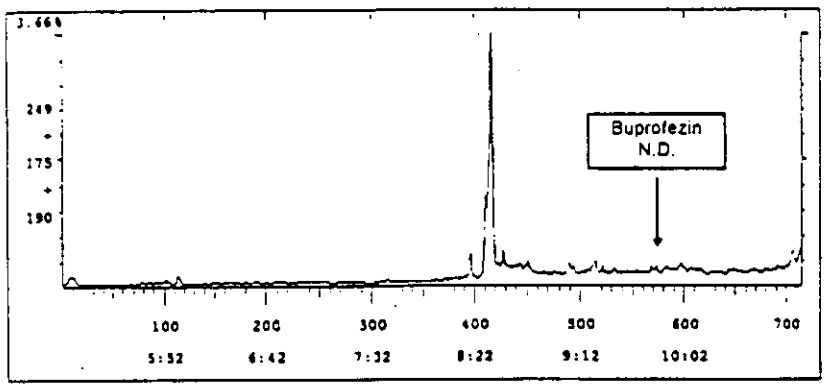
Sample Description:	151-030 39 + 0 05 ppm	Beef liver control fortified at 0 05 ppm		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
9.78	Buprofezin (BF01)	66881	0.043	85



Appendix IV (continued)

Figure 5 Treated Beef Liver

Sample Description:	151-030.41 T-I-2	Treated beef liver (T-I-2)		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
N/A	Buprofezin (BF01)	N.D.	N/A	N/A



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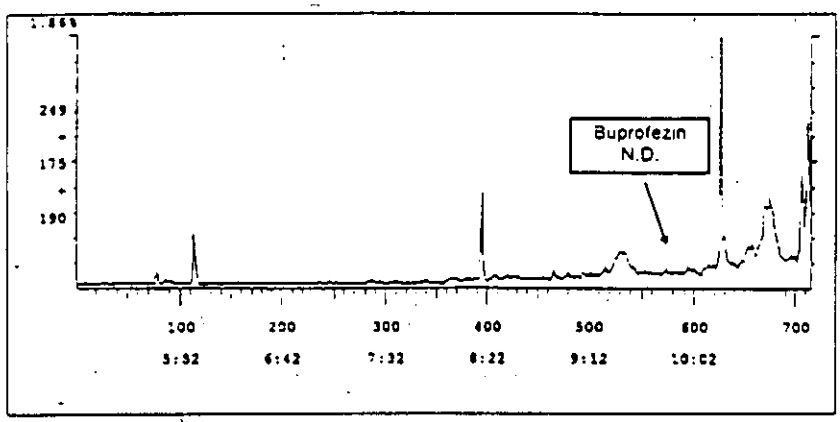
Appendix IV (continued)

Figure 6 Beef Kidney Control

Sample Description:	151-030.74		Beef kidney control	
Retention Time (min)	**Analyte of Interest**	**Peak Area (Counts)**	**Determined Residue (ppm)**	**Analytical Recovery (%)**
N/A	Buprofezin (BF01)	N.D.	N/A	N/A



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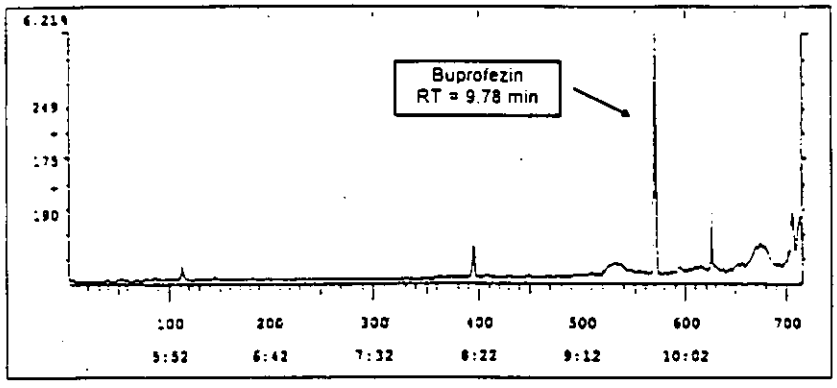
Appendix IV (continued)

Figure 7 Beef Kidney Control Fortified at 0.20 ppm

Sample Description:	151-030.74 + 0.20 ppm	Beef kidney control fortified at 0.20 ppm		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
9.78	Buprofezin (BF01)	292355	0.195	98



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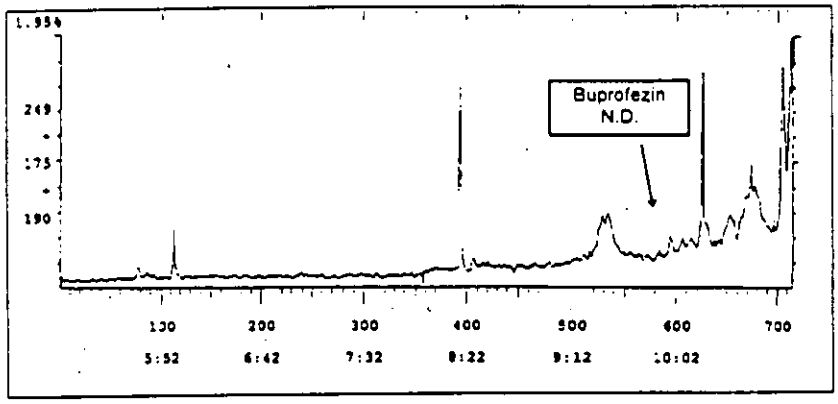
Appendix IV (continued)

Figure 8 Treated Beef Kidney

Sample Description:	151-030.77 T-I-2	Treated beef kidney (T-I-2)		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
N/A	Buprofezin (BF01)	N.D.	N/A	N/A



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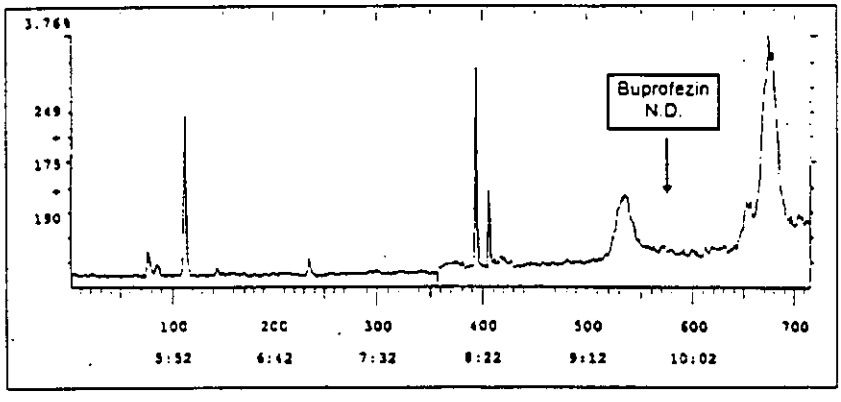
Appendix IV (continued)

Figure 9 Beef Muscle Control

Sample Description:	151-030 62	Beef muscle control		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
N/A	Buprofezin (BF01)	N.D.	N/A	N/A



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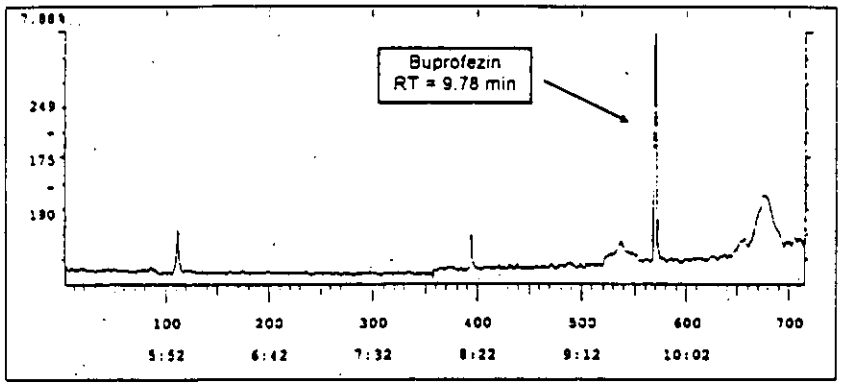
Appendix IV (continued)

Figure 10 Beef Muscle Control Fortified at 0.05 ppm

Sample Description:	151-030.62 + 0.05 ppm	Beef muscle control fortified at 0.05 ppm		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
9.78	Buprofezin (BF01)	65380	0.049	98



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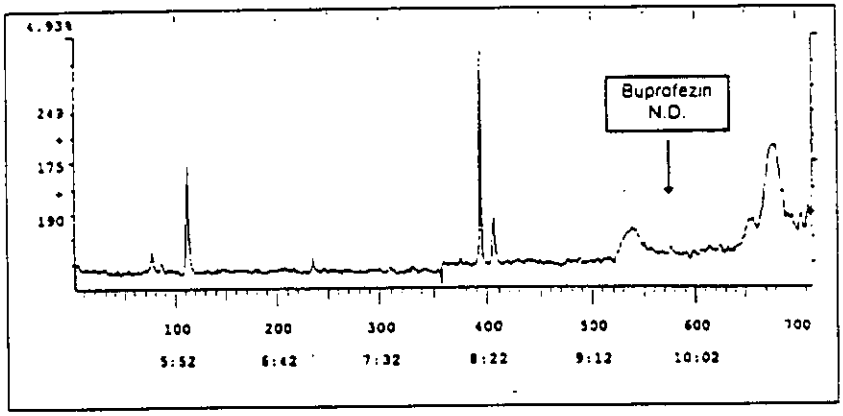
Appendix IV (continued)

Figure 11 Treated Beef Muscle

Sample Description:	151-030.65 T-I-2	Treated beef muscle (T-I-2)		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
N/A	Buprofezin (BF01)	N.D.	N/A	N/A



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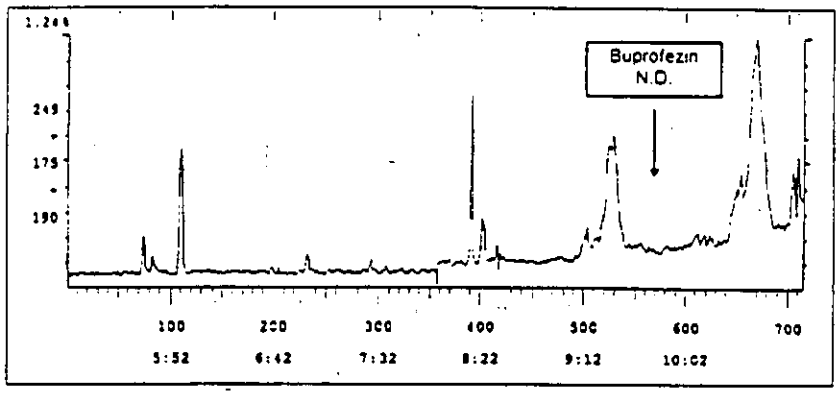
Appendix IV (continued)

Figure 12 Beef Fat Control

Sample Description:	151-030 50		Beef fat control	
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
N/A	Buprofezin (BF01)	N.D.	N/A	N/A



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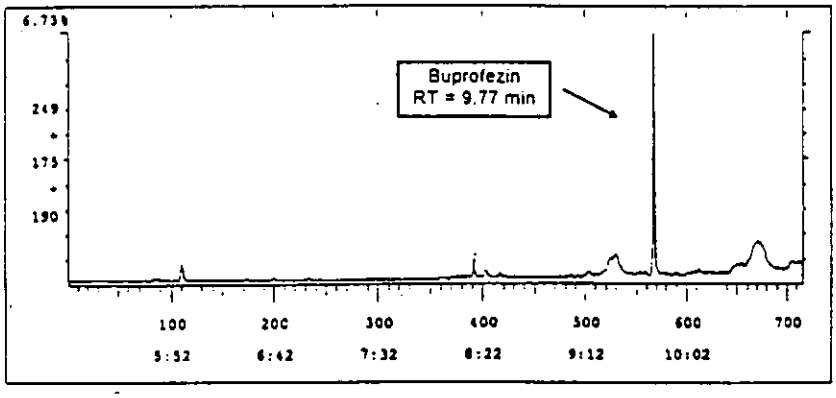
Appendix IV (continued)

Figure 13 Beef Fat Control Fortified at 0.20 ppm

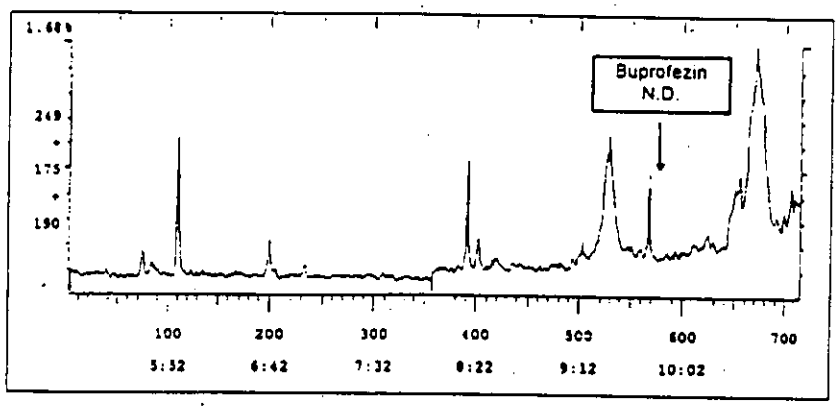
Sample Description:	151-030.50 + 0.20 ppm	Beef fat control fortified at 0.20 ppm		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
9.77	Buprofezin (BF01)	168963	0.170	85



Appendix IV (continued)

Figure 14 Treated Beef Fat

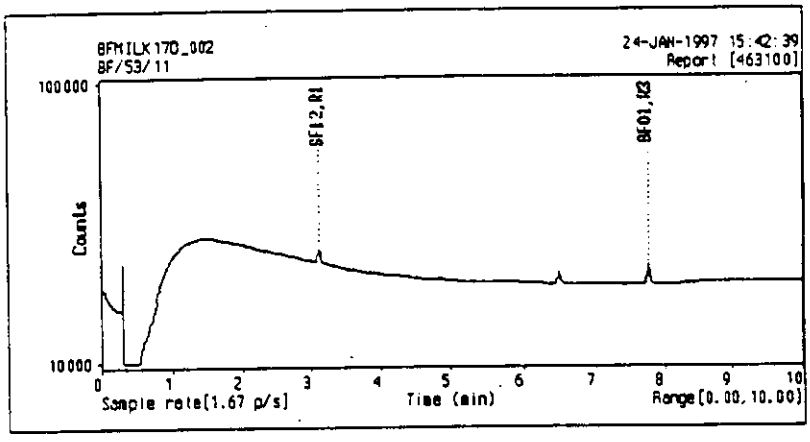
Sample Description:	151-030 53 T-I-2	Treated beef fat (T-I-2)		
Retention Time (min)	Analyte of Interest	Peak Area (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
N/A	Buprofezin (BF01)	N.D.	N/A	N/A



Appendix IV (continued)

Figure 15 0.04 µg/mL Standard of Buprofezin

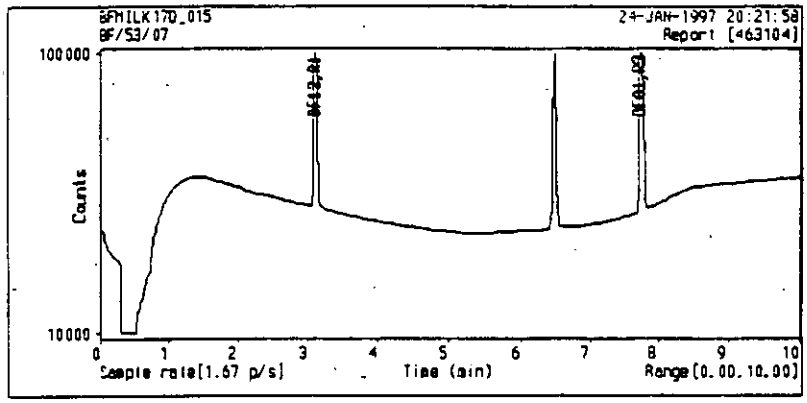
Sample Description:	BF/53/11 | 0.04 µg/mL Standard			
Retention Time (min)	Analyte of Interest	Peak Height (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
7.76	Buprofezin (BF01)	6336	N/A	N/A



Appendix IV (continued)

Figure 16 0.60 µg/mL Standard of Buprofezin

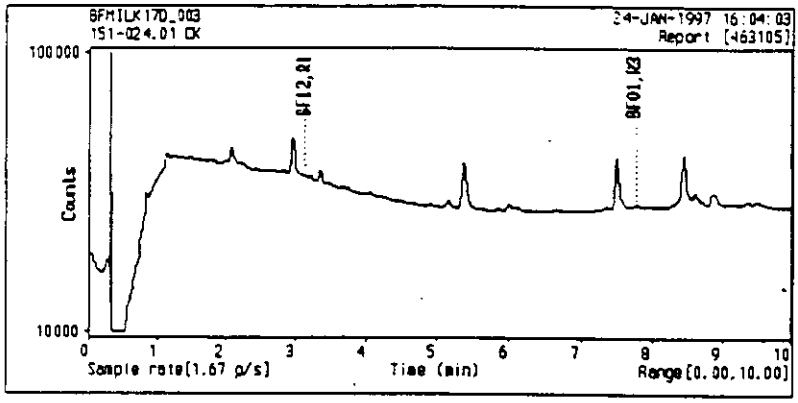
Sample Description:	BF/53/07	0.60 µg/mL Standard		
Retention Time (min)	Analyte of Interest	Peak Height (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
7.75	Buprofezin (BF01)	107361	N/A	N/A



Apoendix IV (continued)

Figure 17 Milk Control

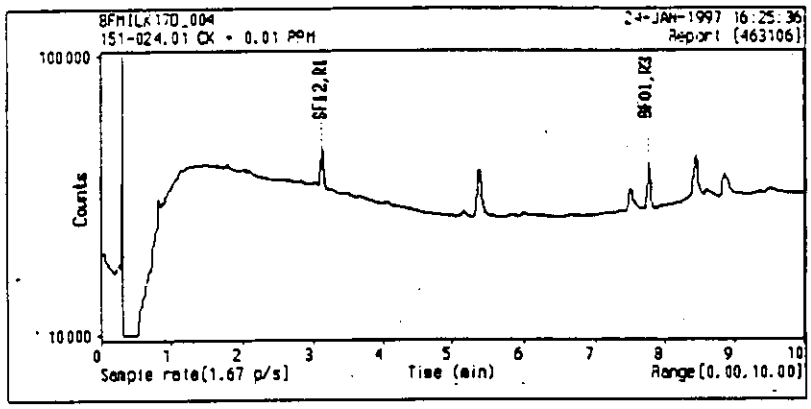
Sample Description:	151-024.01	Milk Control		
Retention Time (min)	Analyte of Interest	Peak Height (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
7.77	Buprofezin (BF01)	1591	<0.01	N/A



Appendix IV (continued)

Figure 18 Milk Control Fortified at 0.01 ppm

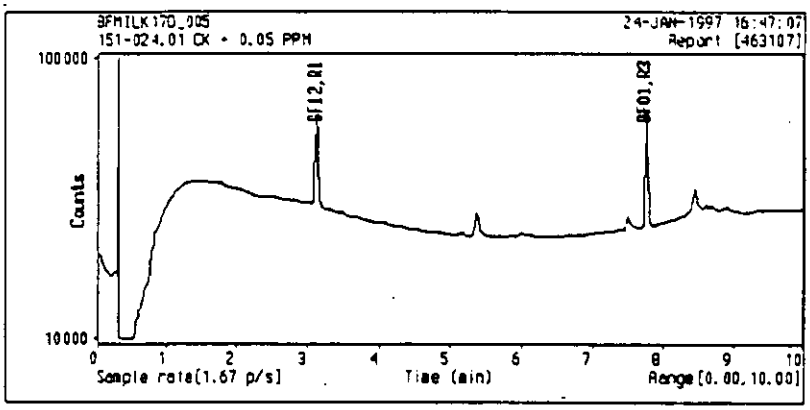
Sample Description:	151-024.01	Milk Control Fortified at 0.01 ppm		
Retention Time (min)	Analyte of Interest	Peak Height (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
7.77	Buprofezin (BF01)	15187	0.008	75



Appendix IV (continued)

Figure 19 Milk Control Fortified at 0.05 ppm

Sample Description:	151-024.01	Milk Control Fortified at 0.05 ppm		
Retention Time (min)	Analyte of Interest	Peak Height (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
7.76	Buprofezin (BF01)	35322	0.039	78



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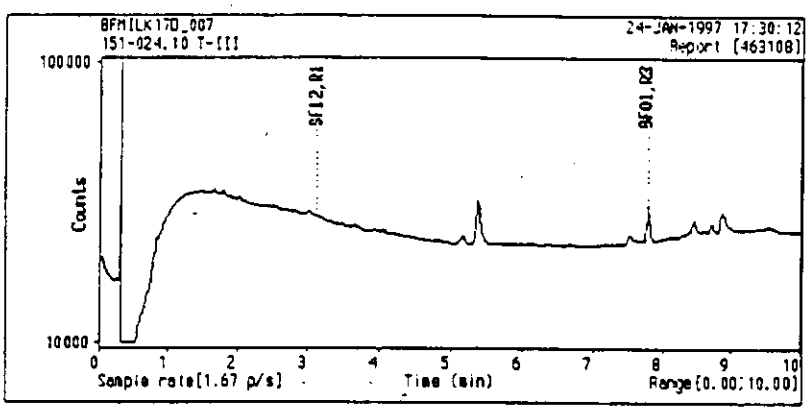
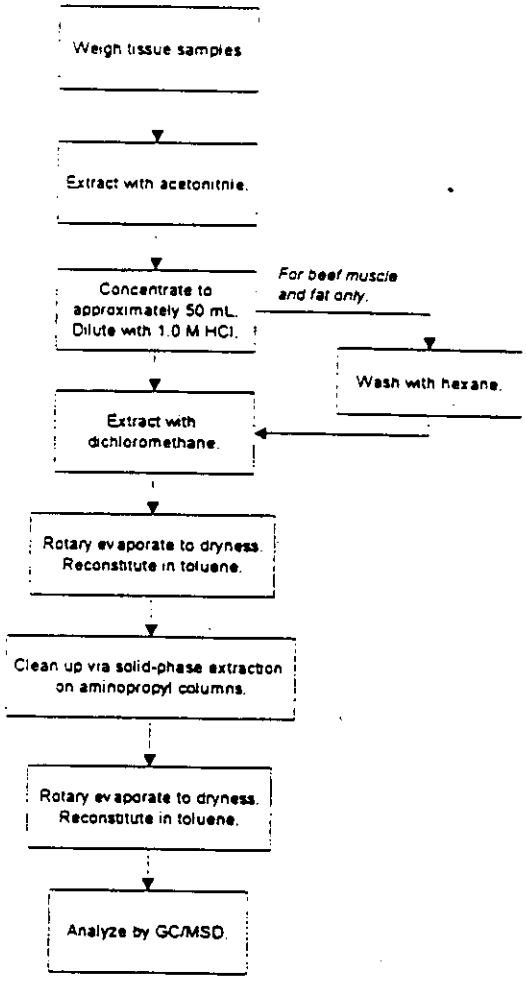
Appendix IV (continued)

Figure 20 Treated Milk

Sample Description: | 151-024.10 | T-III at Day 21				
Retention Time (min)	Analyte of Interest	Peak Height (Counts)	Determined Residue (ppm)	Analytical Recovery (%)
7.76	Buprofezin (BF01)	11137	<0.01	N/A



Appendix V Flow Diagram of Analytical Method BF/05/97



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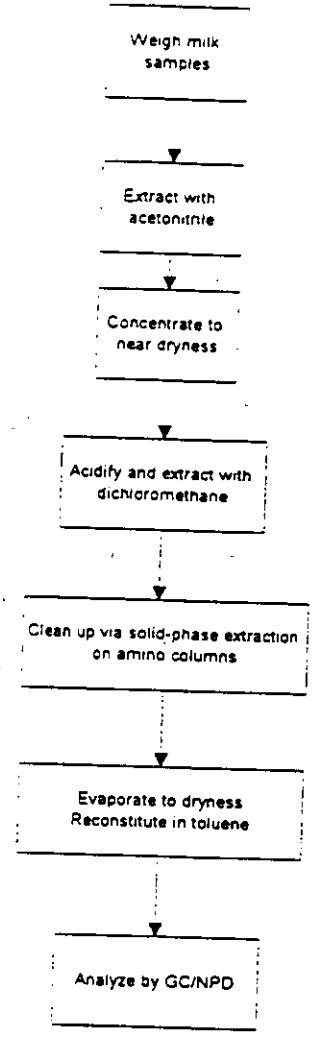
Appendix VI Flow Diagram of Analytical Method BF/04/97



EPA ADDENDUM

1) ACB substituted equivalent equipment and materials.

A) ACB used a Hewlett Packard 6890 GC with nitrogen - phosphorus detector with the following parameters:

Column: DB-1, 15 m x 0.53 mm id, 1.5 um film thickness
Inlet Temperature: 275°
Detector temperature: 300° C
Oven Program: Initial 165° C, hold for 2.0 min.
20° C/min. to 185° C, hold for 2.0 min.
25° C/min. To 300° C, hold for 6.0 min.
Gas Flows:
Carrier: 6.9 ml/min. Helium
Hydrogen: 3.0 ml/min.
Air: 60 ml/min.
Injection Volume: 2 ul

B) For milk, the ACB modified the above program to separate an interference from the analyte.

Oven Program: Initial 165° C, hold for 2.0 min.
20° C/min. to 185° C, hold for 2.0 min.
5° C/min. To 230° C, hold for 3.0 min.
30° C/min. To 300° C, hold for 11.0 min.

C) The ACB used an IKA Works T25 Tissumizer in place of the Omni-Mixer for all commodities except raisins. When using the Tissumizer, the ACB shortened the time to approximately 3 minutes for the first blending, and 2 min. for the second. For raisins, the ACB used a VirTis 23.

D) After checking the elution pattern of Florisil, the ACB increased from the stated 40 ml to 50 ml of 20% e.a./hexane.

E) For the meat and milk method, the ACB collected the eluant from the SPE aminopropyl columns in test tubes. The extracts were evaporated to dryness with a nitrogen evaporator.

2) The ACB suggests changes and clarification to the method as stated below are needed. These should be attached to the current method when distributed and included in any revised method.

A) The petitioner's instructions for calculations do not meet the Residue Chemistry Test Guidelines, OPPTS 860.1340 with respect to analyte found in controls. It is a Residue Chemistry Test Guideline requirement that control values are not subtracted from fortified or unknown samples, but to report the amount found in each sample.

B) The ACB found that sonication aided to dissolve dried extract residues into hexane prior to Florisil cleanup.

C) The ACB obtained clarification from J. Neal, Aventis, concerning the packing of Florisil columns. As per phone conversation (5/3/01), 50 ml of hexane is added to the glass column and 5 gram of dry Florisil is then poured into the glass column.

D) The ACB experienced severe emulsion problems with cottonseed and almond hulls resulting in low recoveries. Using a Tissumizer may have extracted more efficiently, increasing the amount of suspended particles present in the partitioning step. The ACB contacted J. Neal, Aventis, to determine if the petitioner had encountered these problems and discuss any possible solutions. During the third trial of cottonseed, the ACB found that gently rocking the separatory funnel was sufficient during the hexane and acidic aqueous step to achieve partitioning. During the second trial, almond hull solutions still emulsified, even with gentle rocking. The ACB drained the aqueous and emulsion layers together for further partitioning with methylene chloride.

Extraction by a Tissumizer or similar blenders using generators instead of blades, may result in more emulsions and higher background. The beef liver was the only commodity that showed significantly higher background levels in chromatograms than either the petitioner or the ILV.

3) The ACB prepared liver extracts by following the method as written without the hexane/aqueous partitioning and also with the hexane/aqueous partitioning. The ACB also ran a procedural blank with the additional partitioning step, and found that while some background could be attributed to the reagents, much originated from the liver. The ACB found the final extracts to be cleaner and able to be quantitated using GC/NPD in place of GC/MSD.