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

October 17, 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metalaxyl (113501) - Reregistration Case No. 0081.
Addendum to RED Residue Chemistry Chapter
Rotational Crop Studies (GLN 165-1 and 165-2)
Supporting Storage Stability Data
MRIDs 42195401, 42196502, 42196503, 41870308, 43317302, CB Nos. 13740, 14131, DP Barcodes: D203675, D206108

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Ciba Geigy has submitted rotational crop studies to satisfy both Guidelines 165-1 (Confined Rotational Crop Studies) and 165-2 (Field Rotational Crop Studies). Attached is the review of these studies, which was completed by Dynamac Corporation under the supervision of CBRS, HED. It has undergone secondary review in the branch and has been revised to reflect Agency policies.

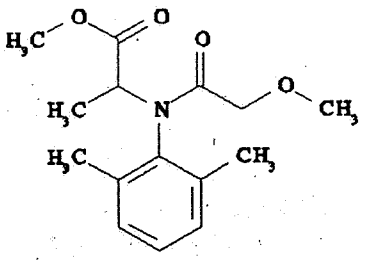
The Confined rotational crop studies are adequate. Two ring hydroxylated metabolites have been reported in immature lettuce in a much greater proportion than reported in primary crops. Additionally, hydroxymethyl methyl aniline metabolites were reported in a much greater proportion in rotated lettuce than reported in primary crops.

The field rotational crop studies are inadequate. Recoveries were reported only for parent compound, metalaxyl. Adequate recoveries are needed for representative metalaxyl metabolites containing the DMA and HMMA moieties.

An upper bound risk assessment can be conducted by assuming that the enforcement method will detect the amount of residue identified as containing the DMA moiety.

cc: Circu, RSF, SF, S. Hummel, RF
RDI:FBS:09/19/94:EZ:10/17/94
7509C:CBRS:SVH:svh:CM#2:RM804:10/17/94
METALAXY.949

METALAXYL



Shaughnessy No. 113501; Case No. 0081

(CBRS No. 13740; DP Barcode D203675)

(CBRS No. 14131; DP Barcode D206108)

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

Task 4

BACKGROUND

Ciba-Geigy Corporation (1992; MRID 42196501) submitted data from a confined rotational crop study in which rotational crops were planted in [^{14}C]metalaxyl-treated soil used to grow tobacco as the primary crop. Storage stability data were also submitted in support of this study (1992; MRID 43317302). In addition, the registrant submitted data from 1977 and 1978 field rotational crop studies in which a variety of crops were grown following metalaxyl-treated potatoes, tobacco, or wheat (1983; MRID 41870308). This submission also includes summaries of previous confined rotational crop studies, which were reviewed in the 1981 Reregistration Standard. In addition, the registrant submitted tobacco and potato metabolism studies (1980-1992; MRIDs 42196501 through 42196503), which were reviewed in the 1981 and 1988 Metalaxyl Reregistration Standards and were resubmitted in support of Guideline Requirements for 165-1. These data are reviewed in this report to determine their adequacy in fulfilling GLNs 165-1 and 165-2. The Conclusions and Recommendations stated in this review pertain only to the nature of the residues in plants and the magnitude of the residue in rotational crops.

The qualitative nature of the residue in plants is adequately understood. The HED Metabolism Committee (9/10/93) has determined that the residues to be regulated are metalaxyl and its metabolites that can be converted to 2,6-dimethyl aniline (2,6-DMA) and those containing 2-hydroxymethyl-6-methylaniline (HMMA). Adequate enforcement methods are available for the determination of residues of metalaxyl and its regulated metabolites in plants. Methods I and II in PAM, Vol. II correspond to Methods AG-348 and AG-349. Method AG-395, an improved modification of AG-348, has undergone successful Agency validation with plant matrices.

Tolerances for residues of metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl) aniline methyl ester] in/on plants are currently expressed as the combined residues of metalaxyl and its metabolites containing the 2,6-dimethylaniline moiety, and N-(2-hydroxy methyl-6-methyl)-N-(methoxyacetyl)-alanine methylester [40 CFR §180.408 (a) and (c)]. Rotational crop tolerances have also been established for residues of metalaxyl in/on RACs of barley, oats, and wheat resulting from the labeled use of metalaxyl to rotated crops.

Codex MRLs and U.S. tolerances for metalaxyl residues in plant commodities are currently incompatible because Codex regulates metalaxyl *per se*, whereas, the HED metabolism committee has concluded that the U.S tolerance definition should include metalaxyl and its metabolites that can be converted to 2,6-DMA and those containing HMMA.

CONCLUSIONS

1. Ciba-Geigy Corp. submitted a confined rotational crop study depicting residues in lettuce, sugar beets, soybeans, and wheat rotated following a primary crop of tobacco treated with [^{14}C]metalaxyl. Complete metabolite analyses were conducted on immature lettuce, sugar beet roots, wheat stalks, and wheat grain planted ~8 months following a preplant incorporated application to the primary tobacco crop, which was also completely analyzed. This study is adequate. The metabolism of [^{14}C]metalaxyl in rotated crops proceeds as in primary crops with the exception of additional metabolites discussed in Conclusion 2 below. However, the proportions of classes of metabolites in rotational crops is substantially different than in primary crops, with greater proportions of hydroxylated and benzoic acid metabolites.

2a. Two metabolites oxidized to the phenol on the 3-position were detected in lettuce. These 3-OH compounds are not covered by the current tolerance definition. Metabolite CGA-100255 is a product of metalaxyl oxidized at the 3-position, and accounted for 11% of the TRR in immature lettuce. A second compound, which would represent an N-dealkylation product of CGA-100255, was detected in hydrolysates of aqueous and insoluble fractions, and was designated N1b aglycone; it constituted 16% of the immature lettuce TRR. Together, these two components comprised 27% of the immature lettuce TRR at a concentration of 0.24 ppm. The registrant also reported these compounds in mature lettuce listing 8% of the TRR as CGA-100255 and 11% as N1b aglycone. Both were released from glucose conjugates.

2b. The target crop tobacco contained metabolite CGA-100255 at low levels, 2.6% of the TRR in immature leaves and 1.6% in mature leaves. Earlier metabolism studies showed low levels of these 3-OH metabolites, except in the inedible commodities, potato foliage (22.1% of CGA-100255), and grape leaves (13.0% of CGA 100255). (See Summary Table 1.)

2c. Rotated crops also had much higher proportions of metabolites containing the hydroxymethyl methyl aniline moiety (HMMA) than previously reported in edible portions of primary crops. (See Summary Table 1)

2d. In rotated wheat grain, the majority of the identified residue (35.2% out of 43.0% identified) was metabolites oxidized to the benzoic acid (containing the 2-amino-3-methylbenzoic acid moiety).

3a. The current enforcement method converts metalaxyl residues to the dimethylaniline moiety (DMA). Samples from the confined rotational crop studies (GLN 165-1) were not analyzed by the current enforcement method. It is unlikely that the current enforcement method will detect the hydroxylated metabolites (either those containing the HMMA moiety, or the 3-OH metabolites) or the benzoic acid metabolites.

3b. TOX is not concerned with the benzoic acid class of metalaxyl metabolites and is less concerned with the 3-OH metabolites than the metabolites containing the HMMA moiety. The benzoic acid and 3-OH containing metabolites will not need to be regulated. The HED Metabolism Committee previously concluded that metalaxyl DMA and HMMA metabolites should be regulated.

3c. Samples from the field rotational crop studies were analyzed by the current enforcement method or a similar method. Recoveries were reported only for metalaxyl, per se. No recoveries were reported for representative metalaxyl metabolites. Recoveries are still needed for representative metabolites containing the DMA and HMMA moieties.

4. The submitted storage stability data adequately support the new confined rotational crop study. Radioactive residues in solvent extracts stored at -20 C are stable for up to 3-4 years, and ^{14}C-residues in homogenized plant samples stored at -20 C are stable for up to 14 months.

5a. Field rotational crop data were submitted pertaining to residues in broccoli, cabbage, corn, lettuce, red beets, rye, soybeans, sugar beets, sweet potatoes, and wheat planted following metalaxyl-treated potatoes, tobacco, and wheat (in one soybean study). Samples were analyzed by the current enforcement method or a similar method. Combined metalaxyl residues, convertible to 2,6-dimethylaniline, in these rotated crops, with the exception of corn, were lower than the established tolerances in 40 CFR §180.408 (a) or indirect tolerances in 40 CFR §180.408 (b).

5b. Combined metalaxyl residues, convertible to 2,6-dimethylaniline, were <0.05 ppm (nondetectable) in corn grain from all studies. Eight to nine months following treatment of potatoes at 2x, combined metalaxyl residues convertible to 2,6-DMA, were <0.05-0.54 ppm in corn forage and <0.05-0.08 ppm in corn fodder. One year following treatment of tobacco at 1x, combined metalaxyl residues, convertible to 2,6-DMA, were <0.05-0.23 ppm in corn forage and <0.05 ppm in corn fodder. Tolerances are needed to cover indirect or inadvertent residues in corn forage and fodder.

5c. A tolerance of 1.0 ppm is pending for metalaxyl residues in forage, fodder, and straw of cereal grains as a result of seed treatment uses (PP#1F3993). CBRS has recommended in favor of establishing this tolerance (CBRS No. 10833; DP Barcode D183914; 9/30/93; D. Miller). If established, this tolerance will be higher than the residues convertible to 2,6-DMA occurring in forage and fodder from rotated corn planted 8 months after treatment with metalaxyl.

6. Field rotational crop studies (GLN 165-2) are inadequate. The studies are potentially upgradable by providing additional recovery data. The registrant must demonstrate that the current enforcement method and the analytical methods used for the field rotational crop studies will detect representative metalaxyl metabolites containing the DMA and HMMA moieties. This may be done by validating the analytical methods used for the field rotational crop studies using representative metabolites from each of these classes.

7. An upper bound risk assessment can be done using the currently available data. We will assume that the enforcement method measures only metalaxyl metabolites containing the DMA moiety.

Crop matrix	%TRR Recovered
grain	2.2%
grain stalks	14.1%
leafy crops	2.2-22%

A summary of the metabolism studies including the confined rotational crop studies is included in Summary Table 1.

The chemical structure of metalaxyl and its metabolites identified in rotational crops are presented in Figure 1.

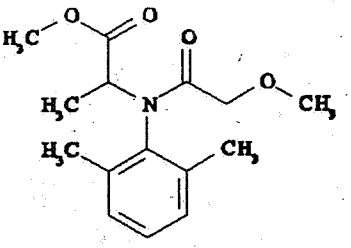
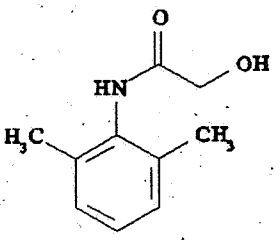
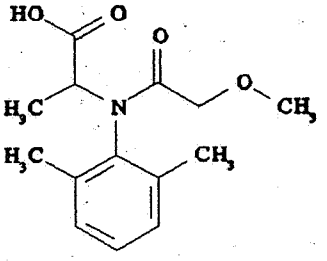
(continued)

Summary Table 1: Summary of Metalaxyl Metabolites by Chemical Class

Commodity	TRR (ppm metalaxyl eq.	Classes of Metalaxyl Metabolites % TRR				
		Total ID	DMA	HMMA	Ring-OH	Benzoic Acid
Plant Commodities (Primary Crops)						
Potato Foliage	25.9	59.9	14.4	23.4	22.1	--
Potato Foliage	1.9	57.4	4.1	50.6	2.7	--
Potato tuber	0.16	40.7	34.6	1.9	4.2	--
Potato tuber	0.14	65.4	52.8	11.2	1.4	--
Potato tuber	0.16	71.5	61.8	4.1	4.4	1.2
Grape Leaves	NR	93.8	25.4	55.4	13.0	--
Grape Presscake	NR	73.1	57.1	13.4	2.6	--
Grape Juice	NR	15.5	8.8	5.0	1.7	--
Head Lettuce	NR	45.2	29.4	10.9	4.9	--
Head Lettuce	NR	76.0	46.5	22.1	6.2	1.2
Rotational Crops						
Immature Lettuce	0.9	89	22	38	28	1
Mature Lettuce	0.6	31.4	2.7	6.6	19.8	2.3
Sugar Beet Roots	0.3	54.9	40.6	11.1	--	3.2
Wheat Grain	0.6	43.0	2.2	--	5.6	35.2
Wheat Stalk	7.2	76.4	14.1	57.7	2.5	2.1
Goat (4X feeding rate)						
Milk	0.07	64.1	49.3	6.1	8.7	--
Liver	1.6	30.0	16.9	8.0	5.1	--
Kidney	1.7	73.7	36.8	34.2	2.7	--
Muscle	0.10	70.6	52.8	14.4	3.4	--
Fat	0.25	63.8	46.3	12.1	5.4	--
Poultry (170x feeding rate)						
Liver	1.4	30.1	19.1	11.0	--	--
Thigh	0.7	76.4	--	76.4	--	--
Whole Egg	0.2	53.9	15.0	38.9	--	--
Fat	0.3	93.2	51.0	42.2	--	--

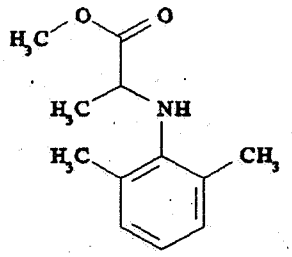
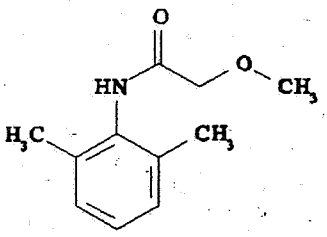
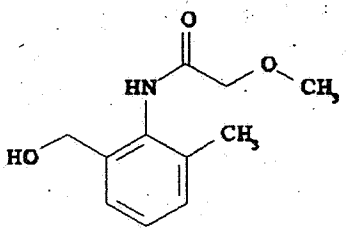
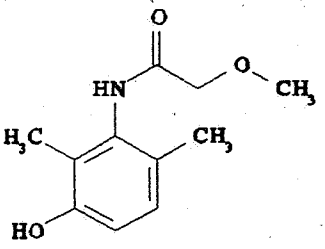
(continued)

Figure 1. Chemical names and structures of metalaxyl and its metabolites in rotational crops.

Common Name Chemical Name	Structure	Substrate
Metalaxyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester		Lettuce-immature Sugar beet roots Wheat stalks Tobacco-immature Tobacco-mature Wheat grain (putative)
CGA-37734 N-(2,6-dimethylphenyl)-2-hydroxyacetamide		Lettuce-immature Wheat stalks Tobacco-immature Tobacco-mature
CGA-62826 N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine		Lettuce-immature Sugar beet roots Wheat stalks Wheat grain Tobacco-immature Tobacco-mature

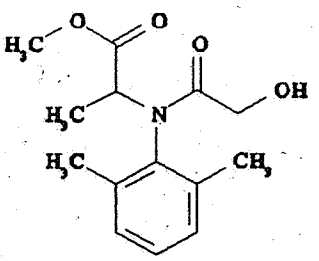
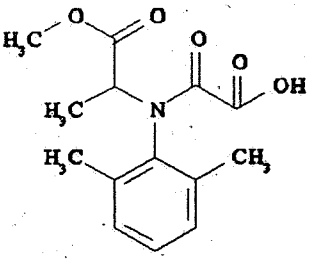
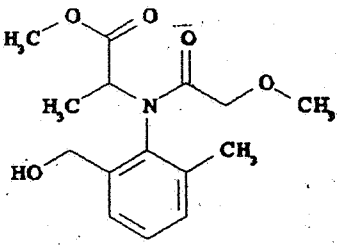
(continued)

Figure 1. (*continued*).

Common Name Chemical Name	Structure	Substrate
CGA-67866 N-(2,6-dimethylphenyl)-2-methoxyacetamide		Wheat grain
CGA-67868 N-(2,6-dimethylphenyl)-2-methoxyacetamide		Wheat stalks Lettuce-immature (putative) Sugar beet roots (putative)
N1a aglycone; benzyl alcohol of CGA-67868 N-[2-(hydroxymethyl)-6-methylphenyl]-2-methoxyacetamide		Wheat stalks
N1b aglycone; phenyl ring hydroxy CGA-67868 N-(3-hydroxy-2,6-dimethylphenyl)-2-methoxyacetamide		Lettuce-immature Wheat stalks (putative)

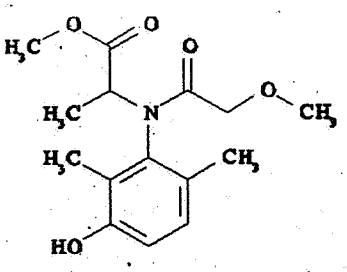
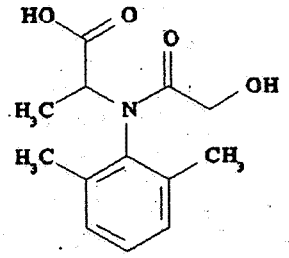
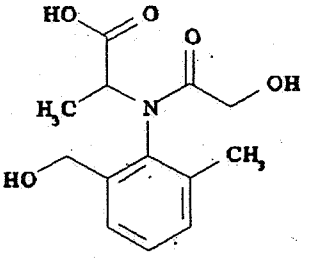
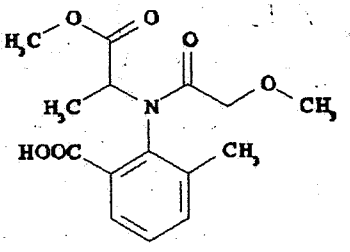
(continued)

Figure 1. (*continued*).

Common Name Chemical Name	Structure	Substrate
CGA-67869 N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)alanine methyl ester		Sugar beet roots (putative) Wheat stalks Wheat grain (putative)
CGA-79353 N-(oxalyl)-N-(2,6-dimethylphenyl)alanine methyl ester		Sugar beet roots Wheat grain
CGA-94689 (isomers A and B) N-[2-(hydroxymethyl)-6-methylphenyl]-N-(methoxyacetyl)alanine methyl ester		Lettuce-immature Wheat stalks Tobacco-immature Tobacco-mature Sugar beet roots (putative)

(continued)

Figure 1. (*continued*).

Common Name Chemical Name	Structure	Substrate
CGA-100255 N(3-hydroxy-2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester		Lettuce-immature Wheat stalks Tobacco-immature Tobacco-mature
CGA-107955 N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)alanine		Lettuce-immature Sugar beet roots Wheat stalks Tobacco-immature Tobacco-mature
B2 (Hen P1); Benzyl alcohol of CGA-107955 N-[2-(hydroxymethyl)-6-methylphenyl]-N-(hydroxyacetyl)alanine		Wheat stalks
CGA-108905 2-[(methoxyacetyl)(2-methoxy-1-methyl-2-oxoethyl)amino]-3-methylbenzoic acid		Sugar beet roots Wheat stalks Wheat grain Lettuce-immature (putative)

(continued)

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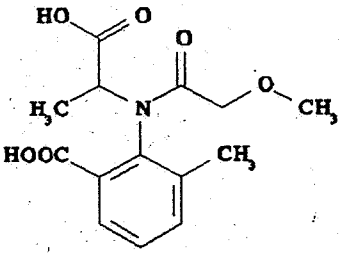
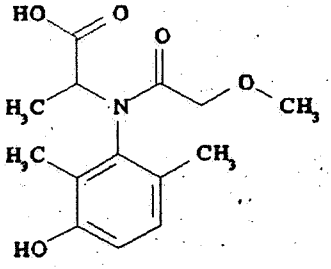
Figure 1. (*continued*).

Common Name Chemical Name	Structure	Substrate
CGA-108906 2-[(1-carboxyethyl)(methoxyacetyl)-amino]-3-methylbenzoic acid		Wheat grain Sugar beet roots (putative)
CGA-119857 N-(3-hydroxy-2,6-dimethylphenyl)-N-(methoxyacetyl)alanine		Sugar beet roots (putative). Wheat stalks Wheat grain (putative) Tobacco-mature (putative)

DETAILED CONSIDERATIONS

Ciba-Geigy (1992; MRID 42196501) submitted data from a greenhouse confined rotational crop study in which rotational crops were planted in [^{14}C]metalaxyl-treated soil used to grow tobacco as the primary crop. The biological and analytical phases of this study were conducted by Ciba-Geigy's Metabolism Dept., Greensboro, NC. [Phenyl-^{14}C]Metalaxyl (specific activity 29.8 μCi/mg; radiochemical purity 98.4%) was applied to a sandy soil as a preplant-incorporation at a rate equivalent to 3 lb ai/A (1x the maximum registered rate for tobacco). Tobacco transplants were planted into the treated soil immediately after application of the [^{14}C]metalaxyl and the mature tobacco plants were harvested 226 days later. The rotational crops, spring wheat, sugar beets, lettuce, and soybeans were planted in the aged soil 232 days (~8 months) after treatment. Rotational crop samples were taken at 25% and or 50% maturity and at maturity; planting to harvest intervals for each crop matrix are specified in Table 1. After harvest, all samples were frozen and stored at -20 C until extraction and analysis.

Storage Stability Data

As a supplement to the confined rotational crop study, Ciba-Geigy (1992; MRID 43317302) submitted data depicting the frozen storage stability of solvent extractable ^{14}C-residues in the mature rotational crops. In the confined rotational crop study, mature samples of lettuce, soybean, sugar beet, and wheat RACs were extracted and the resulting extracts were analyzed by TLC within 1 to 5 months of harvest. These same extracts were also initially analyzed by HPLC within 3.5 to 8 months of harvest. To determine the frozen storage stability of the extracted ^{14}C-residues, the registrant reanalyzed these extracts after an additional ~3 years (38.7-45.2 months after harvest) of storage at -20 C. The registrant provided HPLC radiochromatograms and quantitative data indicating that solvent extractable ^{14}C-residues are stable for up to 3-4 years at -20 C.

In addition, the registrant reextracted and analyzed ^{14}C-residues in immature lettuce, wheat stalks, and sugar beet roots to assess the stability of ^{14}C-residues in plant homogenates stored at -20 C. Immature lettuce was extracted 298 and 432 days after harvest and the solvent extracted ^{14}C-residues were partitioned into aqueous and organosoluble fractions that were analyzed by HPLC. Solvent extracts from wheat stalks (extracted 22 and 285 days after harvest) and sugar beet roots (extracted 45 and 386 days after harvest) were directly analyzed by HPLC. The HPLC radiochromatograms of these extracts indicated that the ^{14}C-residues in these matrices are stable in frozen storage for up 14 months. These data adequately support the confined rotational crop study.

Total Radioactive Residues (TRR)

Quadruplicate subsamples of each plant matrix were combusted and radioassayed by liquid scintillation spectrometry (LSS). The TRR in each plant sample is presented in Table 1.

Table 1. Total radioactive residues (TRR) in crops rotated to [^{14}C]metalaxyl-treated soil 232 days after application to tobacco at 1x.

Crop	Matrix	Postplanting interval (days) [a]	Posttreatment interval (days) [a]	TRR (ppm)
Lettuce	Immature leaves	29	261	0.877
	Mature leaves	60	292	0.564
Wheat	Forage (25% mature)	22	254	5.117
	Forage (50% mature)	47	279	2.578
	Stalks	91	323	7.171
	Grain	91	323	0.593
	Hulls	91	323	7.762
Soybean	Forage (25% mature)	29	261	2.424
	Forage (50% mature)	60	292	2.702
	Stalks	200	432	3.612
	Whole pods	200	432	1.061

Crop	Matrix	Postplanting interval (days) [a]	Posttreatment interval (days) [a]	TRR (ppm)
	Beans	200	432	0.398
Sugar beets	Immature foliage (25% mature)	39	271	1.125
	Immature foliage (50% mature)	75	307	0.856
	Immature roots	75	307	0.291
	Tops	179	411	1.102
	Roots	179	411	0.275

[a] The postplanting interval is the number of days between planting and sampling and the posttreatment interval is the number of days between the soil treatment and sampling.

Extraction and hydrolysis

The registrant presented preliminary analyses of all matrices. However, more extensive analyses and quantitation of ^{14}C-residues were reported only for immature lettuce, mature wheat stalks and grain, and sugar beet roots. The primary tobacco crop was also analyzed.

Radioactive residues in all matrices were extracted in methanol (MeOH):H_2O (9:1, v/v), filtered, concentrated, and partitioned with chloroform ($CHCl_3$) to yield organic and aqueous fractions. Unextracted residues were further fractionated as discussed below for each matrix. For sugar beet roots, immature lettuce, and mature wheat stalks, organosoluble residues were analyzed by HPLC and 2D-TLC. Aqueous soluble residues were further fractionated and hydrolyzed as described below for these matrices. For wheat grain, aqueous and organosoluble residues from the initial MeOH:H_2O extraction were not analyzed further.

Mature sugar beet roots. Table 2 summarizes the extraction and characterization of ^{14}C-residues in mature sugar beet roots. Aqueous soluble residues were cleaned-up on a XAD column, eluted with water and MeOH. The aqueous eluate was repurified using a second XAD column eluted with acidified water and MeOH. The resulting MeOH eluates were combined, concentrated, re-dissolved in water, and hydrolyzed in 6 M HCl at 37 C for at least 12 hours. The 6 M HCl hydrolysate was analyzed using HPLC and 2D-TLC. Anion exchange chromatography was also performed on the purified aqueous extract, however, results were not reported. The purified aqueous residues were also subjected to mild acid hydrolysis (0.1 M HCl) and enzymatic hydrolyses using ß-glucosidase and cellulase. Results from the chromatographic analysis of the cellulase hydrolysate and unhydrolyzed extracts indicated that these fractions contained high proportions of unidentified aqueous components; therefore those results are not reported here.

Two subsamples of the solvent-extracted solids from the original MeOH:H_2O extraction were subjected to 6M HCl (reflux, 17 hours) hydrolysis which solubilized an additional 22.8% of the TRR. The acid hydrolysate was partitioned with ethyl acetate (EtOAc) and the residues were analyzed by HPLC and 2D-TLC as presented in Table 2. Enzyme hydrolyses were also performed on the solvent-extracted solids. Samples were incubated with protease, cellulase, and ß-glucosidase at 37 C for 12 or 16 hours. Control samples were subjected to heated control buffer solutions. The proportions of radioactivity released with enzyme did not differ appreciably from that in the controls; therefore, the enzyme hydrolysates were not analyzed further. In addition, unextracted ^{14}C-residues were subjected to dilute acid (0.1 M HCl) and 6M HNO_3 hydrolyses, which released 10% and 36.5% of the TRR, respectively. These hydrolysates were not analyzed further.

Table 2. Extraction and characterization of ^{14}C-residues in mature sugar beet roots grown in [^{14}C]metalaxyl-treated soil aged 232 days prior to planting.

Commodity Fraction	% TRR [a]	ppm	Characterization/Identification [b]
Sugar beet roots (0.275 ppm)			
MeOH:H_2O	61.5	0.169	Solvent partitioned.
$CHCl_3$	14.7	0.040	HPLC/TLC: Metalaxyl 3.3% (0.009 ppm) CGA-62826 5.4% (0.015 ppm) CGA-108905 2.3% (0.006 ppm) CGA-94689A/B/CGA-107955 1.1% (0.003 ppm) Glucose conj of CGA-107955 1.0% (0.003 ppm)
Aqueous	46.8	0.129	XAD clean-up; 6 M HCl hydrolysis. HPLC/TLC: CGA-62826 20.1% (0.055 ppm) CGA-108905 0.9% (0.003 ppm) CGA-79353 8.1% (0.022 ppm) 4 unknown regions 1.1-7.4% (0.003-0.02 ppm)
Solids	38.5	0.106	6 M HCL solubilized 22.8% of the TRR; solubilized residues were solvent partitioned.
EtOAc	9.4	0.026	2D-TLC: Metalaxyl/CGA-67869/CGA-67868 3.6% (0.01 ppm) CGA-62826/CGA-108905 3.7% (0.01 ppm) CGA-107955/CGA-119857 0.3% (0.001 ppm) CGA-108906/CGA-79353 0.8% (0.002 ppm)
Aqueous	13.4	0.037	HPLC: Metalaxyl/CGA-67869/CGA-67868 1.1% (0.003 ppm) CGA-62826/CGA-108905 1.6% (0.004 ppm) CGA-107955/CGA-119857 0.5% (0.001 ppm) CGA-108906/CGA-79353 1.1% (0.003 ppm)
Solids	15.7	0.043	Not further characterized.

[a] %TRRs were normalized by the registrant for percent recovery.
[b] Percentages reported are given as %TRR.

Immature Lettuce. ^{14}C-Residue extraction from immature lettuce is outlined in Table 3. The aqueous fractions were combined and subjected to anion exchange chromatography resulting in neutral and acidic fractions. The acidic fraction was separated into four components by preparative HPLC and each was characterized by HPLC and 2D-TLC. The neutral fraction was applied to a XAD-4 column and eluted with water, ACN, and MeOH. The eluted residues were subjected to size exclusion chromatography followed by C-18 chromatography. Preparative HPLC analysis of the eluate resolved seven regions (N1-N7). These neutral fractions were hydrolyzed using ß-glucosidase and compared to reference standards using HPLC and 2D-TLC.

Table 3. Extraction and characterization of ^{14}C-residues in immature lettuce grown in [^{14}C]metalaxyl-treated soil aged 232 days prior to planting.

Commodity Faction	% TRR [a]	ppm	Characterization/Identification [b]
Immature Lettuce (0.877 ppm)			
MeOH:H_2O	91.1	0.799	Solvent partitioned.
$CHCl_3$	20.8	0.183	HPLC/TLC: Metalaxyl 15.3% (0.134 ppm) CGA-100255/CGA-67868/CGA-62826 0.9% (0.008 ppm) CGA-108905/gluc conj of CGA-107955 0.9% (0.008 ppm) CGA-94689B 0.5 % (0.004 ppm) CGA-107955 1.3% (0.011 ppm) phenyl ring hydroxy CGA-67868 0.4% (0.004 ppm) 4 unknowns 0.2-0.6% (0.02-0.005 ppm)
Aqueous	69.8	0.612	Anion exchange chromatography.
Neutral	33.7	0.296	Sequentially cleaned-up using XAD; size exclusion; C-18 chromatography. Preparative HPLC resolved 7 regions (N1-N7) which were hydrolyzed by β-glucosidase. HPLC/2D-TLC: glucose conjs of: (N1b) phenyl ring hydroxy CGA-67868 [c] 15.5% (0.136 ppm) (N2) CGA-37344 1.7% (0.015 ppm) (N3) CGA-100255 11.1% (0.097 ppm) (N4) CGA-94689A 0.2% (0.002 ppm) (N5) CGA-94689B 1.9% (0.017 ppm) (N6) CGA-62826 1.5% (0.013 ppm) (N7) CGA-107955 1.8% (0.016 ppm)
Acidic	36.2	0.318	HPLC/2D-TLC: LA1 1.8% (0.016 ppm) [d] LA2 15.4% (0.135 ppm) LA3 13.3% (0.117 ppm) LA4 5.7% (0.05 ppm)
Solids	8.9	0.078	Not further analyzed.

[a] %TRRs were normalized by the registrant for percent recovery.
[b] Percentages reported are given as %TRR.
[c] Identity of component (N1b) confirmed by MS following acetylation.
[d] These four acidic components had similar R_fs to aqueous soluble acidic components identified in wheat stalks as the benzyl alcohol of CGA-107955.

Mature Wheat Stalks. The extraction and characterization of ^{14}C-residues in mature wheat stalks are summarized in Table 4. Residues were solvent extracted, filtered, and concentrated as described above. The concentrated solvent extracted residues were subjected to size exclusion and C-18 chromatography prior to partitioning with $CHCl_3$. The aqueous residues were separated by anion exchange chromatography into neutral and two acidic fractions (designated B and C).

The neutral aqueous residues were purified by XAD chromatography and separated into eight components (N1-N8) by preparative HPLC. Components N1a, N2, N4, and N5 were isolated by repetitive preparative HPLC and TLC and then acetylated. The acetylated components were purified by preparative HPLC and identified by mass spectrometry (MS). Acetylated N5 was also analyzed by NMR. Component N6 was further separated by preparative HPLC and 1D-TLC into N6a and N6b, which were subsequently acetylated and isolated by HPLC and identified by MS. The identities of each of these N-fractions were also confirmed by HPLC and 2D-TLC analyses of ß-glucosidase hydrolysates generated prior to acetylation. Neutral component N3 was purified by preparative HPLC and 1D-TLC then characterized by ß-glucosidase digestion followed by HPLC and 2D-TLC analysis. N7 was hydrolyzed by ß-glucosidase and subsequent HPLC and 2D-TLC analyses.

The acidic B fraction from anion exchange chromatography of aqueous residues was applied to a XAD-4 column and residues were eluted in ACN and MeOH fractions. The ACN fraction was subjected to preparative 1D-TLC yielding three regions of radioactivity. One region, designated B-I, was characterized by HPLC and 2D-TLC. The other two TLC regions were each purified by two preparative HPLC steps then combined with the MeOH XAD eluate. These combined fractions were subsequently separated into fractions designated B-II and B-III. Residues in B-III were identified by HPLC and 2D-TLC. Residues in B-II were identified by HPLC and 2D-TLC following two further preparative HPLC steps.

The non-extractable residues from mature wheat stalks were hydrolyzed with protease (pH 7, at 37 C for 16 hrs) and the resulting hydrolyzed ^{14}C-residues were partitioned into EtOAc and analyzed by HPLC and 2D-TLC. The aqueous fraction was hydrolyzed with ß-glucosidase, and the resulting residues were partitioned into EtOAc and analyzed by HPLC and 2D-TLC.

Table 4. Extraction and characterization of ^{14}C-residues in mature wheat stalks grown in [^{14}C]metalaxyl-treated soil aged 232 days prior to planting.

Commodity Fraction	% TRR [a]	ppm	Characterization/Identification [b]
Mature wheat stalks (7.171 ppm)			
$MeOH:H_2O$	74.5	5.342	Solvent partitioned.
$CHCl_3$	7.83	0.559	HPLC/TLC: Metalaxyl 0.1% (0.007 ppm) CGA-62826 0.7% (0.05 ppm) CGA-108905 1.0% (0.072 ppm) CGA-94689A/B 1.9% (0.136 ppm) CGA-107955 0.4% (0.029 ppm) CGA-37734 1.0% (0.072 ppm) CGA-100255 0.3% (0.022 ppm) CGA-67868 0.2% (0.014 ppm) CGA-67869 0.1% (0.007 ppm) Benzyl alcohol of CGA-67868 (N1a aglycone) 0.8% (0.057 ppm) Glucose conjs of CGA-94689A/B (N4/N5) 1.2% (0.086 ppm) Unknown <0.1% (0.002 ppm)
Aqueous	66.7	4.781	Anion exchange chromatography.
Neutral	46.0	3.299	Following XAD clean-up, prep HPLC isolated 8 components which were further characterized by HPLC, TLC, GC/MS, acylation, enzyme hydrolysis, and/or NMR: glucose conjugates of: (N1a aglycone) Benzyl alcohol of CGA-67868 6.3% (0.452 ppm) (N2) CGA-37734 4.3% (0.308 ppm) (N3) CGA-100255 1.3% (0.093 ppm) (N4) CGA-94689A 12.2% (0.875 ppm) (N5) CGA-94689B 18.4% (1.319 ppm) (N6a,b) CGA-62826 2.0% (0.143 ppm) (N7) CGA-107955 1.5% (0.108 ppm) (N8) Unknown <0.1% (0.003 ppm)
Acidic-B	16.9	1.211	Eluate from XAD column subjected to prep 1D-TLC and HPLC isolating 3 fractions (BI-BIII)
BI	4.62	0.331	HPLC and 2D-TLC: Benzyl alcohol of CGA-67868 (N1a aglycone) 0.5% (0.036 ppm) CGA-94689A/B 3.6% (0.258 ppm) CGA-100255 0.2% (0.014 ppm) 2 Unknowns <0.1% (0.001-0.002 ppm)

(continued; footnotes follow)

Table 4. (*continued*).

Commodity Fraction	% TRR [a]	ppm	Characterization/Identification [b]
Mature wheat stalks (continued)			
BII	4.67	0.335	HPLC and 2D-TLC: Benzyl alcohol of CGA-107955 4.6% (0.330 ppm) Unknown 0.1% (0.007 ppm)
BIII	7.57	0.543	HPLC and 2D-TLC: Benzyl alcohol of CGA-62826 2.6% (0.186 ppm) CGA-119857 0.4% (0.029 ppm) CGA-108905 1.0% (0.072 ppm) CGA-107955 0.7% (0.05 ppm) CGA-94689B 0.1% (0.007 ppm) CGA-62826 0.5% (0.036 ppm) 4 Unknowns 0.4-0.8% (0.029-0.057 ppm)
Acidic-C	3.8	0.272	Not further analyzed.
Solids	25.5	1.829	Protease digestion released 18.2% TRR (1.305 ppm); solvent partitioned.
Protease-released (EtOAc)	7.9	0.567	HPLC and 2D-TLC: Metalaxyl 1.2% (0.086 ppm) CGA-67868 0.1% (0.007 ppm) CGA-62826/CGA-100255 0.2% (0.014 ppm) CGA-94689A/B 5.2% (0.373 ppm) CGA-37734 0.7% (0.05 ppm) N1a/N1b aglycones 0.3% (0.022 ppm)
Protease-released (Aqueous)	10.3	0.739	Hydrolyzed with ß-glucosidase and partitioned into EtOAc; the organic fraction was then analyzed by HPLC and 2D-TLC: Metalaxyl <0.1% CGA-62826/CGA-100255 0.1% (0.007 ppm) CGA-94689A/B 0.3% (0.022 ppm) CGA-107955 0.1% (0.007 ppm) CGA-108905 0.1% (0.007 ppm) CGA-37734 0.1% (0.007 ppm) CGA-109857 <0.1% N1a/N1b aglycones 0.1% (0.007 ppm)
Solids	7.3	0.523	Not further analyzed.

[a] %TRRs were normalized by the registrant for percent recovery.
[b] Percentages reported are given as %TRR.

Wheat Grain. The extraction and characterization of ^{14}C-residues in wheat grain are summarized in Table 5. As for the other matrices, ^{14}C-residues in wheat grain were extracted with MeOH:water, but soluble residues were not analyzed further. Subsamples of the unextracted ^{14}C-residues were hydrolyzed in cellulase or 0.1 M HCl. The hydrolysates were partitioned with EtOAc, and the resulting organic and aqueous fractions were analyzed by HPLC and 2D-TLC.

Table 5. Extraction and characterization of ^{14}C-residues in wheat grain grown in [^{14}C]metalaxyl-treated soil aged 232 days prior to planting.

Commodity Fraction	% TRR [a]	ppm	Characterization/Identification [b]	
Wheat grain (0.593 ppm)				
MeOH:H_2O	22.0	0.130	Not further analyzed.	
Solids	78.0	0.463	Subsamples individually subjected to cellulase or 0.1 M HCl hydrolysis.	
Cellulase	48.1	0.285	Solvent partitioned.	
EtOAc	11.6	0.069	2D-TLC:	
			CGA-108905	10% (0.059 ppm)
Aqueous	36.5	0.216	2D-TLC:	
			CGA-108905	4.8% (0.028 ppm)
			CGA-79353	0.4% (0.002 ppm)
Solids	29.9	0.177	Not further analyzed.	
0.1 M HCl	51.2	0.304	Solvent partitioned; preparative 1D-TLC separated organic fraction into 2 bands (1 and 2).	
EtOAc-band 1	8.4	0.050	HPLC:	
			CGA-119857/CGA-79353	2.8% (0.017 ppm)
			CGA-108906	1.5% (0.009 ppm)
EtOAc-band 2	12.6	0.075	HPLC:	
			CGA-108905	12.5% (0.074 ppm)
Aqueous	30.3	0.180	HPLC:	
			CGA-67866	0.5% (0.003 ppm)
			Metalaxyl/CGA-67869	0.9% (0.005 ppm)
			CGA-62826	0.4% (0.002 ppm)
			CGA-108905	6.4% (0.038 ppm)
			CGA-119857/CGA-79353	2.8% (0.017 ppm)
Solids	26.8	0.159	Not further analyzed.	

[a] %TRRs were normalized by the registrant for percent recovery.
[b] Percentages reported are given as %TRR.

Tobacco. In addition to the rotational crops, the registrant also conducted complete analyses on ^{14}C-residues in immature and mature tobacco, the primary crop. These data are summarized in Table 6. ^{14}C-Residues were extracted in MeOH:H_2O (9:1, v/v), filtered, concentrated, and partitioned between $CHCl_3$ and aqueous fractions. Organosoluble ^{14}C-

residues were analyzed by HPLC and 2D-TLC. Aqueous soluble residues were hydrolyzed with ß-glucosidase and analyzed by HPLC and 2D-TLC.

Table 6. Extraction and characterization of ^{14}C-residues in immature and mature tobacco leaves treated with [^{14}C]metalaxyl (1x) as a preplant soil application.

Commodity Fraction	% TRR [a]	ppm	Characterization/Identification [b]
Tobacco 4-week combined top and bottom leaves (6.223 ppm)			
$MeOH/H_2O$	93.0	5.79	Solvent partitioned.
$CHCl_3$	67.8	4.219	HPLC and 2D-TLC: Metalaxyl 50.3% (3.127 ppm) CGA-107955 4.4% (0.274 ppm) (N7) Glucose conj of CGA-107955 6.3% (0.392 ppm) CGA-94689B 0.8% (0.050 ppm)
Aqueous	32.2	2.00	Hydrolyzed with ß-Glucosidase and analyzed by HPLC and 2D-TLC: aglycones isolated: CGA-94689A 1.7% (0.106 ppm) CGA-94689B/N1a aglycone 3.3% (0.205 ppm) CGA-100255 2.6% (0.162 ppm) CGA-37734 0.5% (0.031 ppm) CGA-62826 0.4% (0.025 ppm) CGA-107955 1.9% (0.118 ppm) [including 1.1% as free CGA-107955]
Solids	7.0	0.436	Not further analyzed.
Tobacco mature combined top and bottom leaves (1.651 ppm)			
$MeOH:H_2O$	91.2	1.505	Solvent partitioned.
$CHCl_3$	56.7	0.936	HPLC and 2D-TLC: Metalaxyl 36.1% (0.596 ppm) CGA-107955 4.3% (0.0.071 ppm) (N7) Glucose conj of CGA-107955 9.1% (0.150 ppm) (N4/N5) Glucose conj of CGA-94689A/B 0.8% (0.013 ppm) CGA-119857 0.5% (0.009 ppm)
Aqueous	43.3	0.715	Hydrolyzed with ß-Glucosidase and analyzed by HPLC and 2D-TLC: aglycones isolated: CGA-94689A 1.8% (0.030 ppm) CGA-94689B/N1a aglycone 3.3% (0.205 ppm) CGA-100255 1.6% (0.026 ppm) CGA-37734 1.4% (0.023 ppm) CGA-62826 1.4% (0.023 ppm) CGA-107955 1.7% (0.028 ppm) [including 1.1% as free CGA-107955]
Solids	8.8	0.145	Not further analyzed.

[a] %TRRs were normalized by the registrant for percent recovery.

[b] Percentages reported are given as %TRR.

Characterization of Residues

Solubilized ^{14}C-residues were isolated and characterized by normal-phase 1D- and 2D-TLC analyses using five solvent systems, reverse-phase 2D-TLC using two solvent systems, and reverse-phase HPLC analysis using numerous solvent systems. Radioactive residues on TLC plates were quantified using a ß-emission TLC analyzer. For quantifying ^{14}C-residues from HPLC analysis, eluant fractions were collected and radioassayed by LSS. Selected components were also characterized by enzymatic hydrolysis, acetylation followed by MS analysis, and NMR.

Radioactive residues identified/characterized in the rotational crops and the target tobacco crop are summarized in Table 7. The proportion of Phase I metabolites in the extractable residue was higher in immature tissues than in mature ones, which contained higher proportions of polar metabolites and glucose conjugates. Chromatograms from the preliminary analyses were provided for all matrices, although quantitative data were not reported. Comparison of immature and mature soybean stalks indicated a predominance of metalaxyl in immature stalks, which appreciably decreased in mature stalks with concomitant increases in polar conjugates and the appearance of the acidic metabolite CGA-62826. Mature soybean pods contained CGA-62826 as the major residue. Mature lettuce showed a decrease in parent compared to the immature lettuce, although in both, the predominant residues were the glucose conjugates of CGA-100255 and the N-dealkylated derivative of CGA-100255 (3-OH phenyl form of CGA-67868). Immature wheat stalks contained metalaxyl, CGA-62826, and CGA-67868, whereas polar acidic components and glucose conjugates were more abundant in mature stalks. The major conjugates in mature stalks and hulls were the glucose conjugates of CGA-94689A/B. In wheat grain, the major residue was free CGA-108905. Metalaxyl and the Phase I metabolites, CGA-67868, CGA-107955, and CGA-94689A/B, predominated in immature sugar beet foliage, and decreased considerably in mature foliage and roots, with a corresponding increase in polar conjugates.

ß-Glucosidase hydrolysis was used to characterize polar neutral components in all tissues. The report stated that the enzyme does not release all of the aglycones and is more effective in breaking sugar-alcohol linkages than sugar-ester bonds. CGA-94689 isomer B was present in all crops and its A isomer was in all crops except lettuce. CGA-94689 isomers were the major conjugates in mature wheat stalks and hulls, whereas CGA-62826 was the major glucose conjugate in mature soybean tissues. CGA-100255, its N-dealkylated derivative (N1b), CGA-62826, and CGA-94689B were released from conjugation in lettuce.

Metalaxyl is metabolized in rotated crops via one or more pathways. In wheat, the primary pathway is by oxidation of a ring methyl and subsequent oxidation to acidic metabolites. In sugar beets, demethylation of the methyl ether and methyl ester lead to the formation of acidic metabolites. The predominant pathway in lettuce is via oxidation to 3-hydroxyphenyl metabolites. In all substrates, metabolites form glucose conjugates and more complex conjugates.

This confined rotational crop study is adequate. The results indicate that metabolism in rotated crops proceeds by the same pathways as seen in primary crops, with the exception of the formation of 3-hydroxyphenyl metabolites in lettuce. Two metabolites oxidized to the 3-hydroxyphenyl were detected in lettuce. These 3-OH compounds are not covered by the current tolerance definition. Metabolite CGA-100255 is a product of metalaxyl oxidized at the 3-position, and accounted for 11% of the TRR in immature lettuce. A second compound, which would represent an N-dealkylation product of CGA-100255, was detected in hydrolysates of aqueous and insoluble fractions, and was designated N1b aglycone; it constituted 16% of the immature lettuce TRR. Together, these two components comprised 27% of the immature lettuce TRR at a concentration of 0.24 ppm. The registrant also reported these compounds in mature lettuce with 8% of the TRR as CGA-100255 and 11% as N1b aglycone. Both were released from glucose conjugates. The target crop tobacco contained metabolite CGA-100255 at low levels, 2.6% of the TRR in immature leaves and 1.6% in mature leaves. CBRS will determine the significance of these 3-OH metabolites in rotated crops and whether or not a modification of the tolerance definition is warranted.

Table 7. Summary of metalaxyl metabolite analysis of rotated crop matrices.

Metabolite/component	% TRR (ppm)					
	Lettuce (immature)	Sugar beet roots	Wheat stalks	Wheat grain	Tobacco (immature)	Tobacco (mature)
Identified residues						
Metalaxyl	15.3 (0.134)	3.3 (0.009)	1.3 (0.093)		50.3 (3.127)	36.1 (0.596)
CGA-37734	**1.7 (0.015)** [a]		**6.1 (0.437)**		0.5 (0.031)	1.4 (0.023)
CGA-62826	**1.5 (0.013)**	25.5 (0.070)	3.2 (0.229)	0.4 (0.002)	0.4 (0.025)	1.4 (0.023)
Benzyl alcohol of CGA-62826			2.6 (0.186)			
CGA-67866				0.5 (0.003)		
CGA-67868			0.3 (0.021)			
(N1a) Benzyl alcohol of CGA-67868			7.6 (0.545)			
(N1b) Phenyl ring hydroxy CGA-67868	15.9 (0.140)					
CGA-67869			0.1 (0.007)			
CGA-79353		8.1 (0.022)		0.4 (0.002)		
CGA-94689A	**0.2 (0.002)**		**12.2 (0.875)**		1.7 (1.06)	1.8 (0.03)
CGA-94689B	**2.4 (0.021)**		**18.5 (1.326)**		0.8 (0.05)	
CGA-100255	11.1 (0.097)		**1.8 (0.129)**		2.6 (1.62)	1.6 (0.026)
CGA-107955	**3.1 (0.027)**	1.0 (0.003)	**2.7 (0.194)**		12.6 (0.784)	15.1 (0.249)
(B1/B2 or P1/P2) Benzyl alcohol of CGA-107955			4.6 (0.330)			
CGA-108905		3.2 (0.009)	2.1 (0.151)	33.7 (0.199)		
CGA-108906				1.5 (0.009)		
CGA-119857			0.4 (0.029)			
Total Identified	**51.2 (0.449)**	**41.1 (0.113)**	**63.5 (4.552)**	**36.5 (0.215)**	**68.9 (6.697)**	**57.4 (0.947)**
Characterized residues [b]						
Metalaxyl/CGA-67869/CGA-67868		4.7 (0.013)				
Metalaxyl/CGA-67869				0.9 (0.005)		
N1a/N1b aglycones			0.4 (0.029)			
CGA-94689 AB/CGA-107955		1.1 (0.003)				
CGA-94689A/B			11.0 (0.789)			0.8 (0.013)
CGA-62826/CGA-108905		5.3 (0.014)				
CGA-100255/CGA-67868/ CGA-62826	0.9 (0.008)					
CGA-107955/CGA-119857		0.8 (0.002)				0.5 (0.009)
CGA-108905/CGA-107955	**0.9 (0.008)**					
CGA-108906/CGA-79353		1.9 (0.005)				
CGA-119857/CGA-79353				2.8 (0.017)		
CGA-62826/CGA-100255			0.3 (0.021)			
Total Identified/Characterized	**53.0 (0.465)**	**54.9 (0.150)**	**75.2 (5.391)**	**40.2 (0.237)**	**68.9 (6.697)**	**58.7 (0.969)**

[a] **Bolded** values include both free and sugar conjugates of the metabolites listed.

[b] The metabolites were not fully resolved by the TLC/HPLC system used.

Metabolism of Metalaxyl in Tobacco and Potatoes

Metabolism studies on tobacco and potatoes, which were previously reviewed for residue chemistry data requirements, were resubmitted in support of the current study for Guideline 165-1. The data on potatoes (1981; MRID 42196502) were reviewed in the Residue Chemistry Chapter for the 1988 Guidance Document (MRID 00071603); no new data from this study were submitted. The tobacco study (1980; MRID 42196503) was originally reviewed in the 1981 Registration Standard (MRIDs 00100467 and 00100478). Additional data are provided in the current submission and are discussed below.

Ciba-Geigy Corporation submitted data (1980; MRID 42196503) pertaining to the metabolism of [^{14}C]metalaxyl in tobacco. Samples of field bright, greenhouse bright, and greenhouse burley cured tobacco obtained from previously submitted studies (MRIDs 00100467 and 00100478; Metalaxyl Registration Standard, 12/81) were reanalyzed to confirm the identities of four glucose conjugated metabolites.

Extraction and Hydrolysis of Residues

Radioactive residues were extracted with MeOH:H_2O (80:20, v/v), concentrated, and partitioned with hexane. The aqueous soluble ^{14}C-residues were lyophilized, redissolved in MeOH, and cleaned-up on a silica gel column. TLC of the eluate isolated 5 radioactive zones. One of the zones isolated was extracted with MeOH, and residues were acetylated using acetic anhydride and methane sulfonic acid. The acetylated residues were partitioned with EtOAc, concentrated, and cleaned up on a silica gel column. The eluate was analyzed by TLC. Identification and confirmation analyses were performed by TLC, HPLC, and MS.

Characterization of Residues

The TLC analysis of the silica gel column eluate resolved four radioactive zones (A through D). HPLC analysis of zone A identified the acetylated derivative of the β-D-glucoside ether conjugate of CGA-107955; its identity was confirmed by TLC. Zone B was subjected to preparative HPLC followed by TLC and further analysis by HPLC. The acetylated derivative of CGA-114522 was identified in zone B, and the identification was confirmed by MS and TLC. HPLC and TLC analyses of zone C identified CGA-114522 and the acetylated derivative of the β-D-glucoside of CGA-100255. The %TRRs of each of these metabolites in all of the samples were as follows: CGA-114522 (6.9-9.2% TRR), β-D-glucoside of CGA-100255 (2.8-4.1% TRR), and β-D-glucoside ether conjugate of CGA-107955 (3.2% TRR; only in greenhouse bright tobacco). The registrant stated that metabolite CGA-114522 may be present as two atropisomers (A and B). The β-D-glucoside of CGA-62826 was also found by TLC in all samples at ~1% TRR. To further confirm the identities of these metabolites, the purified acetylated residues were deacetylated, cellulase hydrolyzed, and co-chromatographed with standards using 2D-TLC.

Field Rotational Crop Studies

Ciba-Geigy (1983; MRID 41870308) submitted residue data from field rotational crop studies conducted in 1977-1978 on broccoli, cabbage, corn, lettuce, red beets, rye, soybeans, sugar beets, sweet potatoes, and wheat that were planted following metalaxyl-treated potatoes, tobacco, or wheat. Potatoes were treated six times with either the 6 lb/gal EC or 50% WP at 0.5 or 1.0 lb ai/A (2x and 4x the registered maximum rate). Tobacco received one treatment of the 50% WP at 3 or 6 lb ai/A (1x or 2x). Plant-back intervals for rotated crops ranged from ~6-12 months, with the exception of winter wheat and rye which were planted 1-6 weeks after treatment of potatoes and 3-19 months after treatment of tobacco. Immature and mature RAC samples were harvested and stored frozen until analysis. Sample storage information was not provided. A single treated and control sample were analyzed from each test using either Ciba-Geigy Method AG-348 (Method I in PAM Vol. II) or a version of AG-330, in which the extraction solvent and aliquot size were changed. The reported limit of detection for both methods for all RACs analyzed was 0.05 ppm. Recovery data from control RAC samples fortified with metalaxyl were analyzed for each test; these data are presented in Table 8. Field study parameters and residue data for each test are summarized by rotational crop in Table 9.

The submitted field rotational crop studies (GLN 165-2) are inadequate. The studies are potentially upgradable by providing additional recovery data. The registrant must demonstrate that the current enforcement method and the analytical methods used for the field rotational crop studies will detect representative metalaxyl metabolites containing the DMA and HMMA moieties. This may be done by validating the analytical methods used for the field rotational crop studies using representative metabolites from each of these classes.

Combined metalaxyl residues, convertible to 2,6-dimethylaniline, in these rotated crops were lower than the established tolerances in 40 CFR §180.408 (a) or indirect tolerances in 40 CFR §180.408 (b).

Combined metalaxyl residues, convertible to 2,6-dimethylaniline, were <0.05 ppm (nondetectable) in corn grain from all studies. Eight to nine months following treatment of potatoes at 2x, combined metalaxyl residues convertible to 2,6-DMA, were <0.05-0.54 ppm in corn forage and <0.05-0.08 ppm in corn fodder. One year following treatment of tobacco at 1x, combined metalaxyl residues, convertible to 2,6-DMA, were <0.05-0.23 ppm in corn forage and <0.05 ppm in corn fodder. Tolerances are needed to cover indirect or inadvertent residues in corn forage and fodder.

A tolerance of 1.0 ppm is pending for metalaxyl residues in forage, fodder, and straw of cereal grains as a result of seed treatment uses (PP#1F3993). CBRS has recommended in favor of establishing this tolerance (CBRS No. 10833; DP Barcode D183914; 9/30/93; D. Miller). If established, this tolerance will be higher than the residues convertible to 2,6-DMA occurring in forage and fodder from rotated corn planted 8 months after treatment with metalaxyl.

Table 8. Method recovery data from rotational crop RACs fortified with metalaxyl, per se.

Commodity (RAC analyzed)/ Method	Fortification levels (ppm)	Number of samples	Method recovery (%)
Lettuce (leaves, heads)			
AG-348	0.1-0.8	5 (1) [a]	67-99
AG-330 modified	0.1, 0.5	3 (1)	66-90
Cabbage and broccoli			
AG-348	0.05-0.8	10 (8)	48-79
Corn (forage, fodder, grain)			
AG-348	0.1-1.0	32 (18)	53-113
AG-330 modified	0.05-0.5	6 (4)	64-80
Wheat and Rye (forage, straw, grain)			
AG-348	0.05-2.0	47 (27)	47-94
AG-330 modified	0.05-1.0	9 (5)	58-95
Soybeans (forage, straw, grain)			
AG-348	0.05-2.0	39 (22)	42-130
AG-330 modified	0.05-0.5	6 (2)	62-97
Sugar and red beet (tops and roots)			
AG-348	0.1-1.0	16 (12)	37-100
AG-330 modified	0.05-1.0	3 (1)	66-82
Sweet potatoes (foliage and roots)			
AG-348	0.05-0.8	8	70-116
AG-330	0.05-0.2	3 (3)	60-68

[a] Values in parentheses are the number of samples with recoveries outside the acceptable (70-120%) range.

Table 9. Metalaxyl residues in rotational crops planted following metalaxyl-treated potatoes and tobacco.

Primary crop	State	Plant-back Interval	Formulation	Rate (lbs ai/A)	PTI (days)	RAC	Combined Residues (ppm) [a]
Broccoli							
Tobacco	GA	11 months	50WP	3.0	420	forage	0.59
					448	heads	0.21
				6.0	420	forage	0.82
					448	heads	0.24
Cabbage							
Potatoes	MI	9 months	2E	6 x 0.5	319	forage	<0.05
					319	heads	<0.05
	NY	8 months	2E	6 x 0.5	307	forage	<0.05
					321	heads	<0.05
	WA	8 month	5W	6 x 0.5	303	forage	0.30
					322	heads	<0.05
Tobacco	NC	10 months	50WP	3.0	375	forage	0.14
					420	heads	0.09
				6.0	375	forage	0.47
					420	heads	0.06
Lettuce							
Potatoes	PA	6 months	2E	6 x 0.5	279	forage	0.12
					307	heads	0.13 (0.09) [b]
	NY	9 months	50WP	6 x 0.5	326	immature leaves	0.37
					355	mature leaves	0.20
				6 x 1.0	326	immature leaves	1.4
					355	mature leaves	0.47
Tobacco	GA	11 months	50WP	3.0	420	heads	0.20
				6.0	420	heads	0.29 (0.06)
	KY	12 months	50WP	3.0	109	leaves	<0.05
				6.0	109	leaves	<0.05
Red Beets							
Potatoes	PA	7-8 months	--	6 x 0.5	307	early forage	0.10
					370	tops	<0.05
					370	roots	<0.05

(continued; footnotes follow)

Table 9. (*continued*).

Primary crop	State	Plant-back Interval	Formulation	Rate (lbs ai/A)	PTI (days)	RAC	Combined Residues (ppm) [a]
Sugar Beets							
Potatoes	IA	8 months	2E	6 x 0.5	324	forage	<0.05
					379	fodder	<0.05
					379	roots	<0.05
	NY	8 months	2E	6 x 0.5	307	forage	0.08 (0.26)
					390	tops	<0.05 (0.18)
					390	roots	<0.05
	NY	9-10 months	50WP	6 x 0.5	326	early forage	0.21 (0.13)
					414	late forage	0.08
					414	roots	<0.05
				6 x 1.0	326	early forage	0.73 (0.13)
					414	late forage	0.33
					414	roots	<0.05
Sweet Potatoes							
Tobacco	NC	12 months	50WP	3.0	437	forage	0.32
					498	tops	0.13 (0.09)
					498	roots	<0.05
				6.0	437	forage	0.55
					498	tops	0.17 (0.09)
	GA	12 months	50WP	3.0	481	early foliage	0.12
					569	roots	<0.05
					569	tops	<0.05
				6.0	481	early foliage	0.06
					569	roots	<0.05
					569	tops	<0.05
	KY	12 months	50WP	3.0	404	forage	0.11
					474	tops	0.05
					474	roots	<0.05
				6.0	404	forage	0.19
					474	tops	0.20
					474	roots	<0.05

(continued; footnotes follow)

Table 9. (*continued*).

Primary crop	State	Plant-back Interval	Formulation	Rate (lbs ai/A)	PTI (days)	RAC	Combined Residues (ppm) [a]
Soybeans							
Potatoes	IA	8 months	2E	6 x 0.5	324	forage	0.41 (0.14)
					379	fodder	0.10 (0.12)
					379	beans	0.07
	MI	8 months	2E	6 x 0.5	319	forage	0.18, 0.19 [c] (0.1, 0.11)
					371	fodder	0.14, 0.17 [c] (1.1, 0.83)
					371	beans	<0.05, <0.05 [c] (0.18, 0.17)
	NY	8 months	2E	6 x 0.5	335	fodder	0.46 (0.29)
					335	beans	0.08 (0.07)
	WA	8-9 months	2E	6 x 0.5	351	forage	0.53
					426	fodder	0.16
					426	fodder	0.05
	PA	8 months	2E	6 x 0.5	326	forage	0.18 (0.52)
					405	fodder	0.14
					405	beans	<0.05
	NY	9 months	50WP	6 x 0.5	326	forage	0.83 (0.24)
				6 x 1.0	326	forage	2.7 (0.24)
Tobacco	GA	14 months	50WP	3.0	481	forage	0.29
					552	fodder	1.4 (0.80)
					552	beans	0.35 (0.47)
				6.0	481	forage	0.25
					552	fodder	0.40 (0.80)
					552	beans	0.49 (0.47)
	NC	11 months	50WP	3.0	437	forage	0.45
					539	fodder	0.15 (0.06)
					539	beans	0.05
				6.0	437	forage	1.3
					539	fodder	0.54 (0.06)
					539	beans	0.14
	KY	12 months	50WP	3.0	399	forage	0.31 (0.12)
					501	fodder	0.45
					501	beans	<0.05
				6.0	399	forage	0.82 (0.12)
					501	fodder	0.33
					501	beans	0.07

(continued; footnotes follow)

Table 9. (*continued*).

Primary crop	State	Plant-back Interval	Formulation	Rate (lbs ai/A)	PTI (days)	RAC	Combined Residues (ppm) [a]
Soybeans (continued)							
Wheat	CA	7 months	2E	1.0	298	forage	0.32, 0.34 [c] (0.10)
					375	fodder	0.14, 0.22 [c]
					375	beans	<0.05, <0.05 [c]
				2.0	298	forage	1.3 (0.10)
					375	fodder	0.50
					375	beans	<0.05
	MD	8 months	2E	1.0	386	fodder	0.06, 0.06 [c]
					386	beans	<0.05, 0.07 [c]
				2.0	386	fodder	0.08
					386	beans	<0.05
Corn							
Potatoes	IA	8 months	2E	6 x 0.5	324	forage	0.16
					379	fodder	0.05
					379	grain	<0.05
	MI	10 months	2E	6 x 0.5	319	forage	<0.05
					371	fodder	0.08
					371	grain	<0.05
	NY	8 months	2E	6 x 0.5	335	forage	0.09 (0.44)
					390	fodder	0.06 (0.47)
					390	grain	<0.05
	WA	9 months	5W	6 x 0.5	351	forage	0.54
					435	fodder	0.08
					435	grain	<0.05
	PA	8 months	2E	6 x 0.5	307	forage	0.06
					405	fodder	<0.05
					405	grain	<0.05
	NY	9 months	50WP	6 x 0.5	326	forage	<0.05
					447	fodder	0.05
					447	grain	<0.05
				6 x 1.0	326	forage	0.20
					447	fodder	0.14
					447	grain	<0.05
Tobacco	KY	12 months	50WP	3.0	399	forage	0.05
					501	stalks	<0.05
					501	grain	<0.05

(continued; footnotes follow)

Table 9. (*continued*).

Primary crop	State	Plant-back Interval	Formulation	Rate (lbs ai/A)	PTI (days)	RAC	Combined Residues (ppm) [a]
Corn (continued)							
Tobacco (*continued*)	KY	12 months	50WP	6.0	399	forage	0.14
					501	stalks	<0.05
					501	grain	<0.05
	GA	12 months	50WP	3.0	430	silage fodder	<0.05
					526	fodder	<0.05
					526	grain	<0.05
				6.0	530	silage fodder	0.06
					526	fodder	<0.05
					526	grain	<0.05
	NC	12 months	50WP	3.0	437	forage	0.23
					498	stalks	<0.05
					498	grain	<0.05
				6.0	437	forage	0.33
					498	stalks	0.15
					489	grain	<0.05
Winter Wheat							
Potatoes	MI	3 weeks	2E	6 x 0.5	199	spring forage	0.05
					294	straw	0.06
					294	grain	0.08
	NY	1 week	2E	6 x 0.5	231	forage	0.10 (0.58)
					307	straw	0.17 (1.0)
					307	grain	<0.05 (0.66)
	NY	6 weeks	50WP	6 x 0.5	649	forage	0.11
					711	straw	0.28
					711	grain	<0.05
				6 x 1.0	649	forage	0.58
					711	straw	1.4
					711	grain	0.15
	NY	6 weeks	50WP	6 x 0.5	68	early forage	1.0
					287	spring forage	0.27
					336	straw	0.56
					336	grain	0.19
				6 x 1.0	68	early forage	1.5
					287	spring forage	0.61
					336	straw	1.1
					336	grain	0.44

(continued; footnotes follow)

Table 9. (*continued*).

Primary crop	State	Plant-back Interval	Formulation	Rate (lbs ai/A)	PTI (days)	RAC	Combined Residues (ppm) [a]
Winter wheat (continued)							
Potatoes	NY	15 months (2nd crop)	50WP	6 x 0.5	448	early forage	0.09
				6 x 1.0	448	early forage	0.09
	WA	6 weeks	5W	6 x 0.5	240	forage	0.55
					329	straw	0.53 (0.13)
					329	grain	<0.05
	PA	1 month	2E	6 x 0.5	84	fall forage	0.90
					243	spring forage	0.12
					307	straw	0.13
					307	grain	0.06
Tobacco	NC	5 months	50WP	3.0	375	spring forage	0.30
					420	straw	0.19
					420	grain	0.05
				6.0	375	spring forage	1.1
					420	straw	0.25
					420	grain	0.09
		17 months (2nd crop)		3.0	731	spring forage	0.07
					766	straw	0.10
					766	grain	<0.05
				6.0	731	spring forage	0.08
					766	straw	0.18
					766	grain	<0.05
	KY	3 months	50WP	3.0	175	fall forage	<0.05
					354	spring forage	<0.05
					370	straw	<0.05
					370	grain	<0.05
				6.0	175	fall forage	0.35
					354	spring forage	0.12
					370	straw	<0.05
					370	grain	<0.05

(continued; footnotes follow)

Table 9. (*continued*).

Primary crop	State	Plant-back Interval	Formulation	Rate (lbs ai/A)	PTI (days)	RAC	Combined Residues (ppm) [a]
Winter wheat (continued)							
Tobacco	KY	15 months (2nd crop)	50WP	3.0	515	fall forage	0.06 (0.06)
					703	spring forage	0.07
					772	straw	0.05 (0.07)
					772	grain	0.05
				6.0	515	fall forage	0.13 (0.06)
					703	spring forage	0.06
					772	straw	0.11 (0.07)
					772	grain	<0.05
	GA	7 months	50WP	3.0	242	fall forage	0.18
					422	straw	0.36, 0.52 [c] (0.32, 0.24)
					422	grain	0.11 (0.09)
				6.0	242	fall forage	0.16
					422	straw	1.0, 0.99 [c]
					422	grain	0.11
		19 months (2nd crop)		3.0	603	fall forage	<0.05
					720	spring forage	0.38, 0.51 [c]
					751	straw	0.08 (0.08)
					751	grain	<0.05
				6.0	603	fall forage	0.06
					720	spring forage	0.05, 0.11 [c]
					751	straw	0.08 (0.08)
					751	grain	<0.05
Rye							
Potatoes	IA	2 weeks	2E	6 x 0.5	48	forage	0.28
					224	spring forage	0.19
					301	straw	<0.05
					301	grain	0.06 (0.06)

[a] Combined residues of metalaxyl and its metabolites determined as 2,6-dimethylaniline and expressed in metalaxyl equivalents.

[b] Residue values in parentheses are for apparent residues detected in control samples; residues in all other control samples were nondetectable (<0.05 ppm).

[c] Duplicate analysis of a single sample.