13544

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Chemical:	**Metalaxyl; D-Alanine, N-(2,6-dimethylphenyl)-N-(met**
PC Code:	**113501; 113502**
HED File Code	**11000 Chemistry Reviews**
Memo Date:	**09/13/2001**
File ID:	**DPD275477**
Accession Number:	**412-02-0006**

HED Records Reference Center
12/10/2001

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

EPA SERIES 361
SCIENTIFIC DATA REVIEWS
HEALTH EFFECTS DIVISION
OPP OFFICIAL RECORD

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: September 13, 2001

MEMORANDUM

SUBJECT: Metalaxyl/Mefenoxam Analytical Method Recovery Data in Livestock Commodities.

DP Barcode:	D275477	PRAT Case:	819456
Submission No.:	S547725	Caswell No.:	None
PC Code:	113501 and 113502	Class:	Fungicide
Trade Name:	Not Applicable	EPA Reg. No.:	Not Applicable
40 CFR:	180.408 and 180.546		
MRID Nos.:	44208101		

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09/14/2001

TO: Mary Waller/Thomas Ellwanger, PM Team #21
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and

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INTRODUCTION

Syngenta (formerly Novartis Crop Protection, Inc. and formerly Ciba Crop Protection) submitted additional method validation data for the fungicides metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester] and mefenoxam [(R)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester; CGA-329351] in livestock commodities. The method validation data were requested under the metalaxyl reregistration process (D172350, S. Hummel, 9/15/93). Mefenoxam (CGA-329351) is the R isomer; metalaxyl (CGA-48988) is a mixture of the R and S isomers.

Tolerances have been established for metalaxyl on livestock commodities (40 CFR §180.408) at 0.4 ppm in the fat, kidney, and liver of cattle, goats, hogs, horses, poultry, and sheep; 0.05 ppm in meat and meat byproducts (except kidney and liver) of cattle, goats, hogs, horses, poultry, and sheep; 0.02 ppm in milk, and 0.05 ppm in eggs.

Tolerances for residues of metalaxyl in/on raw and processed plant commodities and livestock commodities are currently expressed in the CFR in terms of the combined residues of metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester] and its metabolites containing the 2,6-dimethylaniline moiety, and *N*-(2-hydroxymethyl-6-methylphenyl)-*N*-(methoxyacetyl)-alanine methyl ester, each expressed as metalaxyl equivalents. However, the HED Metabolism Committee (now called the Metabolism Assessment Review Committee) determined in a meeting on 9/8/93 that the residues to be regulated in livestock commodities are metalaxyl, metabolites that can be converted to 2,6-dimethylaniline (2,6-DMA), and those metabolites containing the 2-hydroxymethyl-6-methylaniline (HMMA) moiety (S. Hummel, 9/10/93).

The Metabolism Assessment Review Committee (D269910, N. Dodd, 10/27/00) discussed mefenoxam on 10/24/00 and concluded that the residues to be regulated for the tolerance expression and for dietary risk assessments would be the following:

Plants

(R)- and (S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester, its metabolites containing the 2,6-dimethylaniline moiety, and *N*-(2-hydroxymethyl-6-methylphenyl)-*N*-(methoxyacetyl)alanine methyl ester*, each expressed as mefenoxam equivalents

[* *N*-(2-hydroxymethyl-6-methylphenyl)-*N*-(methoxyacetyl)alanine methyl ester is CGA-94689.]

Livestock

(R)- and (S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester, its metabolites containing the 2,6-dimethylaniline moiety, and its metabolites containing the 2-hydroxymethyl-6-methylaniline moiety, each expressed as parent equivalents

Rotational Crops

(R)- and (S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester, its metabolites containing the 2,6-dimethylaniline moiety, and *N*-(2-hydroxymethyl-6-methylphenyl)-*N*-(methoxyacetyl)alanine methyl ester, each expressed as parent equivalents, except that 2-[(methoxyacetyl)(2-methoxy-1-methyl-2-oxoethyl)amino]-3-methylbenzoic acid (CGA-108905) and *N*-(3-hydroxy-2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine methyl ester (CGA-100255) will be considered in risk assessments involving the foliar use of mefenoxam.

Metalaxyl is a List A chemical. A Metalaxyl Reregistration Standard and Guidance Document was issued on 12/81. Product and Residue Chemistry Chapters of the Metalaxyl Registration Standard were issued on 6/22/87. The Metalaxyl Final Reregistration Standard and Tolerance Reassessment (FRSTR) Guidance Document was dated 9/88. The Metalaxyl Product Chemistry and Residue Chemistry Reregistration Standard Updates were issued on 3/13/91. There is a Metalaxyl Product Chemistry and Residue Chemistry Registration Standard Update dated 4/92. The Product and Residue Chemistry Chapters for the Metalaxyl Reregistration Eligibility Decision Document (DP Barcodes D197037 and D197066, CBRS #12906 and 12907) were issued on 6/16/94.

The structure of metalaxyl/mefenoxam is shown in Figure 1 below.

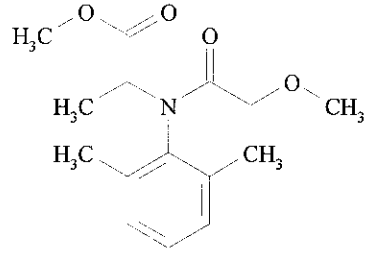


Figure 1. Metalaxyl/Mefenoxam

BACKGROUND

An adequate enforcement method (Method II in PAM II; Method AG-349) is available for metalaxyl/mefenoxam, its metabolites containing the 2,6-dimethylaniline moiety (as represented by combined CGA-67869, CGA-107955, and CGA-62826), and CGA-94689 in livestock tissues, milk, and eggs. Method AG-349 is a modification of Method AG-348. Method AG-349 converts residues of metalaxyl and its metabolites to 2,6-dimethylaniline (DMA), which is analyzed by GLC with alkali flame ionization detection (AFID) in the nitrogen-specific mode. An alternative procedure is gas-liquid chromatography/mass spectroscopy (GC/MS) in the chemical ionization mode with selected ion monitoring (SIM) of the M+1 ion at m/z 268. A successful EPA petition method validation has been conducted on beef liver and milk, which were fortified with metalaxyl, CGA-94689, and a mixture of CGA-62826, CGA-67869, and CGA-107955. The method was validated at 0.4 ppm for beef liver and 0.02 ppm for milk. Although recoveries of CGA-94689 in the EPA method validation of AG-349 were low (i.e., 45-48% in beef liver and 48-50% in milk), EPA determined that the method was adequate as an enforcement method (PP#2F2732, K.H. Arne, PhD, 12/28/82). This conclusion was based on a toxicology determination that metalaxyl and its metabolites are not acutely toxic and show little or no propensity towards mutagenicity or carcinogenicity (PP#1F2500, W. Dykstra, 10/8/82).

Method AG-576 (MRID 42115807) is a combination of methods AG-349 (Method II, PAM II) and AG-395. Method AG-395, which is a modification of Method AG-348 (Method I, PAM II), was forwarded to FDA for inclusion in PAM II as Method III. Method AG-576 converts residues of metalaxyl/mefenoxam and its metabolites to 2,6-dimethylaniline (DMA), which is analyzed by GLC and detected by a nitrogen/phosphorus detector (NPD). The limit of quantitation is 0.05 ppm (in parent equivalents). Recovery data for metalaxyl ***per se*** using method AG-576 indicate that the method adequately recovers residues of parent ***per se*** from livestock tissues (D172350, S. Hummel, 9/15/93).

Radiovalidation data using poultry and goat metabolism samples have been provided for AG-576, including total percent extracted and percent recovered as DMA. The data show a fairly good correlation between total metalaxyl recoveries and the amount of metabolites containing the dimethylaniline moiety for poultry liver and eggs, and goat muscle and milk. Less correlation was observed for poultry fat and muscle, and goat liver and fat. The data from the radiolabel validation imply that Method AG-576 will not adequately recover metalaxyl metabolites containing the hydroxymethyl methyl aniline moiety (ex. CGA-94689) (D172350, S. Hummel, 9/15/93).

CONCLUSIONS

OPPTS GLN 860.1340: RESIDUE ANALYTICAL METHOD

1. Adequate recoveries were not obtained for CGA-94689 using Method AG-576 for beef liver, poultry thigh muscle, poultry skin/attached fat, and eggs since 1) recoveries should lie between 70% and 120% and should not vary significantly from sample to sample and/or 2) control values should be reasonably low in relation to the proposed tolerance/spiking level, preferably less than 20% of the proposed tolerance/spiking level.

2. Adequate recoveries were generally not obtained for CGA-62826 using Method AG-576 for beef liver, poultry skin/attached fat, and eggs since 1) recoveries should lie between 70% and 120% and should not vary significantly from sample to sample and/or 2) control values should be reasonably low in relation to the proposed tolerance/spiking level, preferably less than 20% of the proposed tolerance/spiking level. Exceptions are 1) the control and CGA-62826 recoveries for poultry thigh muscle at the 0.10 ppm fortification level and possibly 2) CGA-62826 controls and recoveries in beef liver and poultry thigh muscle at the highest reported level (0.50 ppm), but only one recovery value was reported for each matrix at that level.

3. The registrant should attempt to improve recoveries for CGA-94689 and CGA-62826 and provide recovery data for P1 and P2 using Method AG-576 for all livestock commodities.

4. An independent laboratory method validation and an EPA method validation are needed for method AG-576 on liver, milk, and eggs so that it can be included in PAM II as an enforcement method.

5. Radiovalidation data using poultry and goat metabolism samples have been provided for AG-576, including total percent extracted and percent recovered as DMA. The data show a fairly good correlation between total metalaxyl recoveries and the amount of metabolites containing the dimethylaniline moiety for poultry liver, eggs, goat muscle, and milk. Less correlation was observed for poultry fat and muscle, and goat liver and fat. The data from the radiolabel validation imply that Method AG-576 will not adequately recover metalaxyl metabolites containing the hydroxymethyl methyl aniline moiety (ex. CGA-94689). Additional radiovalidation will be required for any improved enforcement method.

RECOMMENDATION

The registrant should be informed of the status of the validation data for Method AG-576 on livestock commodities as stated in Conclusions 1, 2, 3, 4, and 5 above.

DETAILED CONSIDERATIONS

See Attachment 1 for the chemical names and structures of metalaxyl/mefenoxam and its metabolites which are discussed in this review.

OPPTS GLN 860.1340: RESIDUE ANALYTICAL METHOD

Livestock Commodity Method AG-576 (MRID 44208101)

Additional method validation data for method AG-576 for representative metalaxyl metabolites, including at least one metabolite containing the 2,6-dimethylaniline (DMA) moiety, the hydroxy metabolite CGA-94689, and P1 and P2, were requested for all livestock commodities (D172350, S. Hummel, 9/15/93). In response, method validation data for Method AG-576 for CGA-94689 and CGA-62826 (which contains the 2,6-dimethylaniline moiety) in meat and eggs were submitted (MRID 44208101, citation below). The registrant indicated that validation data on P1/P2 would be submitted later.

MRID 44208101 Eudy, L.W., PhD (1997) Method Validation Trial for the Determination of CGA-94689, P1/P2, and CGA-62826 in Meat and Eggs Using Analytical Method AG-576, Laboratory Project Number ABR-96108, Study Number 77-95, unpublished study sponsored by Ciba-Geigy Corporation, 63 p.

In MRID 44208101, control samples of beef liver, poultry thigh, poultry skin/attached fat, and eggs were fortified with either CGA-94689 or CGA-62826 or metalaxyl at 0.05 ppm, 0.10 ppm, and 0.50 ppm. Analytical Method AG-576 was used to determine residues. In Method AG-576, residues are extracted from muscle, liver and egg tissues with acetonitrile containing 20% water, from fat and skin with hexane, and from eggs with acetonitrile. The samples are homogenized and filtered. Residues in the hexane extracts are partitioned into acetonitrile, and residues in acetonitrile extracts are partitioned into hexane and back into acetonitrile. The acetonitrile fractions are concentrated. The residues are refluxed with methanesulfonic acid. The solution is made basic. The DMA which is formed is distilled and further cleaned up using silica cartridges. The DMA is analyzed by GLC/NPD. The limit of quantitation is 0.05 ppm (in metalaxyl equivalents). DMA results were calculated in ppm and converted to CGA-94689 or CGA-62826 equivalents by multiplying by the molecular weight ratios of 2.437 or 2.189, respectively. Recoveries for CGA-94689 and CGA-62826 are tabulated below:

Table 1. Recovery Data for Method AG-576 for CGA-94689 and CGA-62826 in Beef Liver					
Commodity	Chemical Added	PPM Added	PPM Found	% Recovery (corrected)[1]	% Recovery (uncorrected)[2]
beef liver	CGA-94689	0.00	0.082	NA[3]	NA
		0.05	0.124	84	248
		0.05	0.088	12	176
		0.10	0.132	50	132
		0.10	0.118	36	118
		0.50	0.170	18	34
	CGA-62826	0.00	0.070	NA	NA
		0.05	0.118	96	236
		0.05	0.081	22	162
		0.10	0.142	72	142
		0.10	0.148	78	148
		0.50	0.527	91	105

[1] Recoveries were corrected for controls.

[2] These recoveries were not corrected for controls.

[3] Not applicable

Table 2. Recovery Data for AG-576 for CGA-94689 and CGA-62826 in Poultry Thigh Muscle					
Commodity	Chemical Added	PPM Added	PPM Found	% Recovery (corrected)[1]	% Recovery (uncorrected)[2]
poultry thigh muscle	CGA-94689	0.00	<0.05 (0.016)	NA[3]	NA
		0.05	0.048	64	96
		0.05	0.048	64	96
		0.10	0.061	45	61
		0.10	0.069	53	69
		0.50	0.264	50	53
	CGA-62826	0.00	<0.05 (0.010)	NA	NA
		0.05	0.061	102	122
		0.05	0.068	116	136
		0.10	0.099	89	99
		0.10	0.108	98	108
		0.50	0.412	80	82

[1] Recoveries were corrected for controls.

[2] These recoveries were not corrected for controls.

[3] Not applicable

Table 3. Recovery Data for AG-576 for CGA-94689, CGA-62826, and Metalaxyl in Poultry Skin/Attached Fat					
Commodity	Chemical Added	PPM Added	PPM Found	% Recovery (corrected)[1]	% Recovery (uncorrected)[2]
poultry skin/attached fat	CGA-94689	0.00	<0.05 (0.000)	NA[3]	NA
		0.05	0.004	8	8
		0.05	0.004	8	8
		0.10	0.011	11	11
		0.10	0.008	8	8
		0.50	0.047	9	9
	CGA-62826	0.00	<0.05 (0.007)	NA	NA
		0.05	0.007	0	14
		0.05	0.008	2	16
		0.10	0.007	0	7
		0.10	0.007	0	7
		0.50	0.008	0.2	2

[1] Recoveries were corrected for controls.

[2] These recoveries were not corrected for controls.

[3] Not applicable

Table 4. Recovery Data for AG-576 for CGA-94689 or CGA-62826 in Eggs					
Commodity	Chemical Added	PPM Added	PPM Found	% Recovery (corrected)[1]	% Recovery (uncorrected)[2]
eggs	CGA-94689	0.00	<0.05 (0.004)	NA[3]	NA
		0.05	0.026	44	52
		0.05	0.058	108	116
		0.10	0.025	21	25
		0.10	0.057	53	57
		0.50	0.221	43	44
	CGA-62826	0.00	<0.05 (0.004)	NA	NA
		0.05	0.033	58	66
		0.05	0.030	52	60
		0.10	0.058	54	58
		0.10	0.060	56	60
		0.50	0.291	57	58

[1] Recoveries were corrected for controls.

[2] These recoveries were not corrected for controls.

[3] Not applicable

Conclusions

Adequate recoveries were not obtained for CGA-94689 using Method AG-576 for beef liver, poultry thigh muscle, poultry skin/attached fat, and eggs since 1) recoveries should lie between 70% and 120% and should not vary significantly from sample to sample and/or 2) control values should be reasonably low in relation to the proposed tolerance/spiking level, preferably less than 20% of the proposed tolerance/spiking level.

Adequate recoveries were generally not obtained for CGA-62826 using Method AG-576 for beef liver, poultry skin/attached fat, and eggs since 1) recoveries should lie between 70% and 120% and should not vary significantly from sample to sample and/or 2) control values should be reasonably low in relation to the proposed tolerance/spiking level, preferably less than 20% of the proposed tolerance/spiking level. Exceptions are 1) the control and CGA-62826 recoveries for poultry thigh muscle at the 0.10 ppm fortification level and possibly 2) CGA-62826 controls and recoveries in beef liver and poultry thigh muscle at the highest reported level (0.50 ppm), but only one recovery value was reported for each matrix at that level.

The registrant should attempt to improve recoveries for CGA-94689 and CGA-62826 and provide recovery data for P1 and P2 using Method AG-576 for all livestock commodities.

An independent laboratory method validation and an EPA method validation are needed for method AG-576 on liver, milk, and eggs so that it can be included in PAM II as an enforcement method.

Radiovalidation data using poultry and goat metabolism samples have been provided for AG-576, including total percent extracted and percent recovered as DMA. The data show a fairly good correlation between total metalaxyl recoveries and the amount of metabolites containing the dimethylaniline moiety for poultry liver, eggs, goat muscle, and milk. Less correlation was observed for poultry fat and muscle, and goat liver and fat. The data from the radiolabel validation imply that Method AG-576 will not adequately recover metalaxyl metabolites containing the hydroxymethyl methyl aniline moiety (ex. CGA-94689). Additional radiovalidation will be required for any improved enforcement method.

Attachment 1: Names and Structures of Mefenoxam, Metalaxyl, CGA-94689, CGA-62826, P1, and P2

cc: N. Dodd (810C), PM#21, PM#53, M. Rust (810J)
RDI: ChemTeam:7/18/01:S. Dapson:8/31/01
7509C:RAB3:CM#2:Rm810C:305-5681:N. Dodd:nd:9/13/01

ATTACHMENT 1

Table 5. Names and Structures of Mefenoxam, Metalaxyl, CGA-94689, CGA-62826, P1, and P2

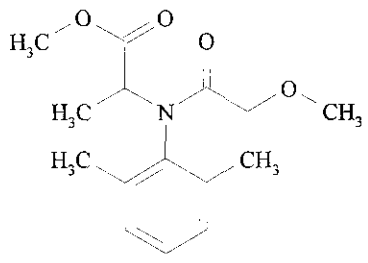
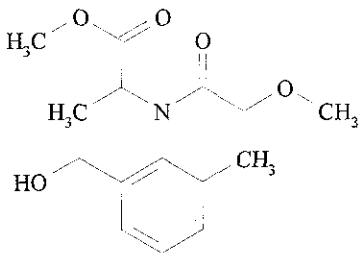
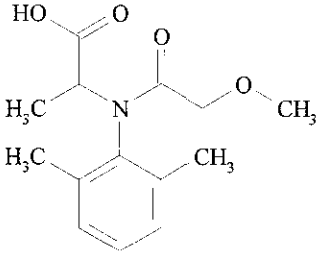
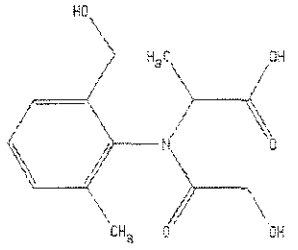
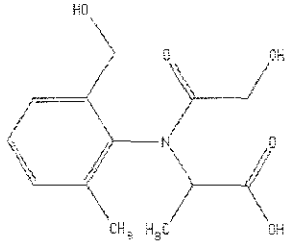
Chemical Name Common Name (Company Code)	Structure
(R)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester mefenoxam (CGA-329351) and *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester metalaxyl (CGA-48988)	
N-(2-hydroxymethyl-6-methylphenyl)-N-(methoxyacetyl)alanine methyl ester (CGA-94689)	
N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine (CGA-62826)	

Table 5. Names and Structures of Mefenoxam, Metalaxyl, CGA-94689, CGA-62826, P1, and P2	
Chemical Name Common Name (Company Code)	Structure
N-[2-(hydroxymethyl)-6-methylphenyl-N-(hydroxyacetyl)alanine[1] (P1)	
N-[2-(hydroxymethyl)-6-methylphenyl]-N-(hydroxyacetyl)alanine[1] (P2)	

[1] P1 and P2 are isomers.