Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Fenbutatin Oxide & Metab

MRID #: 428968-01

Matrix: Soil

Analysis: GC/FPD

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McKenzie Laboratories, Inc., Phoenix
Modification of AMR-720-87 (MMS-R-494-Z)

Residue Determination of Vendex Miticide and Its Organotin Metabolites, SD 31723 and SD 33608 in Soils and Agriculture Commodities

Modified by Kathryn Koktavy and Diana Price on 4/1/88.

Reference: "Residue Determination of Vendex Miticide and Its Organotin Metabolites, SD 31723 and SD 33608 in Agricultural Commodities." AMR-720-87 (MM-R-494-S)

Apparatus and Reagents:

- Boiling flasks, 500 mL and 250 mL
- 250 mL Nalgene Bottles (HDPE)
- Separatory Funnels, 125 mL and 500 mL capacity equipped with Teflon stopcocks
- 50 mL Centrifuge Tubes with Teflon lined lids
- 5.0 mL Gas Tight Syringe (or 2.5 mL)
- Wrist Action Shaker
- N-Evap Analytical Evaporator
- Buchi Rotary Evaporator
- Centrifuge
- Gas Chromatograph
- Chromatographic Column 15 m x 0.53 mm x 0.1 μ dimethyl polysiloxane
- Reference Standards:
  - Vendex Miticide: DPX-CG296 (SD14114), Generic-Fenbutatin oxide
  - IN-CG200 (SD31723) metabolite of Vendex Miticide
  - IN-DP387 (SD33608) metabolite of Vendex Miticide
- Ethyl Acetate, N-Hexane, Chloroform, Acetonitrile, Isopropyl Alcohol, "Distilled in glass" Solvents, Omni-Solv.
- Hydrochloric Acid, EM Science
- Diethyl Ether, Burdick and Jackson High Purity Solvent
- Dry Chemicals, Florisil, Sodium Sulfate
- Methyllithium in Diethyl Ether, 1.4 (CH_3Li) (from Aldrich Chemical Company)
- Tropolone, 98% (from Aldrich Chemical Company)

PROCEDURE:

Isolation

The day before:

1. Weigh 10 g of sample into a 250 mL Nalgene bottle
2. Add 50 mL of concentrated HCl to each sample.
3. Add 150 mL of chloroform to each sample

4. Fortify controls where necessary with a mixed spiker (containing SD 33608, SD 31723, SD 14114) at appropriate level.

5. Tightly cap bottles and place them on their sides on a wrist action shaker and shake vigorously for two hours. If the shaker is not capable of vigorous shaking, double the shaking time.

6. Allow samples to sit for at least 20 hours.

The next day:

1. Shake samples vigorously, by hand, for about 30 seconds.

2. Centrifuge for 3 minutes at setting of 1300 RPM.

3. Pour entire contents into 500 mL or 1 L separatory funnel.

Solvent Partition Cleanup

4. Measure an aliquot of the lower chloroform layer into a 250 mL boiling flask. (60 mL aliquot for crop and 90 mL aliquot for soils). Add 3 drops of keeper (1% mineral oil in toluene) and a boiling chip. Evaporate off the chloroform on a Buchi equipped with a 40°C bath.

5. Remove the residual chloroform traces using a stream of N_2.

6. Add 20 mL of hexane and swirl the sample.

7. Prepare hexane saturated acetonitrile containing 0.5 g/L tropolone. (It is important to add the tropolone just before it is going to be used)

8. Add 20 mL of the hexane saturated acetonitrile solution containing tropolone to the sample and swirl

9 Transfer the sample to a 125 mL separatory funnel and shake for 1 minute.

10. Drain the lower acetonitrile phase into a clean 500 mL boiling flask.

11 Partition 3 additional times using 20 mL of the saturated acetonitrile solution containing tropolone for each partition. Shake for two minutes between each partition and combine all four partitions.

12. Add approximately 3 drops of keeper and concentrate on a Buchi at 40°C to approximately 50 mL.

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13. Add 150 mL ethyl acetate and concentrate to approximately 20 mL.

14. Quantitatively transfer samples with ethyl acetate into a 50 mL centrifuge tube with Teflon lined lids that do not leak.

15. At this point make the methylated standard. The best way is to combine SD 14114 and SD 33608 for one standard and prepare a separate standard containing only SD 31723.

16. Add approximately 2-3 drops of keeper and evaporate all samples (including methylated standards) to near dryness (there should be a small drop left in the tube) on N-evap with a water bath set below 40°C.

Methylation

1. Immediately add 4 mL of diethyl ether, tightly cap centrifuge tubes with Teflon lined lids and swirl.

2. Add methyllithium in diethyl ether DROPWISE (a vigorous reaction may occur!). Use 5 mL of methyllithium for crops and 2.5 mL for soils. Swirl gently to ensure thorough mixing and let stand for 20 minutes.

3. Add 3 mL isopropyl alcohol DROPWISE to destroy any excess reagent. Note that if no reaction occurs the methylation is not complete!

4. Add [illegible] mL of D.I. water — 10(?)

5. Add 20 mL hexane and gently invert several times to mix. Concentrate on N-evap until upper phase is approximately 5 mL.

6. Add more hexane to bring upper phase to 10 mL.

7. Tightly cap centrifuge tube and shake vigorously for 1 minute and remove hexane phase into a clean 50 mL tube. (Tubes without lids are used here).

8. Partition the aqueous solution two additional times with 10 mL of hexane and combine all three hexane extracts into a 50 mL centrifuge tube.

9. Discard aqueous solution and concentrate hexane extract to approximately 4-5 mL for methylated standards, crops, oil and dislodgeables just prior to column cleanup.

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Column Cleanup

1 Weigh up 5 grams Florisil (heated at least 16 hours at 120°-160°F) and make a slurry with hexane.

2 Put a plug of glass wool into a 2.5 x 30 cm column and fill the column with hexane.

3 Add 2 cm of anhydrous sodium sulfate (approximately 1 tsp).

4 Add Florisil slurry and tap to remove air bubbles.

5 Add another teaspoon of sodium sulfate.

6 Drain hexane to the top of the sulfate.

7 Prepare a 5% diethyl ether in hexane solution.

8 Add the 4-5 mL sample to column and drain to the sulfate level.

9 Rinse the tube with 10 mL of the eluting solution (5% diethyl ether in hexane) and add to the column. Drain to the sulfate level into a 50 mL centrifuge tube.

10 Elute three additional times rinsing the tube each time. Columns should be run with the stopcock wide open when draining the column.

Dry down on N-evap to approximately 5 mL.

12 Transfer to 15 mL centrifuge tube previously marked at 2 mL and dry samples down to 2 mL. Transfer methylated standards to 100 mL volumetric flasks and bring to volume.

Helpful Hints for Running Vendex

Night before analysis:

Set up all glassware (separatory funnels, boiling flasks, and 100 mL graduated cylinders).

2 Saturate acetonitrile with hexane. Do not add tropolone until night before this solution is to be used.

3 Take the samples off the shaker and place by the centrifuge.

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G.C. Conditions

Instrument type:	Hewlett Packard 5890
Column.	15 m 0.53 mm x 0.1 µ
Column packing:	Dimethyl Polysiloxane
Detector type:	Flame Photometric Detector Sn Mode
He:	9.5 psi
Air:	50 psi
H_2:	40 psi
Attenuation:	2^2
Oven:	SD 14114 - 208°C SD 31723 - 174°C SD 33608 - 74°C
Inlet:	290°C

Make sure of the final volume and detection limit as they vary with matrix e.g. crops, soils and dislodgeables are different. The final sample weight and volume will determine your detection limit.

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RECOVERY STUDY

Vendex Soil

Sample	ppm Spiked	SD14114 ppm Found	SD14114 % Recovery	SD31723 ppm Found	SD31723 % Recovery	SD33608 ppm Found	SD33608 % Recovery
29532 (10-20)	0.4	0.347	87	0.360	90	0.293	73
	0.5	0.450	90	0.508	102	0.492	98
	5.0	4.17	83	4.42	88	4.08	82
	1.0	0.967	97	0.834	83	0.834	83
	0.2	0.197	98	0.240	120	0.210	105
	0.5	0.458	92	0.458	92	0.525	105
	1.0	0.734	73	1.10	110	0.984	98
59015 (20-30)	3.0	2.85	95	2.30	77	2.30	77
	1.0	0.864	86	0.784	78	0.834	33
	2.0	1.73	86	1.83	92	1.97	98
	4.0	3.14	78	3.87	97	3.34	84
59146 (20-30)	0.5	0.383	77	0.558	112	0.508	102
	1.0	1.07	107	1.12	112	0.967	97
59194 (10-20)	0.2	0.238	119	0.220	110	0.167	84
	0.4	0.418	104	0.400	100	0.380	95
59194 (20-30)	0.04	0.0350	88	0.0283	71	0.0317	79
	0.2	0.170	85	0.210	105	0.220	110
	0.2	0.163	82	0.203	102	0.207	104
	2.0	1.53	76	2.09	104	1.97	98
	1.0	0.950	95	1.07	107	1.03	103
	2.0	1.89	94	2.13	106	1.67	84
	0.5	0.442	88	0.542	108	0.400	80
	1.0	0.884	88	1.07	107	0.917	92
	0.04	0.0300	75	0.0450	112	0.0350	88
	0.4	0.313	78	0.387	97	0.400	100
	4.0	4.67	117	4.40	110	4.20	105
30550 (10-20)	0.1	0.0767	77	0.0917	92	0.0950	95
	20.0	14.7	74	21.7	108	18.0	90
30562 (10-20)	0.02	0.0217	108	0.0167	84	0.0233	117
	0.2	0.225	112	0.167	84	0.223	112
	0.3	0.215	72	0.245	82	0.250	83
	0.4	0.320	80	0.333	83	0.313	78
	1.0	0.867	87	0.934	93	1.10	110
	2.0	1.63	82	1.70	85	2.00	100

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RECOVERY STUDY

Vendex Soil

Sample	ppm Spiked	SD14114 ppm Found	SD14114 % Recovery	SD31723 ppm Found	SD31723 % Recovery	SD33608 ppm Found	SD33608 % Recovery
63586 (10-20)	0.02	0.0150	75	0.0183	92	0.0150	75
	0.2	0.165	82	0.160	80	0.177	88
	2.0	1.83	92	1.97	98	1.70	85
	3.0	3.00	100	3.35	112	2.30	77
63598 (10-20)	2.0	1.73	86	1.73	86	1.80	90
	4.0	3.53	88	3.20	80	3.73	93
	0.5	0.408	82	0.508	102	0.425	85
	1.0	0.800	80	1.05	105	0.934	93
	3.0	3.15	105	3.05	102	2.55	85
68132 (20-30)	0.05	0.0367	73	0.0467	93	0.0467	93
	0.5	0.455	91	0.467	93	0.558	112
	0.1	0.0834	83	0.0834	83	0.0900	90
	0.3	0.220	73	0.285	95	0.285	95
59374 (20-30)	1.0	0.731	73	0.984	98	0.850	85
	2.0	1.73	86	1.93	96	2.17	108
Reagent Blank	- -	<0.02	- -	<0.02	- -	<0.02	- -
Reagent Spike	0.2	0.197	98	0.190	95	0.220	110
Reagent Blank	- -	<0.02	- -	<0.02	- -	<0.02	- -
Reagent Spike	0.2	0.223	112	0.197	98	0.237	118

Table 1 Summary of the Recovery for Vendex® and It's Metabolite Residues in Fortified Soil Controls.

Sample # S000	ppm Added	SD14114 ppm Found	SD14114 % Recovery	SD31723 ppm Found	SD31723 % Recovery	SD33608 ppm Found	SD33608 % Recovery
68144 (10-20 cm)	--	<0.020	--	<0.020	--	<0.020	--
	0.020	0.0217	108	0.0233	117	0.0167	83
	0.20	0.190	95	0.187	94	0.163	82
68144 (0-10 cm)	--	0.0123	--	<0.020	--	<0.020	--
	10.0	10.2	102 Net	10.0	100	0.0400	80
	0.050	0.0377	75 Net	0.0533	107	8.67	87
68144 (10-20 cm)	--	<0.020	--	0.0167	--	0.00734	--
	0.10	0.0750	75	0.0833	83 Net	0.0843	84 Net
	15.0	14.8	99	16.2	108 Net	14.5	97 Net
70006 (10-20 cm)	--	<0.020	--	0.00800	--	0.00300	--
	1.0	0.933	93	0.992	99 Net	0.864	86 Net
	3.0	2.85	95	2.59	86 Net	2.50	83 Net
	3.0	2.60	87	--	--	--	--
70006 (10-20 cm)	--	0.0388	--	0.00618	--	0.00465	--
	--	--	--	0.00667	--	--	--
	0.50	0.419	84 Net	0.403	81 Net	0.487	97 Net
	0.50	--	--	0.397	79 Net	--	--
	2.0	1.72	86 Net	1.56	78 Net	1.68	84 Net
63658 (10-20 cm)	--	<0.020	--	<0.020	--	<0.020	--
	0.10	0.0800	80	0.0733	73	0.0933	93
	3.0	2.20	73	2.85	95	2.15	72
63658 (10-20 cm)	--	<0.020	--	<0.020	--	<0.020	--
	0.10	0.0817	82	0.0767	77	0.0933	93
	5.0	4.83	97	4.92	98	3.58	72
66341 0-10 cm	--	<0.020	--	<0.020	--	<0.020	--
	0.20	0.148	74[6]	0.154	77	0.155	78
	20.0	15.7	78	16.9	84	14.6	73
63682 (10-20 cm)	--	<0.020	--	<0.020	--	<0.020	--
	0.06	0.055	92	0.0500	83	0.0483	81
	1.0	0.817	82	1.0	100	0.830	83

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Table 1 Summary of the Recovery for Vendex® and It's Metabolite Residues in Fortified Soil Controls, continued.

Sample # S000	ppm Added	SD14114 ppm Found	SD14114 % Recovery	SD31723 ppm Found	SD31723 % Recovery	SD33608 ppm Found	SD33608 % Recovery
68305 (10-20 cm)	--	<0.020	--	<0.020	--	<0.020	--
	0.50	0.390	78	0.460	92	0.430	86
	2.0	1.48	74	1.83	92	1.83	92
54112 (10-20 cm)	--	<0.020	--	<0.020	--	<0.020	--
	0.40	0.333	83	0.367	92	0.365	91
	4.0	3.67	92	3.67	92	3.67	92
07018 (10-20 cm)	--	<0.020	--	<0.020	--	<0.020	--
	1.0	0.883	88	0.967	97	0.917	92
	2.0	1.83	92	1.90	95	1.87	94
63694 (10-20 cm)	--	0.00956	--	<0.020	--	<0.020	--
	1.0	0.750	75 Net	0.800	80	0.900	90
	2.0	2.06	103 Net	1.57	78	1.83	92
63694 (10-20 cm)	--	<0.020	--	<0.020	--	<0.020	--
	2.0	1.97	98	2.40	120	1.73	86
	3.0	2.53	84	2.60	87	3.15	102
68329 (10-20 cm)	--	<0.020	--	<0.020	--	<0.020	--
	0.20	0.0146	73	0.0166	83	0.0147	73
	2.0	0.144	72	0.169	84	0.195	98
68329 (10-20 cm)	--	<0.020	--	<0.020	--	<0.020	--
	0.10	0.104	104	0.0863	86	0.0868	87
	10.0	11.3	113	11.4	114	9.24	93
61716 (20-30 cm)	--	<0.020	--	<0.020	--	<0.020	--
	0.050	0.0494	99	0.0375	75	0.0403	81
	2.0	1.56	78	1.43	72	1.46	73
61716 (20-30 cm)	--	0.0198	--	<0.020	--	<0.020	--
	0.20	0.191	96 Net	0.193	96	0.185	92
	2.0	--	--	2.15	108	--	--
	2.0	2.38	119 Net	2.17	108	2.14	107

NOTE Reagent blanks accompanied some intervals of data. All blanks were acceptable. An acceptable reagent blank will have no measurable peak greater than 1x noise. Although not required by the protocol, reagent fortifications accompanied some of the analyses. Reagent fortification recoveries were acceptable. An acceptable reagent fortification will have a recovery in the range of 70% to 120%.

Appendix 4:

Sample Calculations

Calculations:

1. The parts per million Vendex® residues were calculated as follows:

$$ppm = \frac{ng\ found}{mg\ inj} \qquad mg\ inj = \frac{\mu L\ injected \times final\ weight\ (g)}{final\ volume\ (mL)}$$

The ng found was calculated from the standard curve's regression line.

2. The spike recoveries were calculated as follows:

$$percent\ recovery = \frac{ppm\ found}{ppm\ fortified} \times 100$$

Example:

1. Control S000 54112 + 0.40 ppm Vendex®, SD14114, analyzed on July 25, 1991.

The ppm calculated as follows:

$$ppm = \frac{ng\ found}{mg\ inj} \qquad ppm = \frac{1.00\ ng}{3.0\ mg\ inj} = 0.333$$

$$mg\ inj = \frac{2\ \mu L \times 6\ g}{4\ mL} = 3.0$$

The spike recovery was calculated as follows.

$$\frac{0.333\ ppm}{0.40\ ppm} \times 100 = 83\%$$

2. Control, S000 54114 + 0.40 ppm Vendex® SD31723 analyzed on July 26, 1991.

The ppm calculated as follows:

$$ppm = \frac{ng\ found}{mg\ inj} \qquad ppm = \frac{1.10\ ng}{3.0\ mg\ inj} = 0.367$$

$$mg\ inj = \frac{2\ \mu L \times 6\ g}{4\ mL} = 3.0$$

Appendix 4:

Sample Calculations, continued.

The spike recovery was calculated as follows:

$$\frac{0.367 \text{ ppm}}{0.40 \text{ ppm}} \times 100 = 92\%$$

3. Control, S000154112 + 0.40 ppm Vendex®, SD33608 analyzed on July 25, 1991.

The ppm calculated as follows:

$$\text{ppm} = \frac{\text{ng found}}{\text{mg inj}} \qquad \text{ppm} = \frac{0.730 \text{ ng}}{2.0 \text{ mg inj}} = 0.365$$

$$\text{mg inj} = \frac{2 \text{ uL} \times 6 \text{ g}}{6 \text{ mL}} = 2.0$$

The spike recovery calculated as follows:

$$\frac{0.365 \text{ ppm}}{0.40 \text{ ppm}} \times 100 = 91\%$$

CALIBRATION CURVE FOR VENDEX (SD 14114)