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

September 15, 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metalaxyl (113501) Additional Ruminant and Poultry Metabolism Data
Poultry Feeding Study
[MRID Nos. 42115801 to -10; CB No. 9102; DP Barcode D172350]

FROM: Susan V. Hummel, Chemist
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THRU: Francis B. Suhre, Section Head
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TO: C. Peterson/W. Waldrop, PM#71
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Special Review and Reregistration Division (H7508C)

Attached please find the review of additional ruminant and poultry metabolism data and a poultry feeding study, submitted by Ciba Geigy in response to the Metalaxyl FRSTR (9/88). These data were reviewed by Acurex Environmental Corporation under the supervision of CBRS. The review has undergone secondary review in CBRS and has been revised to be consistent with Branch policies.

The ruminant and poultry metabolism studies are adequate. The metabolites of concern are those convertible to 2,6-dimethyl aniline and those containing the 2-hydroxymethyl-6-methyl-aniline moiety. Additional data are needed to determine if the analytical method (AG-576) is adequate, including recoveries for representative metalaxyl metabolites, including the hydroxy metabolite CGA-94689 and P1 and P2. Storage stability data for metalaxyl and its metabolites (including the hydroxy metabolite CGA-94689) are still needed for livestock commodities. Existing feeding studies will be adequate, provided adequate recovery data and adeqyate storage stability data are provided. If you need additional input, please advise.

cc w/ attachment: addressee, S. Hummel, Metalaxyl RSF, R.F., circu, metalaxyl S.F.
RDI:FBS:09/03/93:BZ:09/15/93
H7509C:CBII:SVH:svh:RM:810:CM#2:09/15/93
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METALAXYL
(Chemical Code 113501)
(CBRS No. 9102; DP Barcode D172350)

TASK 3

Registrant's Response to Residue Chemistry Data Requirements

September 15, 1993

Contract No. 68-DO-0142

Submitted to:

U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:

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METALAXYL

(Chemical Code 113501)

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REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

Task 3

BACKGROUND

The Metalaxyl Final Registration Standard and Tolerance Reassessment (FRSTR) Guidance Document dated 9/88 required data on animal metabolism. In response, Ciba-Geigy Corporation submitted data concerning the nature of the residue in goats (1990; MRID 41664503) and hens (1990; MRID 41664504). These data were reviewed in the Metalaxyl Residue Chemistry Reregistration Standard Update dated 3/91. The Agency concluded that the nature of the residue in animals is not adequately understood. The major component in milk was an unidentified "metabolite A" that accounted for 40% of the total radioactive residue (TRR). The major metabolites identified in goat tissues were: N-(2,6-dimethyl-phenyl)-N-(hydroxy-acetyl)-alanine (CGA 107955) accounting for 13.5-31.5% of the TRR in goat liver, kidney, leg muscle, and fat; N-(2-hydroxy-methyl)-6-methylphenyl]-N-(methoxy acetyl)-alanine methyl ester (CGA 94689; isomers A and B) accounting for 34.2% of the TRR in kidney, and 12.1 and 14.4% of the TRR in perirenal fat and leg muscle, respectively; and N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-alanine methyl ester (CGA 67869) accounting for 13.3% of the TRR in leg muscle. The update specified that "metabolite A" isolated from milk and residues in hen liver, muscle, skin and fat required further characterization. The registrant was required to release unextracted residues from hen liver solids using acid hydrolysis, to further separate and identify aqueous soluble residues released following collagenase treatment, to employ additional chromatographic techniques to resolve and identify a co-eluting unknown from hen metabolite CGA-79353, and to further characterize unresolved residues in hen breast muscle, and unknowns in skin with attached fat, breast, and thigh muscle.

A letter dated 6/13/91 from Ciba-Geigy to the Agency relates the outcome of a meeting of the registrant with CBRS on 6/12/91 where animal metabolism requirements were discussed. The letter indicated that CBRS agreed with the registrant that only one poultry fat needed to be considered, not both skin and peritoneal fat and that efforts could be concentrated on thigh muscle only, instead of both liver and thigh because the TLC profiles were similar. In addition, the Agency indicated that additional characterization was required of egg residues, and that results should be expressed in terms of whole egg, not yolk and white.

The update also reiterated the Guidance Document requirements for the analysis of representative livestock metabolism samples by enforcement methodology, and reserved judgement on the available data concerning animal commodities and the adequacy of the established tolerances due to the outstanding requirement for data on animal metabolism.

Ruminant feeding studies were submitted and reviewed in the Residue Chemistry Chapter of 6/87. The Agency concluded that the adequacy of the data on magnitude of the residue in meat, milk, poultry, and eggs would be determined upon receipt of the required metabolism studies. In addition, the update stated that following receipt and review of the additional data requested from the livestock metabolism studies and from crop field studies and processing studies, the need for additional animal feeding studies will be determined, and the established tolerances for residues in animal commodities will be assessed.

In response to these requirements, Ciba-Geigy Corporation submitted the following data (CBRS No. 9102): supplemental data pertaining to the metabolism of metalaxyl in goats (1991; MRIDs 42115802, 42115805-6), additional poultry metabolism data (1991; MRID 42115804), a hen feeding study (1991; MRID 42115809), a residue analytical method, AG-576 (1982; MRID 42115810), and studies (1991; MRIDs 42115807 and 42115808) concerning analyses of representative samples from the livestock metabolism studies using an update of the current enforcement methods, all of which are reviewed here for their adequacy in fulfilling the outstanding data requirements. In addition, the registrant submitted protocol amendments (1990; MRIDs 42115801 and 42115803) for the animal metabolism studies. These were GLP submissions and have no bearing on this review.

The Metalaxyl FRSTR Residue Chemistry Chapter and FRSTR Guidance Document concluded that the qualitative nature of metalaxyl residues in plants is adequately understood. The data indicate that metalaxyl and its metabolites containing the 2,6-dimethylaniline moiety and N-(2-hydroxy methyl-6-methyl)-N-(methoxyacetyl)-alanine methyl ester are the residues of concern in plants. Ciba-Geigy analytical method AG-395, a modification of Method I in PAM, Vol. II (Pesticide Reg. Sec. 180.408) adequately recovers these residues from plant tissues and has undergone successful Agency validation. Ciba-Geigy analytical method AG-395 has been forwarded to FDA for inclusion in PAM II as Method III (M. Bradley letter of 2/18/87 to A. Marcotte, FDA).

As of this review, the nature of the residue in animals is adequately understood. The issue of whether the tolerance expression needs to be revised will be addressed after additional method recovery data are received and reviewed. Tolerances for residues in or on animal commodities are currently expressed in terms of the combined residues of metalaxyl and its metabolites containing the 2,6-dimethylaniline moiety, and N-(2-hydroxy methyl-6-methyl)-N-(methoxyacetyl)-alanine methyl ester (CGA-94689) (40 CFR §180.408[a]). Although Method II in PAM Vol. II has undergone an Agency validation resulting in adequate recoveries of the parent metalaxyl and the metabolites containing the 2,6-dimethylaniline moieties (CGA-67869, CGA-107955, and CGA-62826), and has been deemed an acceptable enforcement method, we note that the method only recovers 45-48% of the hydroxy metabolite CGA-94689. The low recoveries of the hydroxy metabolite CGA-94689 were considered acceptable because the hydroxy metabolite comprised less than 20% of the total residue in plants (K. Arne, 8/2/84, PP#3F2918/3F2919). We note that CGA-94689 comprises a much large portion of the total residue in livestock commodities.

CONCLUSIONS/RECOMMENDATIONS

1a. The qualitative nature of the residue in goats is adequately understood. The supplemental data adequately address the deficiency cited in the 1991 update. In goat milk from goats fed [^{14}C]metalaxyl, the principal residue was "metabolite A," which accounted for 40% of the total radioactive residue (TRR). In the supplemental study "metabolite A" was isolated from goat milk (65.7% of the TRR), and its components were identified as C8 and C10 fatty acid conjugates of CGA-67869 (which contains the 2,6-dimethylaniline moiety), wherein the fatty acids are connected to CGA-67869 through its hydroxyacetyl group. The data from the supplemental study confirm the metabolic pathways of metalaxyl as reported in the original study. That is, metalaxyl in goats may be hydrolyzed to the ester alcohol and the acid alcohol which may then be N-dealkylated. Alternatively, oxidation can lead to either benzylic alcohol or phenolic compounds. No additional ruminant metabolism data are required.

1b. The qualitative nature of the residue in poultry is adequately understood. The additional data submitted indicate that the predominant metabolites are the disubstituted free acid form (P1) of the hydroxy metabolite CGA-94689(B) (37.4% TRR in thigh muscle, and 13.0% TRR in whole egg), the sulfuric acid conjugate of CGA-94689(B) (29.8% TRR in thigh muscle), the disubstituted free acid form (P2) of CGA-94689(A) (9.2% TRR in thigh muscle, and 10.8% TRR in whole egg), CGA-107955 (9.6% TRR in whole egg, and 40.2% TRR in peritoneal fat), and a fatty acid conjugate of P1 and P2 (26.8% TRR in peritoneal fat). The parent, metalaxyl, was isolated only in whole egg (4.3% TRR). Based on these additional data, the registrant proposed a new metabolic pathway indicating metalaxyl is hydrolyzed to either the benzylic alcohol CGA-94689 or the ester alcohol CGA-67869; subsequently the hydroxy metabolite CGA-94689 is converted to the sulfate P4 and CGA-67869 is converted to the fatty acid conjugate U3 or the acid alcohol CGA-107955; and CGA-107955 is subsequently hydrolyzed to the benzylic alcohol. No additional poultry metabolism data are required.

2. The metabolic profiles in ruminant and poultry do not differ substantially. Therefore, the qualitative nature of metalaxyl residues in animals is adequately understood. The issue of whether the tolerance expression needs to be revised to include the sulfuric acid conjugate and substituted forms of the hydroxy metabolite CGA-94689 will be addressed after additional method recovery data are received and reviewed. No additional livestock metabolism data are required.

3a. Analytical method AG-576, used to analyze animal commodities treated with radiolabeled metalaxyl, is a combination of Method II in PAM, Vol II. and Ciba-Geigy method AG-395 each of which has undergone Agency validations. Ciba Geigy has provided data to demonstrate that metalaxyl, per se, is adequately recovered from livestock tissues. Recoveries of metalaxyl metabolites containing the DMA moiety were not reported. Recoveries of CGA-94689 was not reported. Recoveries of newly

reported metabolites containing the hydroxymethyl methyl aniline moiety were not reported. Method recoveries are needed for representative metalaxyl metabolites, including at least one metabolite containing the 2,6-dimethylaniline (DMA) moiety, CGA-94689, and P1 and P2, in all livestock commodities analyzed.

3b. The registrant was requested to provide data on radiovalidation of the metalaxyl enforcement method. Instead, analytical method AG-576 was used. 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, one of which is currently included in the tolerance expression (CGA-94689.

3c. Method AG-395, has been shown to adequately recover Metalaxyl and regulated metabolites in plant commodities. Method II in PAM, Vol II adequately recovers the parent metalaxyl and its metabolites containing the 2,6-dimethylaniline moiety, and recovers 45-48% of the hydroxy metabolite CGA-94689. Because significant levels of metalaxyl residues are expected in ruminant liver and kidney (see ruminant feeding studies, 1987 Residue Chemistry Chapter) and because the hydroxy metabolite CGA-94689 was isolated in ruminant kidney and liver and is included in the established tolerances, the registrant must validate method AG-576 showing adequate recoveries of metabolite CGA-94689 and P1 and P2. If new methodology is required to adequately recover metabolite CGA-94689, additional validation will be required using existing representative samples from radiolabeled metabolism studies.

4a. The results from the submitted poultry feeding study indicate that residues of metalaxyl will not exceed the established tolerances in poultry tissues and eggs. However, judgement of the adequacy of the available data is reserved until the analytical method used is determined to be adequate to recover all metalaxyl residues of concern, and adequate storage stability data for metalaxyl and its major metabolites (including CGA-94689) are provided.

4b. Previously submitted and reviewed ruminant feeding studies indicated that residues were approximately 5 ppm in kidney from a cow dosed at 75 ppm and slaughtered 6.5 hours after the last dose and 0.5 ppm in kidney from a goat dosed at 7 ppm and sacrificed at 4 hours. If the maximum dietary burden for cattle is 16 ppm, based on the 20-ppm tolerance for alfalfa hay at 80% of the diet, residues from a 1x exposure would be expected at about 1 ppm, a level greater than the established 0.4-ppm tolerance for kidney. However, residues in kidney from cows sacrificed after 19-23.4 hours and goats sacrificed after 10-24 hours decreased to <0.1-0.14 ppm. The review of these data (review of PP#1F2500/1H5299 Amendment; P. Errico, dated 7/16/82) concluded that residues in liver and kidney are transient in nature and that

the available data were adequate to support a tolerance of 0.4 ppm in kidney and liver.

DETAILED CONSIDERATIONS

Qualitative Nature of the Residue in Animals

Ruminants. In a previous submission that was discussed in detail in the 1991 Update, Ciba-Geigy presented data (1990; MRID 41664503) pertaining to the metabolism of metalaxyl in goats. In that study, two lactating goats (Alpines) were dosed orally at a feeding level of 76.9 ppm (4x) with uniformly ring labeled [^{14}C]metalaxyl for four consecutive days. The specific activity of the test substance was 37.7 μCi/mg with a radiochemical purity of >97%. During the dosing period, milk samples were taken once in the morning and once in the afternoon. The afternoon and the following morning milk from each goat were combined as a daily milk sample. Residues in milk were highest at the fourth day, 0.121 and 0.415 ppm for goat 1 and goat 2, respectively. Seventy-four percent of the radioactivity in milk was solubilized with the initial acetonitrile (ACN) solvent extraction. The milk extracts were treated with glucuronidase, then extracted with dichloromethane (DCM) followed by ethyl acetate (EtOAc). The resulting DCM and EtOAc fractions were analyzed by TLC. The major component in milk, 40% of the total radioactive residue (TRR), was the unknown "metabolite A."

In the submitted supplemental data (1991; MRIDs 42115802, 42115805-06), the isolation and identification of "metabolite A" from goat 2, day 2, milk samples is presented. The samples were stored for approximately 33 months. Storage stability data and data on recent radioassays of whole samples were not included with the supplemental study. The reviewer is assuming that compounds in the samples have remained stable over time.

Extraction

A sample of milk was extracted with dichloromethane (DCM), cleaned-up by solubilization in acetonitrile (ACN), frozen at -20 °C overnight, and fat and liquid (organic/aqueous) layers separated by filtration. Solid fractions resulting from the extraction of milk with DCM were pooled and washed with methanol (MeOH) resulting in a solid fraction and a MeOH wash fraction. The aqueous fraction of the milk after extraction with DCM was concentrated and dissolved in ACN that resulted in an ACN fraction and a precipitate. The precipitate was pooled with the other solids, dried, combusted, and not analyzed further (12% of the TRR). The ACN fraction was pooled with the MeOH wash and not analyzed further (14.3%). The organic fraction (73.8% of the TRR) was subjected to TLC, silica column chromatography and mass spectral analyses.

Of the 73.8% of the total radioactivity in milk that was extractable with DCM, 65.7% of the TRR represented "metabolite A." In the original study, "metabolite A" reportedly

represented 40% of the TRR. The registrant stated that the higher recovery of "metabolite A" in the supplemental study was obtained through the use of more efficient extraction procedures.

Characterization of residues

Milk fractions were co-chromatographed with standards on normal phase TLC using silica gel plates using 12 solvent systems in addition to the solvent systems described in the initial study. The areas of the TLC plates containing the radioactive components were scraped and eluted with chloroform. One-dimensional reversed-phase TLC was also performed under conditions similar to the initial study.

Thin layer chromatography analysis of the milk DCM soluble fraction isolated "metabolite A" (65.7% of the TRR) as a distinct band. Upon rechromatography, the single band yielded two bands, Aa (56.5%) and Ab (9.2%). Aa was applied to a silica column, eluted with $CHCl_3$ and the resulting fractions rechromatographed on multidimensional TLC resulting in isolation of Aa_1 (43.9% of the TRR), Aa_2 (7.6%), Aa_3 (3.4%) and several components that combined accounted for 1.6% of the TRR. Band Ab was also rechromatographed on multidimensional TLC and resulted in one radioactive area.

"Metabolite A" and each of its components (Aa_1, Aa_2, Aa_3, and Ab), as well as the standards CGA-67869 and CGA 107955, were treated, in parallel experiments, with esterase. Each sample was incubated for 4.5 hours at 37 °C in a mixture containing esterase and potassium phosphate (pH 7.5). After incubation, the samples were centrifuged, extracted with ethyl acetate or chloroform, and the organic extract analyzed by TLC. These hydrolysis reactions led to the recovery of CGA-107955.

Component Aa_1 was treated with lipase in a mixture containing tris buffer (pH 8), 10% solution of gum arabic, and 45% solution of calcium chloride. The samples were incubated for 4.5 hours at 37 °C. After incubation, pH was adjusted to 2 and the samples were applied to a C10 Baker-bond extraction column. The column was washed with acidified water (HCl). The radioactivity was eluted with MeOH and analyzed by TLC.

"Metabolite A" was hydrolyzed in a mixture containing MeOH, water, and sodium hydroxide (NaOH) for half an hour in an ice bath. After evaporation of the MeOH, the sample was neutralized with dilute HCl. After complete removal of solvents, the remaining residue was dissolved in ACN and water and then extracted with ethyl acetate. The extract was analyzed by TLC.

The resulting extracts from the esterase, lipase, and NaOH hydrolyses were cochromatographed by TLC with the standards of CGA-107955, CGA-678669 and 4-(2,6-dimethyl-phenyl)-3-methyl-2,5-morpholinedione (CGA-68125).

The two bands (Aa and Ab) resulting from TLC chromatography of "metabolite A" were enzymatically hydrolyzed with glucuronidase, protease, and sulfatase under the same conditions as described in the initial study.

Components of "metabolite A" (Aa_1, Aa_2, Aa_3, Ab_1, and Ab_2) and the standard for the n-decanoic acid conjugate of CGA-67869 were analyzed by electron impact and/or chemical ionization gas chromatography/mass spectroscopy (GC/MS). The retention time of the standard was close but not identical to the retention times of Aa_{1-3}, and Ab_{1-2}. The spectrum of the standard was similar to the spectra for Aa_1, Aa_3, and Ab_1. The registrant, however, stated that the standard and the components were isomers of each other. The registrant stated that components Aa_1 and Aa_3 were different isomers of the C10 fatty acid conjugate of CGA-67869. Component Aa_2 was identified as a C8 fatty acid conjugate of CGA-67869 and component Ab was identified as a combination of a C10 fatty acid conjugate of CGA-67869 (Ab_1; 8.6% of the TRR) and a C-8 conjugate of CGA-67869 (Ab_2; 0.6% of the TRR).

In summary, "metabolite A" was isolated from goat milk and its components identified as C8 and C10 fatty acid conjugates of CGA-67869. In both types of conjugates, the fatty acids are connected to CGA-67869 through its hydroxyacetyl group. The data from the supplemental study support the metabolic pathways of metalaxyl as reported in the original study. That is, metalaxyl in goats may be hydrolyzed to the ester alcohol and the acid alcohol which may in turn be N-dealkylated. Alternatively, oxidation can lead to either benzylic alcohol or phenolic compounds. Chemical names and molecular structures of metalaxyl metabolites identified in tissues and used as standards are summarized in Figure 1.

Poultry. The registrant performed new analyses on thigh muscle, peritoneal fat, liver, egg whites, and excreta from the original study (1990; MRID 41664504) that was reviewed in detail in the 1991 Update.

In addition, a new study was conducted to generate additional egg and excreta samples for residue characterization. In the new study (1991; MRID 42115804), four White Leghorn laying hens were dosed daily for 5 days with uniformly ring-radiolabeled [^{14}C]metalaxyl (specific activity 49.0 μCi/mg, radiochemical purity of 99.0%). Gelatin capsules containing the [^{14}C]metalaxyl were administered orally at rates equivalent to ca. 100 ppm in feed, representing 71x the maximum theoretical dietary exposure (based on tolerances for soybeans and wheat grain). Excreta and eggs were collected each morning. Excreta samples were stored frozen. The eggs were separated into white and yolk and refrigerated. Approximately 6 hours after the last dose, the test animals were sacrificed, and blood and tissue samples were collected. The tissue samples were stored frozen. Excreta was shipped frozen, and egg whites and yolks were shipped refrigerated. Excreta and egg yolk samples generated in the second study were stored no longer than 101 days before analysis. Samples from the original study may have been stored for as long as 147 weeks. Storage stability data and data on recent radioassays of whole samples were not included with the supplemental study. The reviewer is assuming that compounds in the samples have remained stable over time.

Total radioactive residues (TRR)

Excreta samples generated in the second study were combusted, and the collected $^{14}CO_2$ was measured by liquid scintillation spectrometry (LSS). Egg yolk samples were analyzed directly by LSS. Detection limits were not reported. The total radioactive residue (TRR) in each commodity is presented in Table 1.

Extraction of Residues

Muscle. Samples of thigh muscle were lyophilized, the residues were extracted using MeOH, and the solids were separated and combusted. The MeOH fraction was partitioned with hexane/ether (unspecified ratio). The MeOH layer was cleaned up using a C-18 solid phase extraction (SPE) column, and residues were eluted separately into ACN and water. The aqueous layer was not analyzed further. The ACN and hexane layers were analyzed by 2D-TLC. In addition, the ACN layer was cochromatographed with non-radioactive standards, and residues were further characterized using HPLC.

Table 1. Distribution of radioactive residues in fractions of eggs and tissues from hens dosed with [^{14}C]metalaxyl at 100 ppm.

Commodity (Final Combined Fractions)	Total	Soluble		Insoluble	
	PPM	PPM	%TRR	PPM	%TRR
Thigh Muscle	0.674	0.596	88.5	0.077	11.4
(hexane/ether)[a]		[redacted]	[redacted]		
(ACN)[a]		[redacted]	[redacted]		
(water)		(0.008)	(1.2)		
Peritoneal Fat	0.254	0.244	95.9	0.010	4.1
(MeOH)[a]		[redacted]	[redacted]		
(oily residues/lipids)		(0.011)	(4.4)		
(aqueous)		(0.008)	(3.01)		
Egg Yolk, Day 5	0.360	0.287	79.6	0.047	13.1
(DCM)		(0.014)	(3.9)		
(MeOH)[a]		[redacted]	[redacted]		
($CHCl_3$)[a]		[redacted]	[redacted]		
(aqueous)[a]		[redacted]	[redacted]		
Egg White	0.179	0.157	87.9	0.022	12.1
(MeOH)[a]		[redacted]	[redacted]		
(water)		(0.019)	(10.4)		

[redacted]

(continued)

Egg white. Samples of egg whites were lyophilized, the residues were extracted using methanol (MeOH), the solids were separated and combusted, and the MeOH layer was analyzed by 2D-TLC. The MeOH fraction was cleaned up using a C-18 SPE column, and the residues were eluted and collected separately in water and MeOH. The water layer was not analyzed further; the MeOH fraction was cochromatographed with cold standards, and residues were further characterized using HPLC, and 2D-TLC.

Egg yolk. For egg yolk, an elaborate extraction scheme was employed in which organic-soluble residues, insoluble residues, and a crude phospholipid fraction precipitated from the initial organic solvent fraction were each subjected to various solvent partitioning/extractions and C-18 column chromatography. Then polar and nonpolar fractions from different sources were combined and subjected to additional C-18 and preparative TLC resulting in five final fractions: aqueous, MeOH, DCM, chloroform, and insolubles, each containing ^{14}C-residues from the three initial fractions. The distribution of egg yolk radioactivity in the final fractions is summarized in Table 1. Discrepancies were observed between the text of the submission and the flow chart provided. Although these errors were not serious enough compromise the outcome of the study, they did present difficulties in understanding some details of the extraction scheme. The paragraphs that follow represent this reviewer's interpretation of the egg yolk extraction procedure; the final fractions that appear in Table 1 are indicated below in [redacted].

Residues were extracted from egg yolk samples into chloroform ($CHCl_3$):MeOH (2:1, v,v). A crude phospholipid fraction was precipitated from the soluble residues by the addition of acetone in diethyl ether. The remaining triglycerides and neutral fats were concentrated and mixed with hexane. The acetonitrile-soluble residues were cleaned up using a C-18 SPE column, the neutral fats eluted with DCM, and the remaining residues were eluted with ACN (ACN-1). The DCM fraction was combined with the hexane fraction, further cleaned up using a C18 column, and eluted with ACN/MeOH and [redacted].

The unextracted residues were extracted first into MeOH:water (8:2, v/v) and then Soxhlet extracted with MeOH:water (9:1, v,v); the remaining [redacted] were radioassayed and not analyzed further. These methanol extracts were combined with the crude phospholipid fraction and the ACN-1 fraction from the initial organic extract. This combined fraction was treated with cold acetone to recrystallize the phospholipids. The precipitate and the filtrate were each cleaned up using preparative TLC. Residues in the major TLC bands were eluted and combined with the ACN/MeOH fraction from the organic residue cleanup described above. This combined fraction was cleaned up using a C18 column and residues eluted in [redacted], and [redacted] fractions. Residues in these fractions were analyzed using 2D-TLC and the metabolites scraped, eluted, and co-chromatographed with standards and excreta metabolites on 1D-TLC.

Fat. Residues in peritoneal fat were extracted with hexane, and partitioned into ACN. The ACN fraction was cleaned up using C-18 SPE columns, from which residues were eluted with ACN/MeOH and then with DCM. The collected ACN/MeOH fraction was

radioassayed and analyzed by 2D-TLC. Subsamples of the ACN/MeOH fraction were hydrolyzed with lipase, protease, cholesterol esterase or carboxylic acid esterase, and the released residues were analyzed by 1D-TLC. The DCM fraction was combined with the initial hexane fraction and solids; the resulting fraction was radioassayed and then partitioned with MeOH/water. The solids and oils were separated and the MeOH/water was combined with the ACN/MeOH elute (analyzed by 2D-TLC), and further cleaned up with C-18 SPE columns. The oily/lipid residues were radioassayed. The residues were eluted in water and methanol, after which the MeOH fraction was cleaned up using preparative TLC. The polar residues were collected and cochromatographed with excreta metabolites, and the nonpolar residues were hydrolyzed with esterase for 4.5 hours in potassium phosphate buffer (pH 7.5) at 37 °C, and then partitioned into methanol. The remaining water layer was radioassayed, and the MeOH layer was radioassayed and co-chromatographed with selected standards.

Liver. A subsample of liver was treated with a combination of protease/collagenase in THAM buffer (pH 7.4) at 37°C. The mixture was extracted with MeOH:water (80:20, v/v), and the solids were separated from the extract. The extract was concentrated and cleaned up using a C-18 bond elut column, and the residues were eluted in MeOH. The elute was radioassayed and analyzed by TLC. The solids were dried, combusted, and radioassayed. A subsample of the soluble residues was partitioned with DCM, and analyzed by 2D-TLC. The use of the combined enzymes increased the extractable percent TRR to 96.6% from 69%. The metabolite profile of the liver extract by TLC was similar to that of the thigh muscle.

In addition, residues from excreta samples were extracted and analyzed by TLC. The chromatographic properties of the radioactive excreta components were used to verify the identity of radioactive components in the tissues.

The distribution of radioactivity in the extracts of tissues and eggs is summarized in Table 1.

Characterization of residues

Hexane and ACN fractions from thigh muscle were cochromatographed with excreta metabolites P1-P4 using 2D-TLC. Metabolite letter designations refer to those used in the earlier study discussed in the Metalaxyl Update of 3/91. The ACN fraction was also subjected to preparative HPLC, after which the isolated residue peaks were analyzed by NMR. Trimethylsilyl derivatives of the ACN residues were prepared and metabolites P1 and P2 were assayed by GC/MS. In addition, metabolite P4 was isolated and determined by MS. The spectrum obtained was compared to a spectrum obtained from excreta metabolite P4. Methanol fractions from egg whites and methanol, chloroform, and aqueous fractions from egg yolks were analyzed using 2D-TLC, with non-radioactive standards and excreta metabolites P1-P4. Identities of metabolites were confirmed by cochromatographic 1D-TLC analyses. Peritoneal fat methanol and ACN/MeOH fractions were analyzed by 2D-TLC. Confirmatory analysis was performed on the MeOH fraction using cochromatographic 1D-TLC. Liver DCM and MeOH fractions were analyzed by 2D-TLC; the aqueous fraction was

analyzed by HPLC. However, no results on liver were reported. Excreta EtOAc and MeOH fractions were analyzed by 2D-TLC. The MeOH soluble residues were also subjected to preparative TLC after which they were further characterized by 2D-TLC. The characterization of the residues in eggs and tissues from laying hens is summarized in Table 2. The qualitative nature of the residue in poultry is adequately understood. The predominant metabolites are the disubstituted free acid form (P1) of CGA-94689(B) (37.4% TRR in thigh muscle, and 13.0% TRR in whole egg), the sulfuric acid conjugate of CGA-94689(B) (29.8% TRR in thigh muscle), the disubstituted free acid form (P2) of CGA-94689(A) (9.2% TRR in thigh muscle, and 10.8% TRR in whole egg), CGA-107955 (9.6% TRR in whole egg, and 40.2% TRR in peritoneal fat), and a fatty acid conjugate of P1 and P2 (26.8% TRR in peritoneal fat). The parent, metalaxyl, was only isolated from egg white and yolk (whole egg, 4.3% TRR). Based on this study, the registrant proposed a new metabolic pathway indicating metalaxyl is hydrolyzed to either the benzylic alcohol CGA-94689 or the ester alcohol CGA-67869. Subsequently CGA-94689 is converted to the sulfate P4, and CGA-67869 is converted to the fatty acid conjugate U3 or the acid alcohol CGA-107955. CGA-107955 is subsequently hydrolyzed to the benzylic alcohol isomers P1 and P2 (which may also be formed from the hydrolysis of CGA-94689 thru P0). Chemical names and molecular structures of metalaxyl metabolites identified in tissues and used as standards are summarized in Figure 1.

Residue Analytical Method Validation

The registrant tested analytical method AG-576 (1991; MRIDs 42115807 and 08) using samples from the poultry and ruminant metabolism studies, and samples fortified with the parent metalaxyl. This method is a combination of methods AG-349 (Method II, PAM Vol. II) and AG-395, which is a modification of AG-348 (Method I, PAM Vol. II). Method AG-576 involves converting residues of metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester] and its metabolites to 2,6-dimethylaniline (DMA), which is analyzed by GLC and detected by nitrogen/phosphorus detector (NPD). 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 made basic, the DMA formed is distilled, further cleaned up using silica cartridges, and analyzed by GLC/NPD. The limit of detection is 0.05 ppm (in metalaxyl equivalents). Only recoveries from fortifications of parent compound were provided by the registrant. Recoveries from samples fortified with metalaxyl are summarized in Table 3 below. No recoveries were reported for any metalaxyl metabolites. Not all metalaxyl metabolites contain the DMA moiety.

The registrant analyzed selected tissues obtained from animals dosed with [^{14}C]metalaxyl (representative samples from metabolism studies). The results from method AG-576 are summarized below in Table 4.

Table 2. Characterization of residues in eggs and tissues from laying hens

Metabolites	Thigh Muscle		Whole Egg[a]		Fat	
	%TRR	PPM	%TRR	PPM	%TRR	PPM
Metalaxyl, CGA-48988	-	<0.001	4.3	0.023	-	<0.001
CGA-94689(A)	-	<0.001	1.7	0.009	-	<0.001
CGA-94689(B)	-	<0.001	2.6	0.014	-	<0.001
CGA-67869	-	<0.001	-	<0.001	-	-
CGA-108905	-	<0.001	1.3	0.007	-	<0.001
CGA-107955	-	<0.001	9.6	0.052	40.2	0.102
Fat U3 (fatty acid conjugated P1, P2)	-	<0.001	2.4	0.013	26.8	0.068
P0 monosubstituted free acid of CGA-94689(B)	-	<0.001	3.0	0.016	-	<0.001
P1 disubstituted free acid form of CGA-94689(B)	37.4	0.252	13.0	0.070	5.1	0.013
P2 disubstituted free acid form of CGA-94689(A)	9.2	0.062	10.8	0.058	2.0	0.005
P3a glucuronide of CGA-67869	-	<0.001	1.1	0.006	0.8	0.002
P4 sulfuric acid conjugate of CGA-94689(B)	29.8	0.201	5.4	0.029	8.3	0.021
P3 (unknown)	2.5	0.017	4.5	0.024	0.8	0.002
% Identified	76.4	0.515	55.1	0.297	83.2	0.211

[a]Yolks and whites were analyzed separately. Total whole egg residue levels were calculated by the reviewer.

Table 3. Recoveries from poultry and ruminant tissues fortified with metalaxyl, per se, as determined by method AG-576.

Substrate	Fortification Level	Percent Recovery	Number of Samples
Poultry: Eggs	0.05-1.0	82-118	17
Breast/Thigh	0.05-1.0	75-115	11
Skin	0.05-1.0	82-125	11
Peritoneal Fat	0.05-1.0	92-113	8
Liver	0.05-2.0	64-194	17
Ruminant: Liver	0.03-1.0	52-101	3
Leg Muscle	0.05-0.2	88-101	3
Milk	0.01-0.05	88-159	3
Fat	0.05-0.07	83-127	3

Table 4. Summary of results comparing metabolism results with the results from method AG-576 on samples from animals fed [^{14}C]metalaxyl.

Substrate	Percent Total Extractability		Percent Recovered as DMA	Percent Identified in Metabolism Study	
	Method AG-576	Metabolism	Method AG-576	as DMA moiety [a]	as HMMA moiety [b]
Poultry Liver[c]	58	80.3	19	19.1	11.0
Poultry Skin and Attached Fat[e]	37	87.3	18	51[f]	42[f]
Poultry Breast[e]	98	95.3	26	-	76.4[g]
Eggs[d]	31	82.4	22	15.0	38.9
Goat Liver[e]	38	25.4	30	16.9	8.0
Goat Leg Muscle[e]	71	88.5	58	52.8	14.4
Goat Milk[e]	85	74.4	49	49.3	6.1
Goat Fat[e]	95	96.8	89	46.3	12.1

[a]Percent includes all compounds identified as containing the 2,6-dimethylaniline moiety.
[b]Percent includes all compounds identified as containing the 2-hydroxymethyl-6-methylaniline moiety
[c]Metabolism results from MRID 41664504, and summarized in the 3/91 Update.
[d]Metabolism results from MRID 42115804.
[e]Metabolism results from MRID 41665403, and summarized in the 3/91 Update.
[f]Percentage from fat
[g]Percentage from thigh

Ciba Geigy has provided recovery data to demonstrate that metalaxyl, per se, is adequately recovered from livestock tissues. Recoveries of metalaxyl metabolites containing the DMA moiety were not reported. Recoveries of CGA-94689 was not reported. Recoveries of newly reported metabolites containing the hydroxymethyl methyl aniline moiety were not reported.

The registrant has shown that method AG-576 adequately recovers residues of the parent metalaxyl from animal tissues. The results from the Agency validations of PAM Vol. II, Method II and Ciba-Geigy method AG-395 indicate that method AG-576 will adequately recover metalaxyl metabolites containing the 2,6-dimethylaniline moieties, CGA-67869, CGA-107955 and CGA-62826. It is not clear whether method AG-576 will adequately recover metabolite CGA-94689 (which contains the hydroxymethyl methyl aniline moiety). Method AG-395 was considered adequate to recover CGA-94689 in PP#3F2918. Although recoveries of CGA-94689 were consistently less than 70%, CGA-94689 was a minor part (<20%) of the residue in edible portions of plants (K. Arne memo of 8/2/84, PP#3F2918 and 3F2919). Method II, PAM Vol. II only recovers 45-48% of fortified CGA-94689. We note that the hydroxy metabolite, its isomers and conjugates comprise a significantly greater portion of the total residue in livestock commodities.

Metabolism study results are resummarized in Table 5 by metabolite class.

Because significant levels of metalaxyl residues are expected in ruminant liver and kidney (see ruminant feeding studies, 1987 Residue Chemistry Chapter), and because the metabolite CGA-94689 was isolated in ruminant kidney and liver (see ruminant metabolism study, 1991 Reregistration Standard Update) and is included in the established tolerances, the registrant must validate method AG-576 showing adequate recoveries of metabolite CGA-94689 and P1 and P2. If new methodology is required to adequately recover metabolite CGA-94689, P1 or P2, additional validation will be required using representative samples from radiolabeled metabolism studies.

Table 5: Summary of Metalaxyl Metabolism Studies, including total percentage of radiolabel identified, and summation of identified metabolites by chemical class.

Classes of Metalaxyl Metabolites					
Commodity	Total ID	DMA	HMMA	Ring-OH	Benzoic Acid
Plant Commodities					
Potato Foliage	59.9	14.4	23.4	22.1	--
Potato Foliage	57.4	4.1	50.6	2.7	--
Potato tuber	40.7	34.6	1.9	4.2	--
Potato tuber	65.4	52.8	11.2	1.4	--
Potato tuber	71.5	61.8	4.1	4.4	1.2
Grape Leaves	93.8	25.4	55.4	13.0	--
Grape Presscake	73.1	57.1	13.4	2.6	--
Grape Juice	15.5	8.8	5.0	1.7	--
Head Lettuce	45.2	29.4	10.9	4.9	--
Head Lettuce	76.0	46.5	22.1	6.2	1.2
Goat					
Milk	64.1	49.3	6.1	8.7	--
Liver	30.0	16.9	8.0	5.1	--
Kidney	73.7	36.8	34.2	2.7	--
Muscle	70.6	52.8	14.4	3.4	--
Fat	63.8	46.3	12.1	5.4	--
Poultry					
Liver	30.1	19.1	11.0	--	--
Thigh	76.4	--	76.4	--	--
Whole Egg	53.9	15.0	38.9	--	--
Fat	93.2	51.0	42.2	--	--

Dimethyl aniline (DMA) metabolites include metalaxyl, per se, CGA-67869, CGA-68124, CGA-79353, CGA-37734, CGA-62826, CGA-67867, CGA-107955, CGA-78532.
Hydroxymethyl methyl aniline (HMMA) metabolites include CGA-94689 and its conjugates.
Ring hydroxy (Ring-OH) metabolites include CGA-100255 and its conjugates.
Benzoic acid metabolites include CGA-108905 and CGA-108906.

Magnitude of the Residue in Meat/Milk/Poultry/Eggs

Poultry. Ciba Geigy submitted data (1991; MRID 42115809) from a poultry feeding study in which 45 White Leghorn laying hens (three treatment groups of 15) were given feed fortified with technical grade metalaxyl at 10 ppm, 30 ppm, or 100 ppm. The maximum theoretical dietary intake of residues of metalaxyl by poultry would be 1.4 ppm, based on tolerance level residues in cull potatoes, soybean seed, soybean meal, and processed potato waste. The feeding rates were, therefore, 7x, 21x, and 71x, respectively. The metalaxyl-treated feed was analyzed using method AG-576 to confirm metalaxyl levels. Fifteen additional hens were kept as controls. Eggs were collected at 0 (predose), 1, 3, 7, 14, 21, and 28 days. Animals were sacrificed after 7, 14, 21, and 28 days. Composite samples of breast plus thigh, skin plus attached fat, peritoneal fat, and liver were collected from three hens of each treatment group. Samples were stored for 6-11 months at -15 °C. The registrant stated that metalaxyl residues are stable for 18 months at -15°C, but no data were provided. The registrant must submit supporting storage stability data. Method AG-576 was used to analyze samples; the detection limit was 0.05 ppm. A summary of the residues found in poultry tissues and eggs is provided in Table 6. A summary of method recoveries for metalaxyl, per se, is provided in Table 7.

Apparent residues in controls were nondetectable (<0.05 ppm) in each of seven composite samples of egg, in each of four composite samples of breast/thigh, skin/attached fat, peritoneal fat, and in three of the four composite samples of liver. The day 21 control liver sample was analyzed four times; the results were 0.07, 0.10, 0.10, <0.05 ppm, with 0.07 ppm being the final confirmed analysis result.

The results of this poultry feeding study indicate that residues of metalaxyl will not exceed the established tolerances of 0.4 ppm in fat, kidney, and liver, and 0.05 ppm in meat and meat byproducts in poultry commodities, dosed metalaxyl at 1x. However, judgement of the adequacy of the available data is reserved until the analytical method used is determined to be adequate and adequate storage stability data are provided.

Table 6. Residues in tissues of hens fed metalaxyl-treated feed.

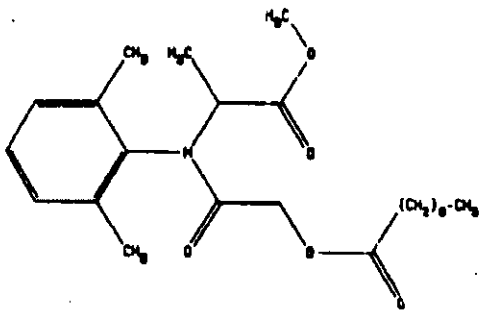
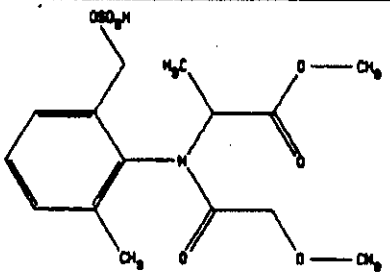
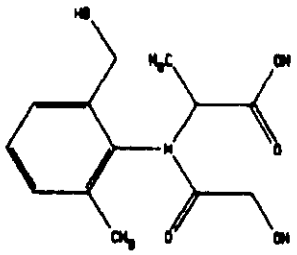
Tissue	Treatment Level (PPM)	Total Metalaxyl Residues (ppm)			
		Fed 7 Days	Fed 14 Days	Fed 21 Days	Fed 28 Days
Eggs[a]	10	<0.05	<0.05	<0.05	<0.05
	30	<0.05	<0.05	<0.05	<0.05
	100	<0.05	<0.05	<0.05	<0.05
Breast/Thigh	10	<0.05	0.06	<0.05	<0.05
	30	0.06	0.10	<0.05	<0.05
	100	0.13	0.13	<0.05	0.12
Skin/Attached Fat	10	<0.05	<0.05	<0.05	<0.05
	30	<0.05	0.07	0.10	0.08
	100	0.12	0.32	0.40	0.34
Peritoneal Fat[b]	10	<0.05	<0.05	<0.05	<0.05
	30	<0.05	0.07	0.08	0.07
	100	0.09	0.27	0.34	0.17
Liver	10	<0.05	<0.05	0.18[c]	0.05
	30	0.07	0.07	0.15[c]	0.10
	100	0.16	0.10	0.12	0.11

[a]Results on egg samples were also provided for 1 and 3 day dose intervals; the results were all <0.05 ppm. [b]Results from analyses of peritoneal fat were provided using both method AG-576 and a modification to method AG-576. The resulting residue levels from method AG-576 were higher and are reported here. [c]Samples analyzed more than once, the highest result is reported.

Table 7. Recovery of metalaxyl from controls of eggs and poultry tissues fortified with metalaxyl, per se.

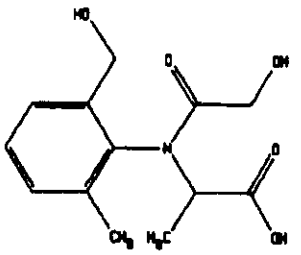
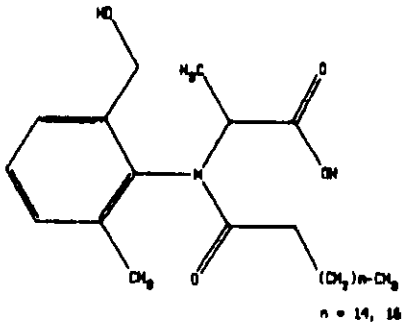
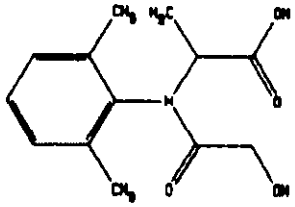
Substrate	Fortification Level	Number of samples	Percent Recovery
Eggs	0.05-1.0	14	82-118
Breast/Thigh	0.05-1.0	8	85-104
Skin	0.05-1.0	8	94-125
Peritoneal Fat	0.05-1.0	8	92-113
Liver	0.10-2.0	14	64-125

Figure 1. (continued)

Chemical Name Common Name (Company code)	Structure	Substrate:	MRID
N-decanoic acid ester of CGA-67869		**Ruminant:** Milk	42115802
Sulfuric acid conjugate of CGA-94689 (P4)		**Poultry:** Thigh muscle Whole egg Peritoneal fat	 42115804 42115804 42115804
N-[2-(hydroxy methyl)-6-methylphenyl-N-(hydroxyacetyl) alanine (P1)		**Poultry:** Thigh muscle Whole egg Peritoneal fat	 42115804 42115804 42115804

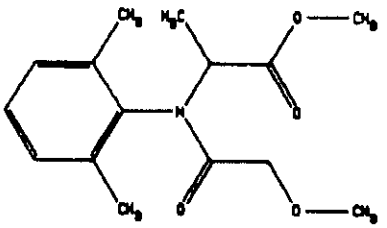
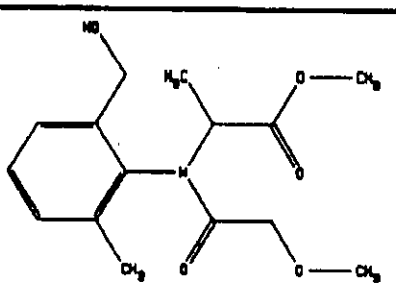
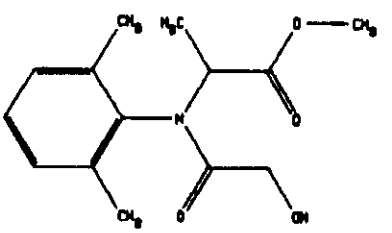
(continued)

Figure 1. (continued)

Chemical Name Common Name (Company code)	Structure	Substrate;	MRID
N-[2-(hydroxy methyl)-6-methylphenyl]-N-(hydroxyacetyl) alanine (P2)		**Poultry:** Thigh muscle Whole egg Peritoneal fat	 42115804 42115804 42115804
Fatty acid conjugates of P1, P2 (Fat U3)		**Poultry:** Whole egg Peritoneal fat	 42115804 42115804
N-(2,6-dimethyl-phenyl)-N-(hydroxyacetyl)-alanine (CGA-107955)		**Ruminant:** Milk Liver Urine Tenderloin Kidney Leg muscle Perirenal fat **Poultry:** Whole egg Peritoneal fat	 41664503 41664503 41664503 41664503 41664503 41664503 41664503 42115804 42115804

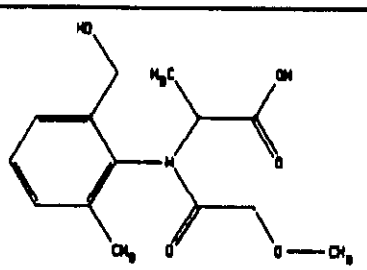
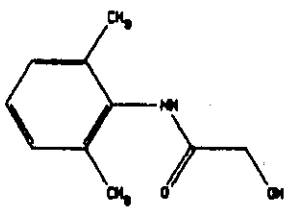
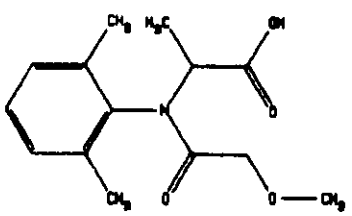
(continued)

Figure 1. Chemical names and molecular structures of metalaxyl metabolites identified in animal tissues and used as standards in metabolism studies.[a]

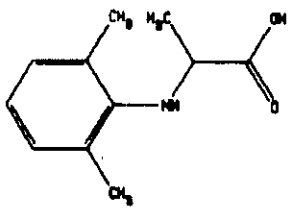
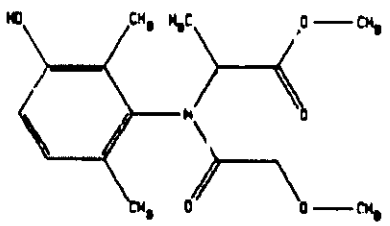
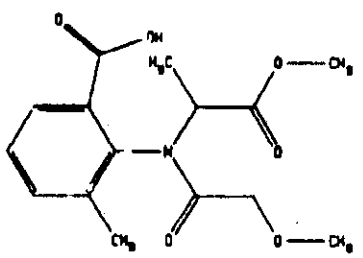
Chemical Name Common Name (Company code)	Structure	Substrate;	MRID
N-(2,6-di-methyl phenyl)-N-(methoxy-acetyl)-alanine methyl ester metalaxyl (CGA-48988)		**Poultry:** Whole egg	 42115804
N-[(2-hydroxy-methyl)-6-methyl-phenyl]-N-(methoxy acetyl)-alanine methyl ester isomers (CGA-94689)		**Ruminant:** Milk Liver Kidney Leg muscle Perirenal fat Urine Tenderloin **Poultry:** Whole egg	 41664503 41664503 41664503 41664503 41664503 41664503 41664503 42115804
N-(2,6-dimethyl-phenyl)-N-(hydroxyacetyl)-alanine methyl ester (CGA-67869)		**Ruminant:** Urine Tenderloin Milk Liver Kidney Leg muscle Perirenal fat	 41664503 41664503 41664503 41664503 41664503 41664503 41664503

(continued)

Figure 1. (continued)

Chemical Name Common Name (Company code)	Structure	Substrate;	MRID
N-[(2-hydroxy methyl)-6-methylphenyl]-N-(methoxyacetyl)-alanine (P0)		**Poultry:** Whole egg	 42115804
N-(2,6-dimethyl-phenyl)-2-hydroxy acetamide (CGA-37734)		**Ruminant:** Tenderloin Milk Liver Urine Kidney Leg muscle Perirenal fat	 41664503 41664503 41664503 41664503 41664503 41664503 41664503
N-(2,6-dimethyl-phenyl)-N-(methoxy acetyl)-alanine (CGA-62826)		**Ruminant:** Tenderloin Milk Liver Urine Kidney Leg muscle Perirenal fat	 41664503 41664503 41664503 41664503 41664503 41664503 41664503

(continued)

Chemical Name Common Name (Company code)	Structure	Substrate;	MRID
N-(2,6-dimethyl-phenyl) alanine (CGA-67867)		**Ruminant:** Urine	 41664503
N-(3-hydroxy-2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester (CGA-100255)		**Ruminant:** Tenderloin Milk Liver Urine Kidney Leg muscle Perirenal fat	 41664503 41664503 41664503 41664503 41664503 41664503 41664503
2-[(methoxy-acetyl) (2-methoxy-1-methyl-2-oxoethyl) amino]-3-methyl-benzoic acid (CGA-108905)		**Poultry:** Whole egg	 42115804

*Additional standards include: N-(3-hydroxy-2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine, 4-(2,6-dimethylphenyl)-1-methyl-2,5-morpholinedione, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine, Fatty acid conjugates of CGA-67869, Inorganic salts of P4, Lactones of P1 and P2, Tris-trimethylsilyl derivative of P1, Tris-trimethylsilyl derivative of P2, Lactone Mono-trimethylsilyl derivative of P1, Lactone Mono-trimethylsilyl derivative of P2, [2,6-dimethyl-phenyl) amino] oxoacetic acid, N-(carboxy-carbonyl)-N-(2,6-dimethylphenyl) alanine methyl ester, 2-[1-carboxyethyl) (methoxy acetyl) amino]-3-methyl benzoic acid, and N-(carboxycarbonyl)-N-(2,6-dimethylphenyl) alanine.

References:

Citations for the MRID documents referenced in this review are presented below. Submissions reviewed in this document are indicated by shaded print.

41664503 Emrani, J. (1990) Metalaxyl: Metabolism of ^carbon 14|-Metalaxyl in Goats: Lab Project Number: ABR/90078. Unpublished study prepared by CIBA-GEIGY Corp., and Others. 205 p.

41664504 Kennedy, E. (1990) Metalaxyl: Metabolism of ^phi-carbon 14|-Metalaxyl in Hens: Lab Project Number: ABR/90077. Unpublished study prepared by CIBA-GEIGY Corp., and Others. 129 p.

42115802 Emrani, J. (1990) Supplemental to: Metabolism of ^phi-carbon 14| Metalaxyl in Goats. Unpublished study prepared by Ciba-Geigy Corp. 74 p.

42115804 Kennedy, E. (1990) Supplemental to: Metabolism of ^phi-carbon 14| Metalaxyl in Hens. Unpublished study prepared by Ciba-Geigy Corp. 176 p.

42115805 Kim, H.; Lindsay, R. (1990) Method for the quantitative analysis of volatile free and total branched-chain fatty acids in cheese and milkfat. J. of Dairy Sci. 73(8): 1988-1999.

42115806 Missart-Leeq, A.; De Pooter, H.; DeCloedt, M.; et al. (1981) Composition and variability of the branched-chain fatty acid fraction in the milk of goats and cows. Lipids 16(5): 286-292.

42115807 Eddy, E. (1990) Improved Analytical Method for the Determination of Total Terminal Residues of Metalaxyl in Poultry Tissues and Eggs as 2,6-Dimethyl Aniline: Lab Project Number: AG-578: Unpublished study prepared by Ciba-Geigy Corp. 45 p.

42115808 [illegible] Validation of Analytical Method AG-578 for the Determination of [illegible] Metalaxyl in Goat Tissues, Milk, Poultry Tissues and Eggs: [illegible] Number: ABR-91008: Unpublished study prepared by Ciba-Geigy [illegible]

42115809 Eddy, E. (1991) Metalaxyl: Three Level/28-Day Poultry Study: Lab Project Number: [illegible]: Unpublished study prepared by Ciba-Geigy Corp. 23 p.

42115810 Chaing, M. (1987) Metalaxyl: Gas Chromatographic Determination of Metalaxyl (Apron) Coatings on Seeds: Lab Project Number: AG-387: Unpublished study prepared by Ciba-Geigy Corp. 11 p.

References not used:

42115801 Ermani, J. (1990) Amendment to: Metabolism of ^phi-carbon 14| Metalaxyl in Goats. Unpublished study prepared by Ciba-Geigy Corp. 4 p.

42115803 Kennedy, E. (1990) Amendment to Metabolism of ^phi-carbon 14| Metalaxyl in Hens. Unpublished study prepared by Ciba-Geigy Corp. 4 p.

Agency Memoranda:

CB No. none
Subject: PP#1F2500/1H5299 Metalaxyl on a Variety of Raw Agricultural Commodities. Amendment of April 1, 1982 and April 7, 1982.
From: P.W. Errico
To: H.M. Jacoby
Dated: 7/16/82
MRID(s): none

CB No. none
Subject: PP#3F2918 and 3F2919 Metalaxyl on Legume Vegetables and Peanuts, Respectively. Results of a Method Trial
From: K. H. Arne
To: H. Jacoby, PM#21
Dated: 8/2/94
MRID(s): none