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Analytical Method

Department: Residue Chemistry

Date: May 28, 1996

RAM Number: BF/02/96

Title: Analytical Method For the Determination of Residues of Buprofezin at Estimated Tolerance Levels in Vegetable Crops (Lettuce and Tomatoes) by Gas Chromatography Using NP Detection

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Approved by: James K. Campbell, Ph.D., Manager, Residue Chemistry

Signed: _____ Date: 28 May 1996

DISTRIBUTION

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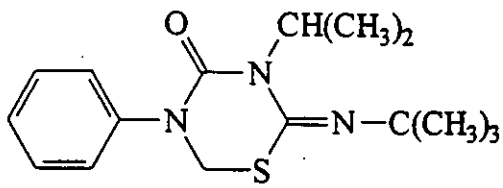
Residue Methods Book

(Total Number of Pages 23)

1. SCOPE

This method is suitable for the determination of the total extractable residues of buprofezin (Figure 1) in vegetable crops at and above the estimated tolerance level of 0.10 ppm. The limit of quantitation for this procedure has been set at 0.10 ppm.

Figure 1



Common Name: Buprofezin (BF1)

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 the crop matrix by blending with acetone. After rotary evaporation to the aqueous phase, the extracts are transferred to separatory funnels with 1M hydrochloric acid. The acidic aqueous extracts are then cleaned up by partitioning with hexane.

Buprofezin is partitioned upon neutralization and buffering of the aqueous phase to pH 7 with 50% (v/v) ethyl acetate / hexane. The organic extracts are then dried through granular anhydrous sodium sulfate, rotary evaporated to dryness and re-dissolved in toluene. Buprofezin is then quantified by gas chromatography using nitrogen phosphorus detection.

3. APPARATUS

Use as a guide; equivalent substitution may be required.

- Blender, Sorvall Omni-Mixer, Model 17105, Omni International
- Blender Blade Assembly, Omni International
- Blending jars (any pint size canning jar)
- Boiling flasks (1000 mL and 250 mL)
- Buchner funnels and filter adapters
- Fused silica megabore column, DB-1, 15 meters x 0.53 mm i.d., 1.5 µm film thickness, J & W Scientific (Cat. No. 125-1012)
- Glass fiber filter paper, Whatman 934-AH (Cat. No 1827-090)
- Glass pipettes, Class A
- Glass powder funnels, Pyrex
- Glass stoppers, 24/40, Pyrex
- Glass wool (silane-treated), Supelco Inc. (Cat. No. 2-0411M)
- Hewlett-Packard 5890A gas chromatograph with capillary split/splitless inlet and nitrogen phosphorus detector equipped with a Model 7673A autosampler
- Hobart VCM 40 or other apparatus suitable for grinding frozen plant tissue
- pH indicator strips, EM Science (Cat. No. 9590)
- Rotary Evaporation Unit (Buchi RE-R1)
- Separatory funnels (250 mL)
- Transfer Pasteur pipettes (9"), Fisher Scientific, (Cat. No. 13-678-7C)
- Ultrasonic Bath, Branson B-22-4 or equivalent
- Volumetric flasks (50 mL and 100 mL)

4. REAGENTS

All solvents should be pesticide grade or better.

- Acetone, pesticide grade
- Analytical standard of buprofezin
- Ethyl Acetate, pesticide grade
- Hexane, pesticide grade
- Hydrochloric Acid, concentrated ACS reagent
- pHydrion Buffer (pH=7), Krackler Scientific, (Cat. No. 47-KP2525-40)
- Sodium Hydroxide, 50% w/w, ACS reagent
- Sodium Sulfate, granular anhydrous, ACS reagent
- Toluene, pesticide grade

5. PROCEDURE

5.1 Extraction

5.1.1 Weigh a finely ground representative crop sample (20.0 grams) into a blending jar.

5.1.2 Fortify the recovery samples with buprofezin as required. Add 100 mL of acetone and blend for 1 minute.

5.1.3 Filter the acetone (leave the crop sample in the blending jar) by suction through a Buchner funnel containing Whatman 934-AH filter paper. Collect the extract in a 1000 mL boiling flask.

5.1.4 Add an additional 100 mL of acetone to the crop sample contained in the blending jar and blend again for 1 minute. Decant the acetone and crop sample into the Buchner funnel and collect the extracts in the same 1000 mL boiling flask. Rinse the filter cake with acetone and collect the rinsings.

5.1.5 Transfer the filter cake to the blending jar. Add 100 mL of acetone and blend just enough to break up the filter paper. Ultrasonicate for 2 minutes, and decant the extracts into the Buchner funnel containing Whatman 934-AH filter paper. Collect the extract in the 1000 mL boiling flask from step 5.1.3. Rinse the filter cake with acetone and collect the rinsings.

5.1.6 Rotary evaporate the organic extracts to the aqueous phase at 40 °C under reduced pressure. Transfer the aqueous extract to a 250 mL separatory funnel. Add 50 mL of 1M hydrochloric acid to the boiling flask and rinse. Transfer the acidic rinse to the same 250 mL separatory funnel.

5.2 Partitioning

5.2.1 Partition the acidic aqueous phase from step 5.1.6 to remove any possible interfering co-extractives by shaking with 3 x 25 mL of hexane. Drain the acidic aqueous phase into the original 1000 mL boiling flask between each partition. Discard the hexane extract after each partition.

NOTE: The 3 x 25 mL hexane volumes should be used to rinse the 1000 mL boiling flask from steps 5.1.6 and 5.2.1 after transfering the acidic aqueous phase back to the separatory

funnel. This will produce a clearer aqueous solution at step 5.2.2

5.2.2 Transfer the acidic aqueous phase to the separatory funnel and neutralize by adding 2.5 mL of 50% (w/w) sodium hydroxide and 50 mL of pH 7 buffer. Adjust the pH to 7 if necessary with sodium hydroxide and/or hydrochloric acid as required.

5.2.3 Partition the neutral aqueous phase with 3 x 25 mL of 50% (v/v) ethyl acetate/hexane. Drain the neutral aqueous phase into the original 1000 mL boiling flasks between each partition. Rinse the boiling flask with a small amount of 50% (v/v) ethyl acetate/hexane prior to the final partition and combine the rinse with the last partition.

5.2.4 Dry each partition through granular sodium sulfate contained in a glass powder funnel plugged with glass wool. Collect the organic extracts in a 250 mL boiling flask. Rinse the sodium sulfate well with 50% (v/v) ethyl acetate / hexane following the last partition and collect the rinsings.

5.3 Concentration

5.3.1 Rotary evaporate the extracts from step 5.2.3 to dryness under reduced pressure at 40 °C and dissolve the residue in 20 mL of toluene to await analysis by GC/NPD.

6. GC/NPD ANALYSIS

6.1 Preparation of Analytical Standards

6.1.1 Prepare a stock solution containing 50 mg of buprofezin in 50 mL of acetone. Make serial dilutions of the stock solution in acetone to yield fortification standards of 100, 10, 1.0, and 0.1 µg/mL.

6.1.2 Prepare GC calibration standards by diluting aliquots of the fortification standards and diluting with toluene to yield a concentrations of 0.60, 0.40, 0.20, 0.10, 0.04, and 0.02 µg/mL of buprofezin.

NOTE: It is recommended to prepare the calibration standards below by making a 1-100 mL dilution in toluene from the stock

fortification solution prepared in acetone. This would yield a 10.0 µg/mL buprofezin standard in toluene which would serve as a stock solution for preparation of the calibration standards.

6.2 Analysis of Samples

6.2.1 Construct a standard curve by plotting standard peak heights or areas vs. standard concentration (µg/mL). Calculate the least-squares regression line.

6.2.2 Determine the concentration of buprofezin in the treated and fortified samples by comparing the peak heights (or areas) to the standard curve.

6.2.3 Calculate the residue R using *Equation 1* as follows:

$$R\ (ppm) = \frac{(Y - b) / m}{C} \qquad \textit{(Equation 1)}$$

Where: Y = peak height (or area) response (cts.)
b = Y-intercept of standard regression line (cts.)
m = slope of standard regression line (cts mL/µg)
C = crop/solvent ratio (g/mL)

The crop/solvent ratio C is defined by the concentration of sample in g/mL at injection using *Equation 2*. This factor incorporates all dilutions made to the sample.

$$C = \frac{W}{20\ mL} \times D \qquad \textit{(Equation 2)}$$

Where: W = sample weight (g)
D = dilution factor (if needed)

The dilution factor D is defined by *Equation 3* below:

$$D = \frac{A}{V} \qquad \textit{(Equation 3)}$$

Where: A = Aliquot taken (mL)
V = Final Volume (mL)

6.3 Fortification Experiments

6.3.1 With each sample set, analyze an untreated control sample and one or more fortified control samples.

6.3.2 Calculate recoveries by *Equation 4* as follows:

$$\text{Recovery (\%)} = \frac{R - S}{T} \times 100 \qquad \textit{(Equation 4)}$$

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

7. DISCUSSION

The control lettuce sample used to generate the recovery data for this method were purchased at a local grocer and assigned a study number of BF-92R-09 and a sample number of 134-006.02. The control tomato sample (134-027.05) was obtained from study BF-94R18. The mean recovery of buprofezin was 92 % on lettuce and 90 % for tomato fruit. All recovery values were between 70 % and 120 % with a standard deviation of less than ± 10 %. On this basis the analytical method was judged to account for buprofezin residues in vegetable samples.

Table 1 Recovery Data for Buprofezin in Vegetable (Head Lettuce & Tomato Fruit)

Target Analyte	Fortification Level (ppm)	Analytical Recoveries (%)	
		Head Lettuce	Tomato Fruit
Buprofezin	0.10	92 92 92	87 87 90
	0.50	93 92 90	91 90 90
	1.00	90 91 93	90 90 91
Number =		9	9
Mean (%)=		92	90
Std. Dev. (%)=		1	2

8. REFERENCES

1. Anonymous, "Analytical Method of Buprofezin and p-Hydroxy Metabolite in Crops and Soil", Nihon Nohyaku Co. Ltd (July, 1985).
Reference: R30 (A-1005)

Appendix I Instrument Conditions (GC/NPD)

Gas Chromatography

Instrument:	Hewlett-Packard 5890A gas chromatograph with capillary split/splitless inlet operated in the direct injection mode, 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 Cat No. 125-1012)
Carrier Gas:	Helium (Ultrapure 99.999%) Head pressure set to 4 psi @ 165 °C Column flow rate @ 165 °C = 6 ml/min.
Split Flow:	0 mL/min. (at 235 °C)
Septum Purge:	= 3.5 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 hold 2.0 min. Ramp A: 20 °C/min to 235 °C hold 2.5 min.
Retention Times:	Buprofezin: ≈ 7.7 minutes

Injection

Autosampler:	Hewlett-Packard 7673A 2 µl injection volume, residence time less than one second (fast injection)

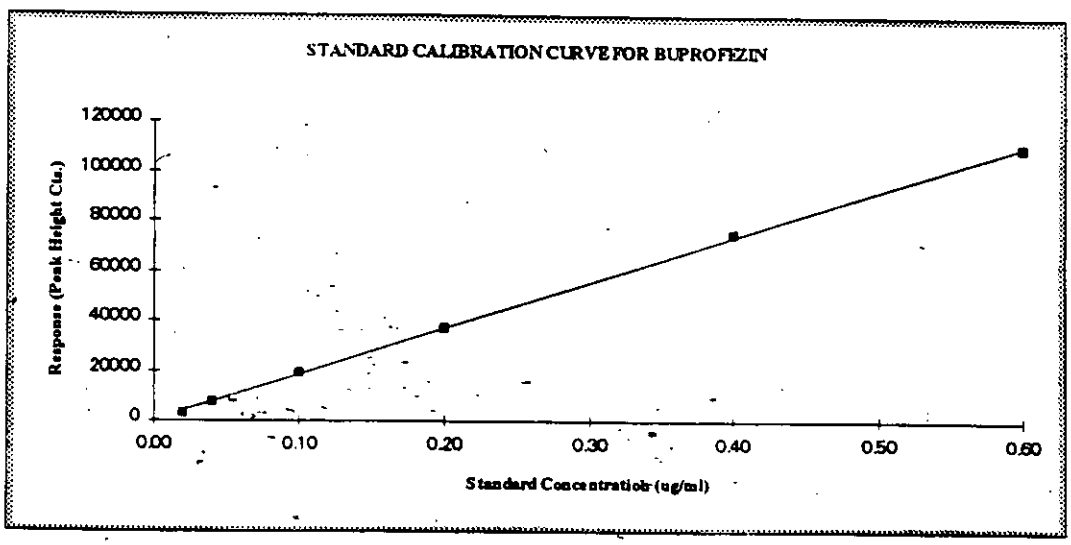
Detector: Hewlett-Packard nitrogen phosphorus detector (NPD)

Appendix I (continued)

Fuel Gases:	Hydrogen:	3.5 mL/min.
	Air:	100 mL/min.
Make Up:	Helium:	24 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	

Appendix II Standard Calibration Data for Buprofezin

Standard Calibration Curve:		Buprofezin		
Retention Time (min.)	Standard Solution Reference Number	Standard Conc. (ug/ml)	Response (Counts)	Statistical Data
7.70	BF/48/12	0.02	3032	Slope 150290
7.71	BF/48/11	0.04	6262	Y-Int. 449.06
7.71	BF/48/10	0.06	15932	
7.70	BF/48/09	0.10	30852	Coeff. 0.9999
7.70	BF/48/08	0.40	60524	
7.71	BF/48/07	0.60	90488	

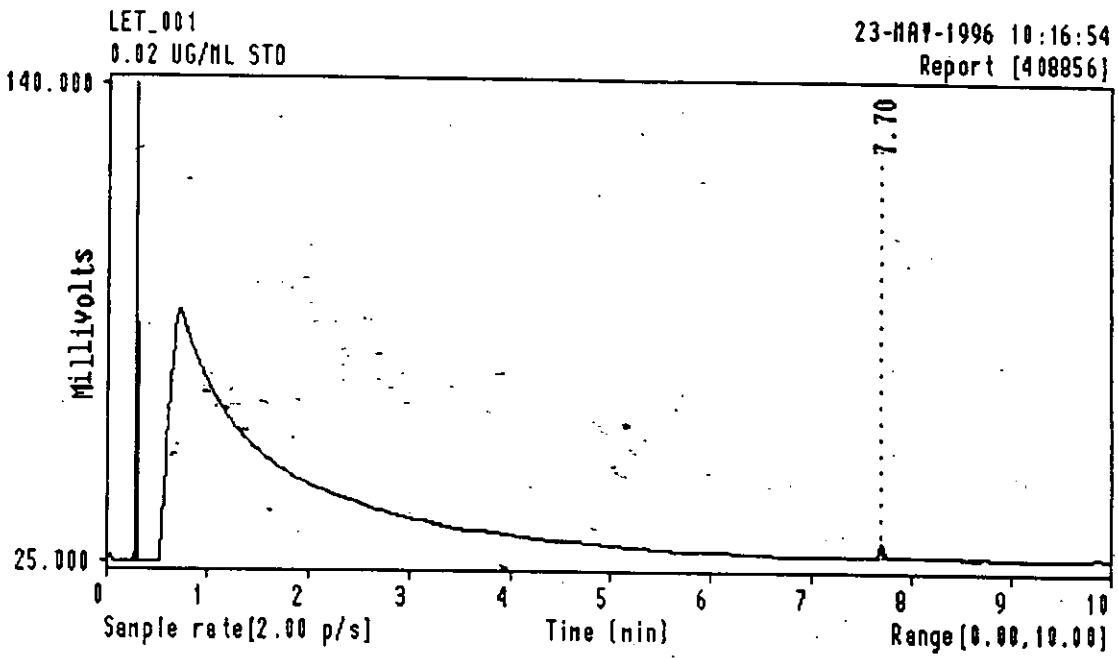


Appendix III Representative Chromatograms (con't) Page

Appendix III Representative Chromatograms (con't)

Figure 1 0.02 ug/ml Buprofezin Standard

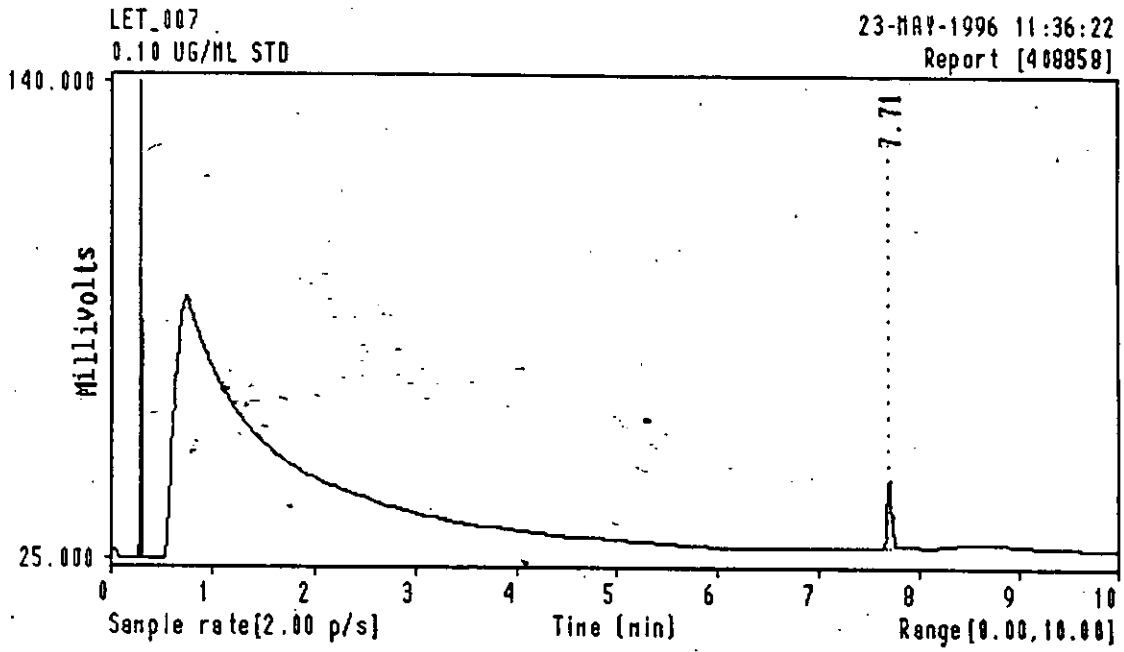
Sample Description:	BF/48/12	0.02 ug/ml Buprofezin Standard		
Retention Time (min.)	**Analyte of Interest**	**Response (Counts)**	**Residue Conc. (ppm)**	**Analytical Recovery (%)**
7.70	Buprofezin	3032	N/A	N/A



Appendix III Representative Chromatograms (con't)

Figure 2 0.10 ug/ml Buprofezin Standard

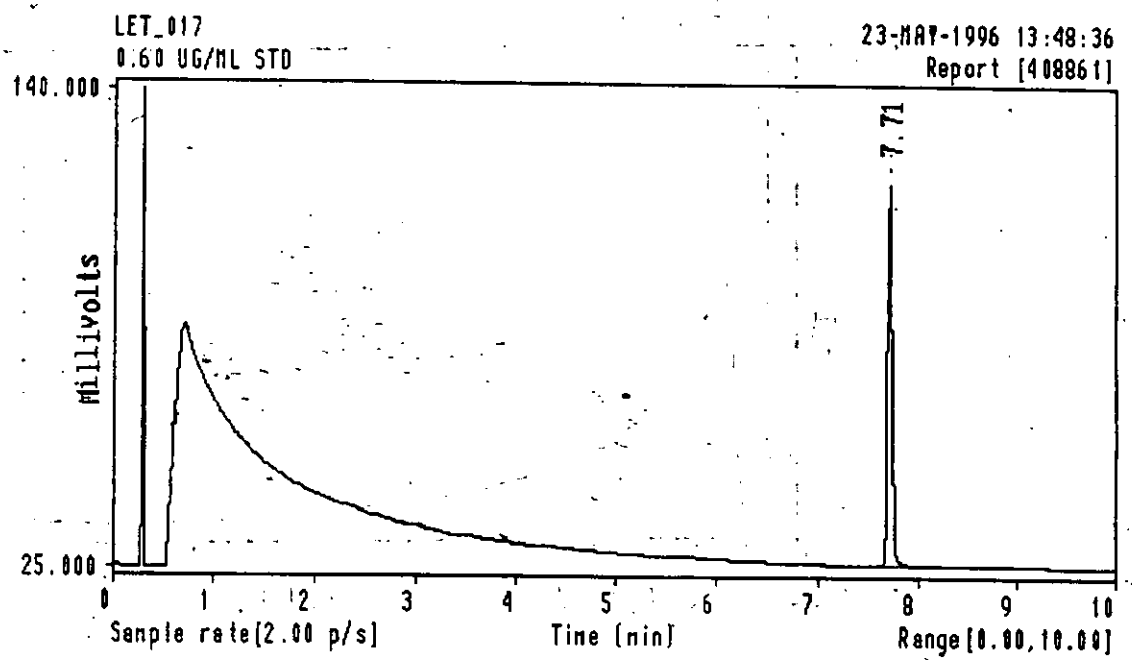
Sample Description:	BF/48/10	0.10 ug/ml Buprofezin Standard		
Retention Time (min.)	**Analyte of Interest**	**Response (Counts)**	**Residue Conc. (ppm)**	**Analytical Recovery (%)**
7.71	Buprofezin	15932	N/A	N/A



Appendix III Representative Chromatograms (con't)

Figure 3 0.60 ug/ml Buprofezin Standard

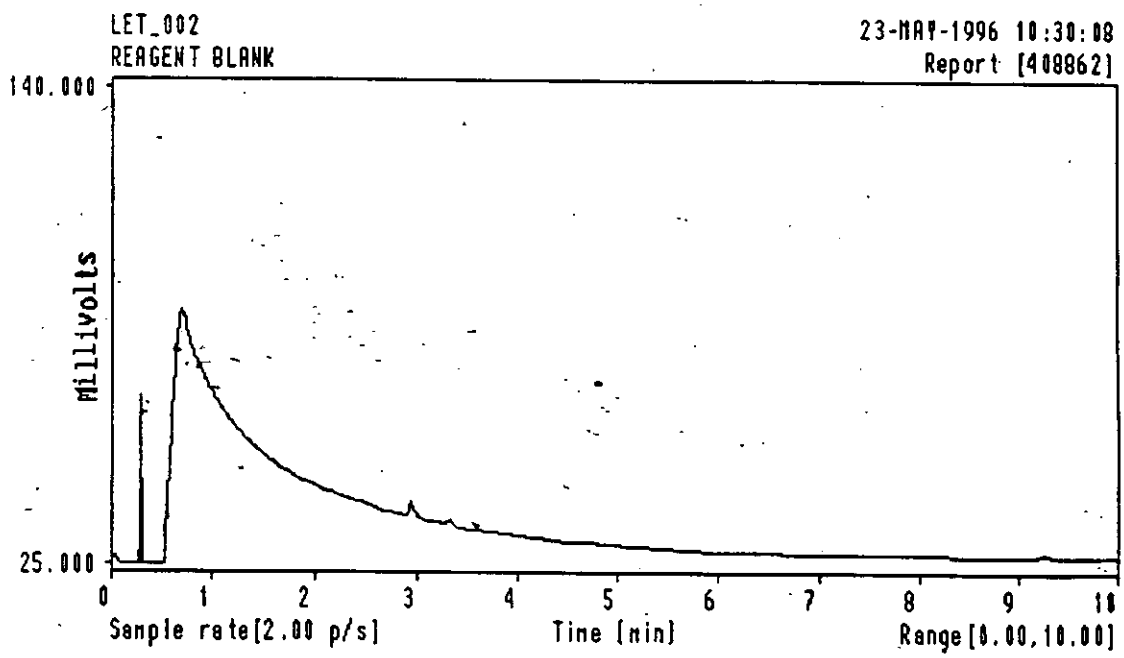
Sample Description:	BF/48/07	0.60 ug/ml Buprofezin Standard		
Retention Time (min.)	**Analyte of Interest**	**Response (Counts)**	**Residue Conc. (ppm)**	**Analytical Recovery (%)**
7.71	Buprofezin	90488	N/A	N/A



Appendix III Representative Chromatograms (con't)

Figure 4 Reagent Blank

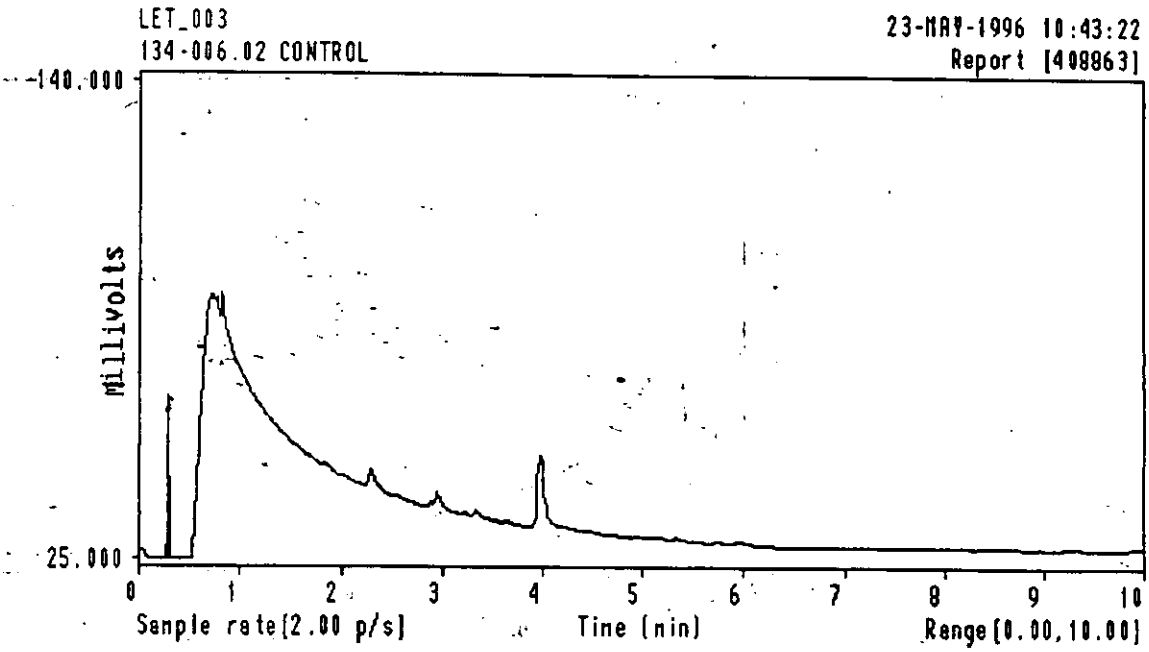
Sample Description:	Reagent Blank			
Retention Time (min.)	Analyte of Interest	Response (Counts)	Residue Conc. (ppm)	Analytical Recovery (%)
N/A	Buprofezin	N.D.	N.D.	N/A



Appendix III Representative Chromatograms (con't)

Figure 5 Lettuce Control

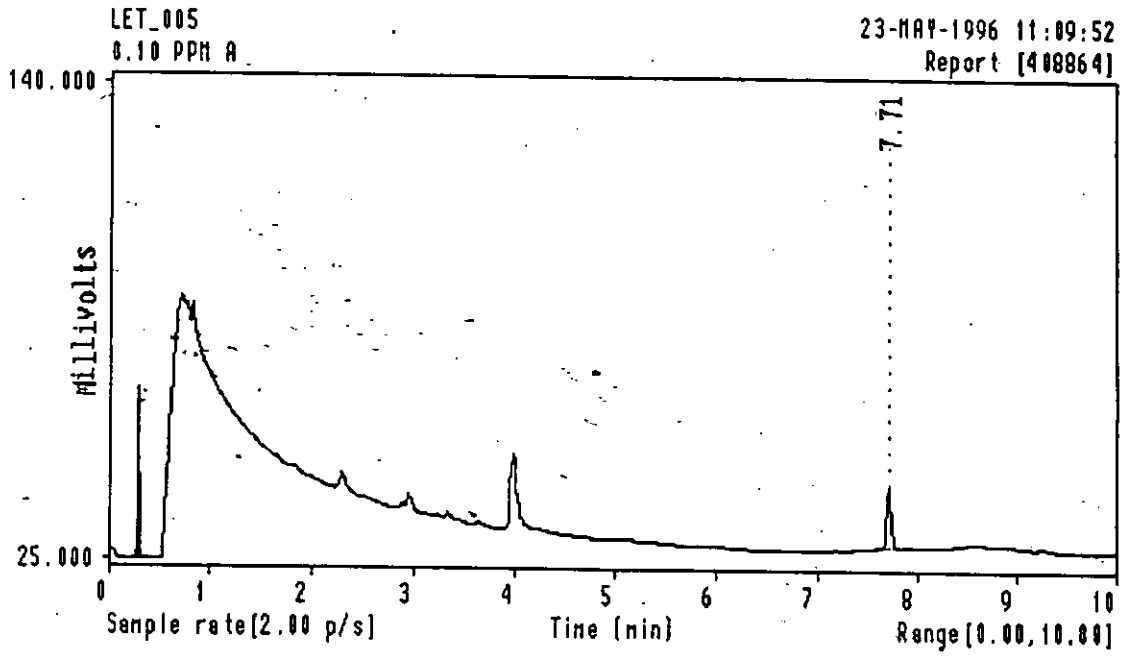
Sample Description:	134-006.02	Lettuce Control		
Retention Time (min.)	**Analyte of Interest**	**Response (Counts)**	**Residue Conc. (ppm)**	**Analytical Recovery (%)**
N/A	Buprofezin	N.D.	N.D.	N/A



Appendix III Representative Chromatograms (con't)

Figure 6 Lettuce Control Fortified at 0.10 ppm

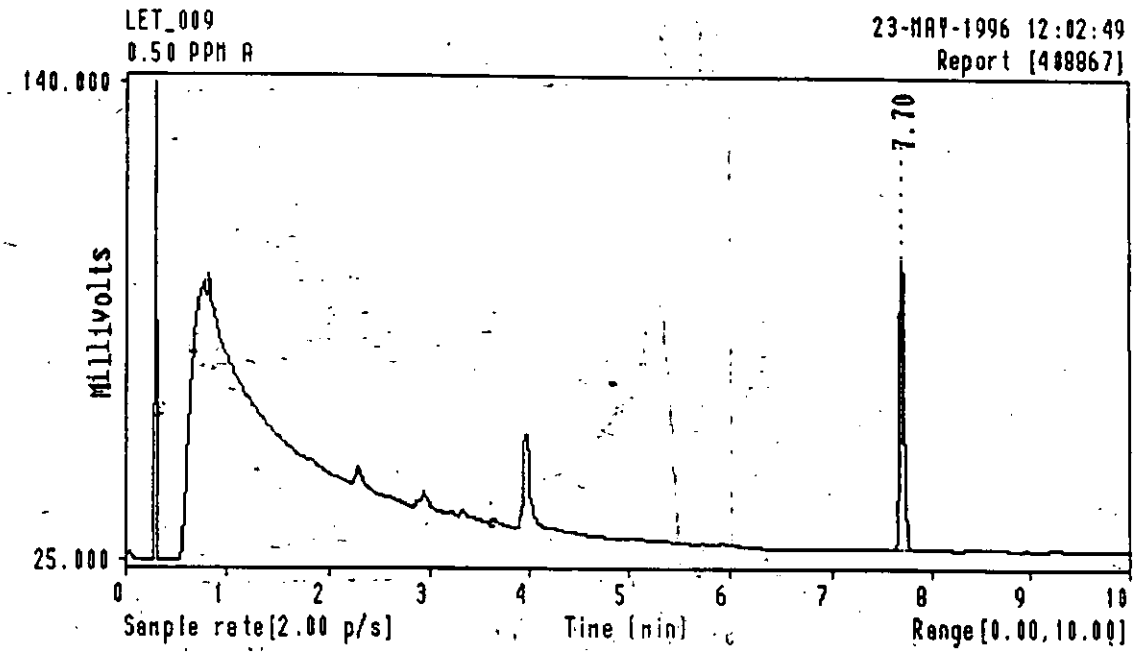
Sample Description:	134-006.02	Lettuce Control Fortified at 0.10 ppm		
Retention Time (min.)	**Analyte of Interest**	**Response (Counts)**	**Residue Conc. (ppm)**	**Analytical Recovery (%)**
7.71	Buprofezin	14329	0.0924	92



Appendix III Representative Chromatograms (con't)

Figure 7 Lettuce Control Fortified at 0.50 ppm

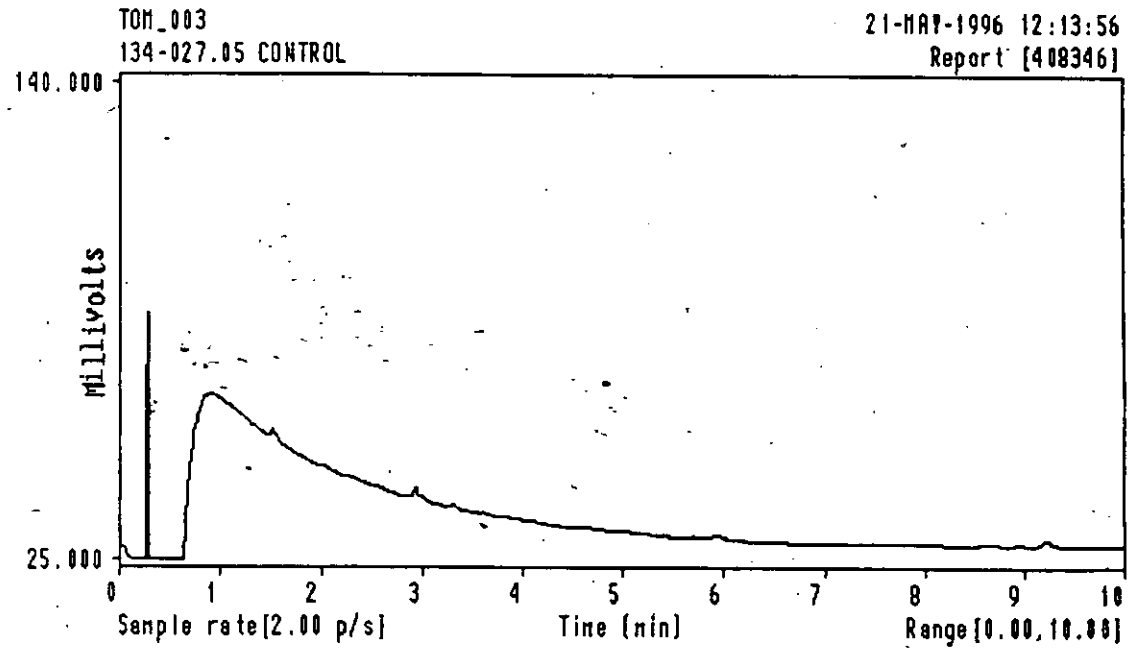
Sample Description:	134-006.02	Lettuce Control Fortified at 0.50 ppm		
Retention Time (min.)	**Analyte of Interest**	**Response (Counts)**	**Residue Conc. (ppm)**	**Analytical Recovery (%)**
7.70	Buprofezin	70028	0.4630	93



Appendix III Representative Chromatograms (con't)

Figure 8 Tomato Fruit Control

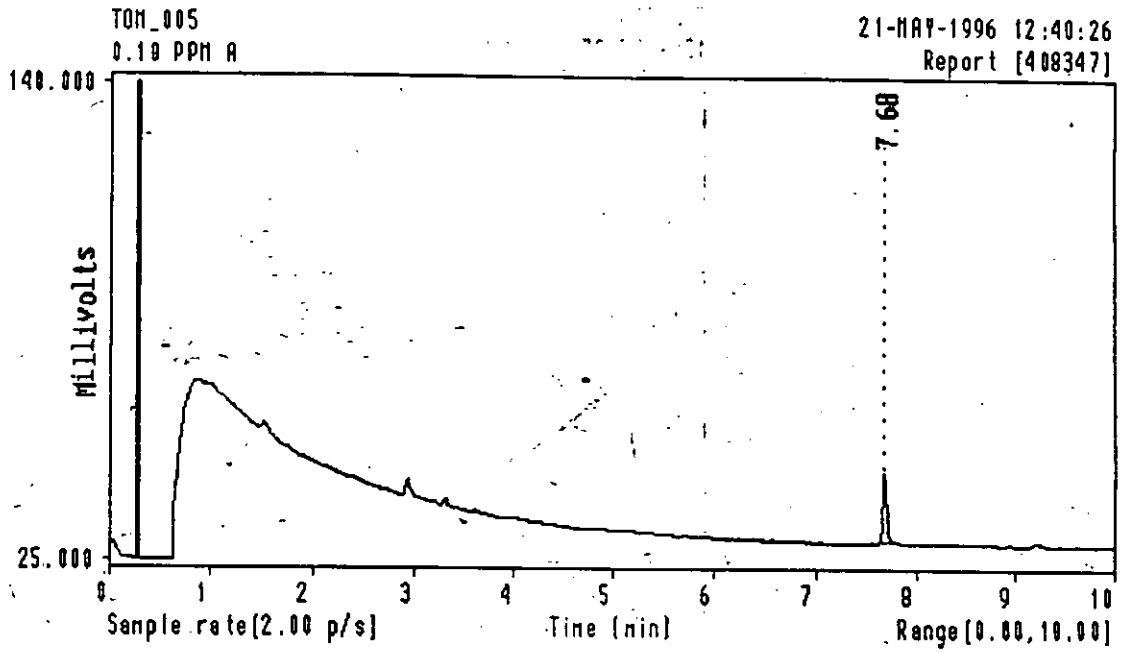
Sample Description:		Tomato Fruit Control		
Retention Time (min.)	**Analyte of Interest**	**Response (Counts)**	**Residue Conc. (ppm)**	**Analytical Recovery (%)**
N/A	Buprofezin	N.D.	N.D.	N/A



Appendix III Representative Chromatograms (con't)

Figure 9 Tomato Fruit Control Fortified at 0.10 ppm

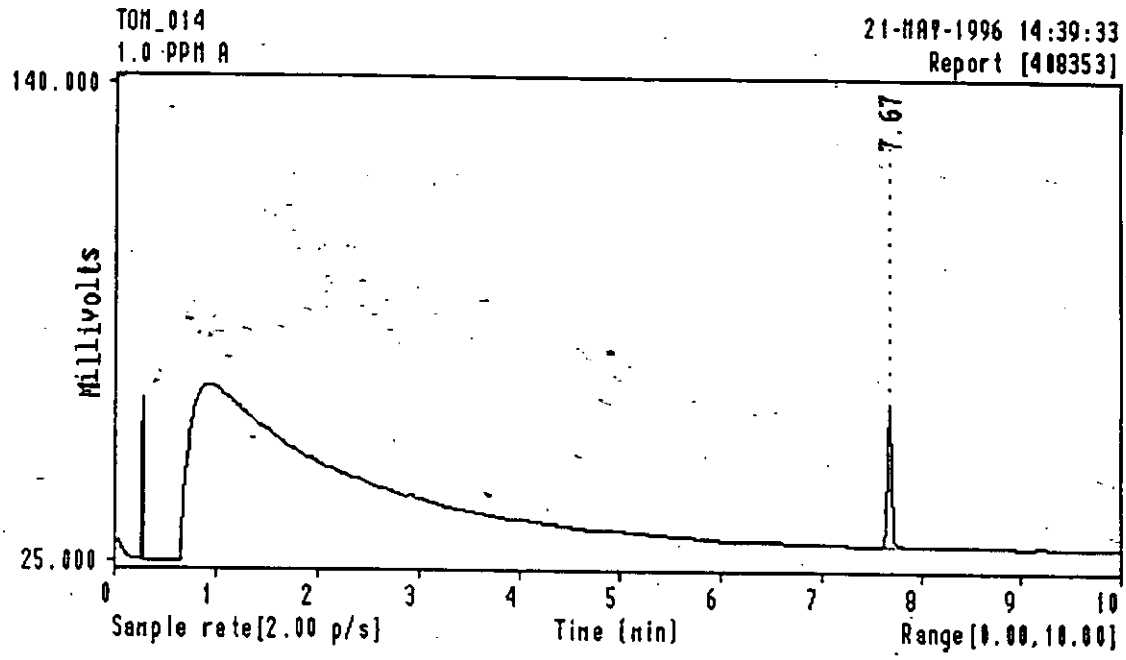
Sample Description:	134-027.05	Tomato Fruit Control Fortified at 0.10 ppm		
Retention Time (min.)	**Analyte of Interest**	**Response (Counts)**	**Residue Conc. (ppm)**	**Analytical Recovery (%)**
7.68	Buprofezin	16323	0.0874	87



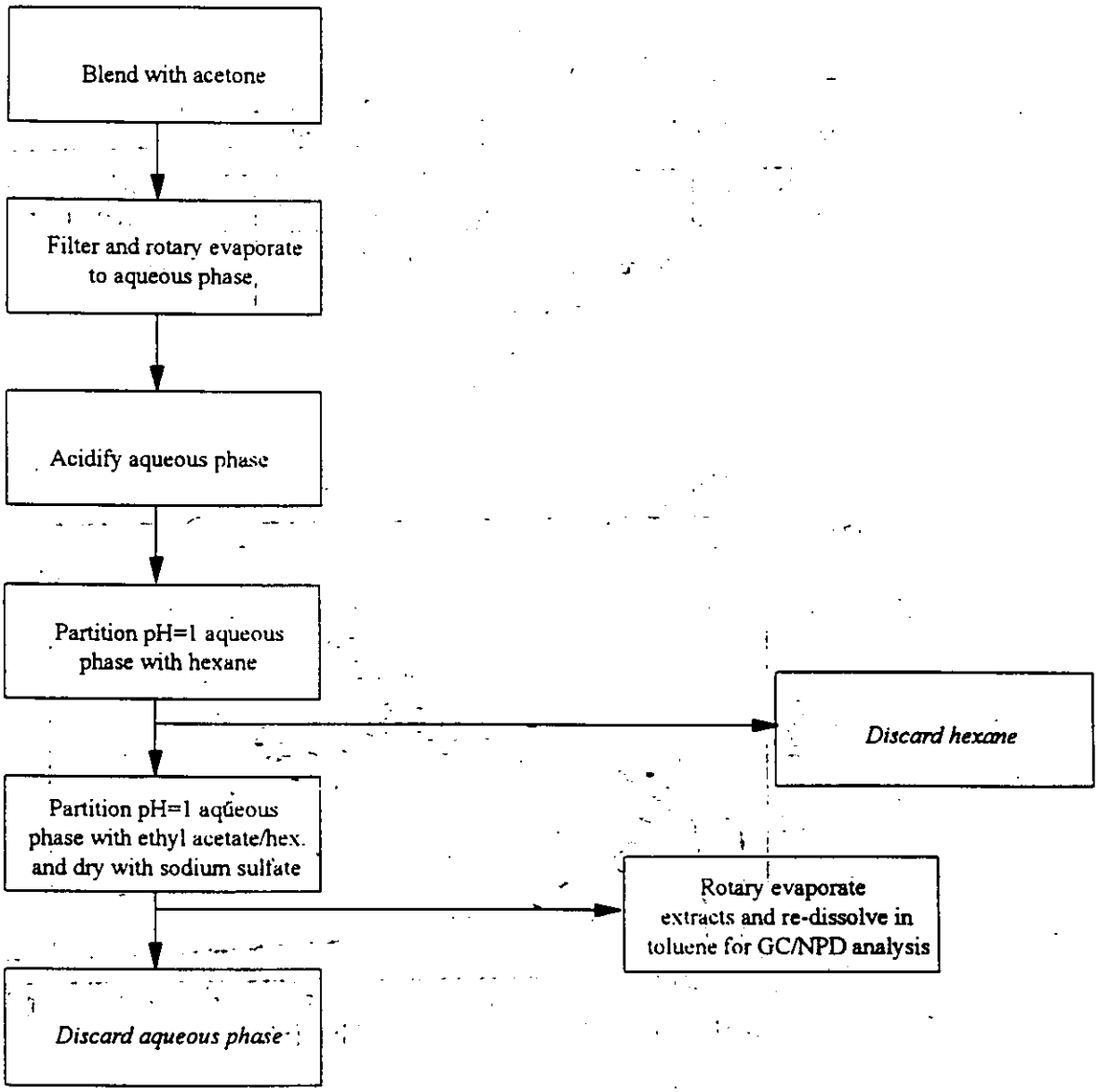
Appendix III Representative Chromatograms (con't)

Figure 10 Tomato Fruit Control Fortified at 1.00 ppm.

Sample Description:	134-027.05	Tomato Fruit Control Fortified at 1.00 ppm. Diluted 1-5 mL		
Retention Time (min.)	**Analyte of Interest**	**Response (Counts)**	**Residue Conc. (ppm)**	**Analytical Recovery (%)**
7.67	Buprofezin	33293	0.8975	90



Appendix IV Flow Diagram of Analytical Method BF/02/96



EPA ADDENDUM

Buprofezin on Lettuce and Cucumber

1) In section 5.1.6 and 5.3.1 of the petitioner's method use of a rotary evaporate system under reduced pressure is suggested. ACB recommends caution when using the vacuum in order to avoid bumping. ACB did not use full vacuum strength and suggest method user do the same..

2) While the method does not provide a overnight stopping points as such ACB found that in section 5.1.6 once you evaporate the organic extracts, the aqueous phase can be refrigerated for three days prior to adding 50 ml of 1M HCl.

3) In section 5.2 (partitioning step) the method does not mention how long the solution should be shaken. ACB shook the sample vigorously for one minute for each partition. Our recoveries are acceptable.

4) In section 5.2.2 a pH meter with a narrow long probe should be used to adjust the solution to pH 7. The method calls for extracting buprofezin three time once you set the pH to 7. ACB found that it is necessary to recheck the pH before doing the second and third extractions to be sure that the pH is still 7.

5) In section 5.2.4 ACB used 20 grams of anhydrous granular sodium sulfate. The method also states "Rinse the sodium sulfate well with..." ACB used another 25 ml of 50% ethyl acetate/hexane (v/v) to rinse the sodium sulfate.

6) Subtraction of control values is not an acceptable procedure in calculations as specified in the Residue Chemistry Test Guidelines (see 860.1340). For enforcement purposes, the formula needs to be changed to eliminate this subtraction of control values.

7) ACB has determined from its standard curve that the response of the compound is linear. Calculations also can be done using bracketing and a single point calibration.

8) ACB used a final sample volume of 7 ml, instead of the 20 ml specified in the method, for controls and samples fortified at the LOQ level.