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

May 12, 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metolachlor (108801) Ciba Geigy Responses to Residue Chemistry Reregistration Requirements
Plant Metabolism Study in Potatoes (and Corn)
Use of Enforcement method for metabolism study samples
[MRID Nos. 42644301, 42652101 to -11; CB No. 11378; DPBarcode D188194]

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Ciba Geigy Corporation has submitted additional Residue Chemistry data in response to the Metolachlor Registration Standard Followup of 5/14/89 (R. S. Quick, DEB No. 4931), consisting of an additional plant metabolism study on potatoes (corn was also included in the study), including analysis of the plant metabolism samples by the enforcement method. Ciba Geigy's earlier responses to the Plant Metabolism data requirements in the FRSTR were reviewed in the Metolachlor Followup (6/89).

Metolachlor is on List A. The structure of metolachlor and its derivatives included in the tolerance expression are shown in Figure 1. A Registration Standard was completed 9/80, and the FRSTR 1/87 (Residue Chemistry Chapter 6/13/86). A Registration Standard Followup (Update) was completed 6/14/89. Tolerances for residues of metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide] in or on raw agricultural commodities are currently expressed in terms of the combined residues (free and bound) of metolachlor and its metabolites, determined as the derivatives, CGA-37913 and CGA-49751, each expressed as metolachlor [40 CFR §180.368(a), (b), and (c)]. Adequate analytical methods are available for enforcing tolerances of metolachlor and its free and bound metabolites in

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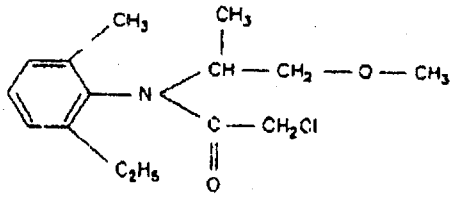
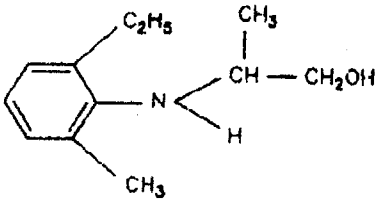
or on plant and animal commodities. The currently preferred method for plant matrices is listed as Method I in PAM, Vol. II and the preferred method for animal matrices is listed as Method II in PAM, Vol. II. As there are no Codex MRLs for residues of metolachlor, there is no question with respect to Codex/U.S. tolerance compatibility.

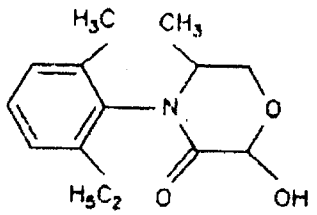
Figure 1: Structure of Metolachlor and its derivatives



Metolachlor



CGA-37913



CGA-49751

The Metolachlor followup concluded that the nature of the residue of metolachlor in potatoes was not adequately understood. Additional data were required as follows. The identity of metolachlor metabolites in potato tubers must be confirmed by a second chromatographic system or by some other suitable method such as mass spectrometry. Representative samples from the metolachlor metabolism studies must be analyzed by the residue analytical methods used for data collection and tolerance enforcement to ascertain that the methods are capable of adequately recovering and quantifying all residues of concern. These submissions address these plant metabolism data deficiencies.

CONCLUSIONS/RECOMMENDATIONS

1. The submitted potato metabolism study is adequate. Metolachlor is extensively metabolized. Although less than 50% of the metabolites were identified, each of the unidentified metabolites accounts for a small portion of the residue.

2. The potato metabolism study and a concurent corn metabolism study have been radiovalidated using a method similar to the metolachlor enforcement method. The method detected about 50% of the TRR in 75% mature potato tubers and corn stalks. Residues were non-detectable in mature tubers and corn grain, and thus, a comparison could not be made. Potato foliage was not tested.

3. For dietary exposure analysis, in cases where finite residue levels are found in crops, residue levels may need to be adjusted to account for the inability of the analytical method to detect more than 50% of the residue.

Detailed considerations

The Metolachlor Registration Standard Guidance Document (1/87) concluded that the metabolism of metolachlor is adequately understood in corn and soybeans, but required additional metabolism data for metolachlor on potatoes following foliar applications. In response to the Registration Standard, Ciba Geigy submitted a metolachlor potato metabolism study where potatoes were treated with preemergence and foliar applications in the green house and preemergence only in the field (1988; Report No. ABR-88110; MRID 40766601). The Agency concluded in the Metolachlor Registration Standard Followup (R. Quick, 6/14/89, DEB NO. 4931) that the submitted potato metabolism study was inadequate because no confirmation of metabolite identities was done, and the samples were not analyzed by the enforcement methods to ascertain that these methods are capable of determining all metabolites of concern.

In response, Ciba Geigy has submitted additional metabolism data for potatoes (Report No. ABR-92090; MRID 42652101) and SOPs for various aspects of conducting plant metabolism studies (MRID 42652102 to -11). In a separate report, Ciba Geigy has reported the results of using Analytical method AG-338 (the enforcement method) to analyze samples from the potato plant metabolism study (MRID 42644301). CBRS (then DEB) reviewed a protocol for this study (F. Toghrol, 2/28/91, DEB No. 7207) and concluded that the submitted protocol was very general. The registrant was advised to address the specific deficiencies in the earlier potato metabolism study.

Potatoes and corn were grown in pails in a greenhouse. For potatoes, the top two inches of soil were treated with ^{14}C-metolachlor, uniformly labeled in the phenyl ring, at 2.02 lb ai/A and incorporated into the soil, followed by a foliar application at 1.24 lb ai/A 31 days after planting and a soil drench application at 1.42 lb ai/A 66 days after planting, for a total seasonal rate of 4.68 lb ai/A. Potato tops and tubers were harvested at 50% maturity (35 days after the second application), 75% maturity (1 day after the third application), and 100% maturity (95 days after the third application). Potatoes were separated from foliage and washed under running tap water to remove adhering soil. After sample collection, the samples were stored in plastic bags at -20 C until transferred for analysis. Subsample extracts were kept refrigerated at about -5 C for up to 20 months.

The corn samples received a preplant incorporated application of metolachlor at 2.02 lb ai/A, followed by two stem injections of metolachlor, one of 3.59 mg metolachlor, and the second, of 4.21 mg metolachlor 101 days after planting. The registrant states that this was an exaggerated rate application, but the equivalent rates in lb ai/A were not stated. Immature stalks and cobs were collected at 75% maturity (24 days after the second application). Stalks, cobs and grain were collected at maturity (54 days after the third application).

Plant samples were homogenized, combusted and quantitated by liquid scintillation counting (LSC). Throughout the extraction procedure described below, aliquots of the extracts and the non-extractables were combusted and counted. Ciba Geigy provided flow charts for their extraction procedures (Ciba Geigy Figures 3, 4, 5, 5a, and 5b).

Crop samples were homogenized with methanol:water (8:2 for potato tubers and 9:1 for foliage) and filtered. The filtrate (aqueous methanol extract) was partitioned with chloroform. The non-extractables from mature potatoes were hydrolyzed (in individual experiments) with the following enzymes: α-amylase, amylglucosidase, β-glucosidase, cellulase, and protease to release aglycones. The conditions for the enzyme hydrolyses were described. The non-extractables were also hydrolyzed (individually) with 1M and 6M HCl and sequentially with various solvents in an experiment designed to separate natural products. Descriptions of the hydrolysis procedures, chromatographic conditions, derivatization procedures, and mass spectrometry follow.

Acid Hydrolysis. For the acid hydrolysis, the non-extractable mature potato tuber filtercake was incubated with 1M HCl at 78 C for one hour, and a second subsample with 6M HCl for two hours. The supernatant was lyophilized, resuspended in water, centrifuged, and partitioned with ethyl acetate. The neutralized

aqueous fraction was then acidified to 1M HCl and partitioned again. The neutral and acidic organic fractions were combined, concentrated by rotary evaporation, and resuspended in acetonitrile. Subsamples of the concentrated, combined organic fractions were analyzed by 2D-TLC.

A subsample of the extract was hydrolyzed with 6M HCl in ethanol for 2 hours at 110 C. The solution was cooled, diluted with water, and the ethanol evaporated under a stream of nitrogen. The sample was then analyzed using a C-18 column (HPLC System VI, described below).

Sequential Hydrolyses. A subsample of the non-extractable filtercake was hydrolyzed sequentially in an experiment designed to separate natural products. The residue was refluxed with sodium chloride solution for 2 hours. The dilute NaCl supernatant should contain polysaccharides. The filtercake residue was refluxed with EDTA solution for one hour. The dilute EDTA supernatant should contain pectic polysaccharides. The resulting filtercake was then incubated with 1.25N NaOH for 6 hours at room temperature. The resulting supernatant, which was neutralized with HCl, should contain hemicellulose fraction I. The resulting filtercake was extracted with sodium chlorite solution for three hours at 75 C. The dilute sodium chlorite supernatant should contain lignin. The remaining filtercake was incubated with 6M NaOH for 6 hours at room temperature. The supernatant, which was neutralized with HCl, should contain hemicellulose fraction II. The supernatants from all steps were combined except those from the NaOH extractions. The supernatants from the NaOH extractions were gelatinous and could not be characterized further. The combined supernatant was lyophilized, resuspended in water, centrifuged, and partitioned with ethyl acetate. The neutralized aqueous fraction was acidified to 1M HCl and partitioned and radioassayed. The neutral and acidic organic fractions were combined, concentrated by rotary evaporation and resuspended in acetonitrile. Subsamples were analyzed by 2D-TLC.

Thin Layer Chromatography (TLC) conditions. Eleven solvent systems were used with silica gel plates.

SS1 = chloroform:methanol:formic acid:water (150:40:8:4)
SS2 = ethyl acetate:isopropanol:water (128:48:24)
SS3 = n-butanol:acetic acid:water (134:33:33)
SS4 = chloroform:acetonitrile (9:1)
SS5 = chloroform:acetonitrile (8:2)
SS6 = chloroform:acetonitrile (7:3)
SS7 = chloroform:methanol:formic acid:water (90:10:1:1)
SS8 = methylene chloride:methanol (192:8)
SS9 = ethyl acetate:isopropanol:water (128:24:12)
SS10= chloroform:acetonitrile (154:46)
SS11= hexane:toluene:methanol:ethyl acetate (36:25:25:14)

For preparative TLC, silica gel plates were also used. The radioactive zones were scrapped (sic) and extracted with an appropriate solvent, such as chloroform, methanol:formic acid, or chloroform:methanol:formic acid:water. Visualization was by autoradiography (AMBIS Radioisotope Imaging System) or using a Berta Spark Chamber. Quantitation was by scraping the entire TLC plate and counting by LSC, with relative ratios of components determined by autoradiography. Standards were visualized by UV at 254 nm. Photocopies of Polaroid photographs of representative TLC separations were included in the report. The registrant states that identities of metabolites were confirmed by cochromatography of the standard (visualized by UV) on 2D TLC plates.

Anion Exchange Chromatography was used to separate basic/neutral components from acidic components. DEAE Sephadex A-25 columns were used. An aliquot of the aqueous extract was placed on the column and eluted with a step-wise gradient of water to 0.1 M Formic Acid. Basic/neutral components eluted first, followed by weakly acidic compounds. More acidic components were removed from the column by stripping with 1M formic acid. The eluant from the column was collected in 5 mL fractions and the fractions radioassayed.

Silica Gel Column Chromatography was used to separate the organic solubles. A subsample of the organic soluble fraction was dissolved in chloroform and placed on the column. The column was eluted with (1) hexane:chloroform (1:1), (2) chloroform, (3) chloroform:methanol (1:1), (3) chloroform:methanol:formic acid: water (100:20:4:1), (4) chloroform:methanol:formic acid:water (75:40:8:2), and (5) methanol:formic acid:water (165:8:2). The eluant was collected in 25 mL fractions and the fractions radioassayed.

Partitioning with XAD-4 Resin was used to separate polar aqueous soluble metabolites. The aqueous solution was placed on the column, which was eluted with water, and then with methanol. The water eluate and the methanol eluate were radioassayed.

HPLC Separations. Nine different HPLC systems were used. All systems used a variable wavelength UV detector, a flow-through radioisotope detector, and a fraction collector. Fractions of 0.5 or 1 minute were collected. HPLC systems I and II were used for the comparison with standards, and the comparison between potatoes and corn.

I. Reverse phase ODS-AQ column eluted with phosphate buffer:acetonitrile (95:5), held for 5 minutes, followed by a linear gradient to 70:30, held for 10 minutes, followed by a linear gradient to 0:100.

II. Same as I, except that initial solvent was 85:15. Retention times for metolachlor metabolite standards were given for this system.

III. Whatman Magnum 9 PAC semi-pr parative column. The mobile phase was 100% acetonitrile, followed by a linear gradient to acetonitrile:1% acetic acid in water (80:20), then to 100% 1% acetic acid in water.

IV. Whatman Magnum 9 silica gel semi-preparative column, eluted with chloroform and acetonitrile in varying ratios and gradients, depending on sample polarity.

V. Reverse phase Hamilton PRP-1 column (polymeric stationary phase), eluted with 0.05% phosphoric acid: acetonitrile:methanol (100:0:0) to (80:20:0), then to (90:10:0), then to (50:50:0), then (0:100:0), then (0:0:100). Fractions were collected at 0.3 minute intervals starting 18 min. after injection.

VI. C-18 column eluted with the same solvents as V.

VII. Whatman Partisil 10 ODS-2 column eluted with water: methanol (70:30) under isocratic conditions.

VIII. Whatman Partisil ODS-2 preparative column eluted with the same gradient as for system V, but with mobile phase of water:acetonitrile:methanol. Fractions were collected by hand.

IX. Semi-preparative ODS-2 Whatman Magnum-9 column, eluted with water:methanol (1:1).

Gas Chromatography was performed using an HP-5890 with a DB-5 column, flame ionization detector (FID) and flow-through radioactivity detector. A temperature gradient was used. Conditions were reported. Gas chromatograms of standards were submitted.

Acetylation. Some of the isolated metabolites were acetylated, by taking the sample to dryness under nitrogen, redissolving in dry pyridine, and acetylating with acetic anhydride. After acetylation, the samples were again taken to dryness under nitrogen, and reconstituted in methanol for analysis by 2D-TLC, HPLC, and MS.

Silylation. Isolated metabolites were taken to dryness under nitrogen, redissolved in acetonitrile, and silylated with dimethyl-t-butylsilyltrifluoroacetamide (MTBSFTA), followed by analysis by 2D-TLC.

Mass Spectrometry was performed using a Finnigan MAT-700 triple quadrupole mass spectrometer using EI, CI, FAB, and Thermospray ionization. Both MS and MS/MS (Daughter ion spectra) were used.

GC/MS was performed using an HP Series 2 gas chromatograph and an HP Mass Selective Detector (MSD). The column and conditions used were not provided in the text of the report.

Identification of Metabolites

The total radioactive residue found in mature potato tubers was 0.10 ppm. In the aqueous fraction, 20 components were separated. Because of the low levels of radioactivity, the potato foliage was used for identification of metabolites. Ten of the metabolites were identified using 2 chromatographic systems, chemical or enzymatic conversion, or mass spectrometry.

The TRR in potato foliage ranged from 1.48 to 1.73 ppm, at the various sample collection times. The TRR in potato tubers declined from 0.35 ppm at 50% maturity to 0.10 ppm at 100% maturity. The hexane extractable and chloroform soluble residues in foliage decreased from 10% and 14% of the residue at 25% maturity to 2% and 9% at maturity, respectively. The hexane soluble and chloroform soluble residues in tubers were 3-4% and 4-7%, respectively, at all sample collection times. The aqueous soluble residues were 72-81% of the TRR in foliage and 60-71% of the TRR in tubers. Non-extractable residues in foliage increased from 3.3% of the TRR at 25% maturity to 13% at maturity. In tubers, the non-extractable residues increased from 20% of the TRR at 50% maturity to 31% of the TRR at maturity. These percentages are tabulated in Ciba Geigy Table IV.

In corn, the TRR in corn stalks increased from 1.4 ppm, 29 days after planting to 8.6 ppm after the stem injection treatment. At maturity, the TRR in corn cobs was 0.15 ppm and in corn grain, 0.06 ppm. Organic soluble residues decreased from 21% of the TRR at 29 days after planting to <8% in mature corn stalks. Aqueous soluble residues were 75-81% of the TRR at all sample collection times. The percentage of non-extractable residues in corn stalks increased from 4% of the TRR at the 29 day sampling point to 11% of the TRR at harvest. The percentage of non-extractable residues in corn cobs increased from 23% of the TRR at the 98 day sampling point to 34% at harvest. The non-extractable residues in corn grain were 49% of the TRR at harvest. These percentages are tabulated in Ciba Geigy Table V.

The Separation and Identification of metolachlor residues in potato tubers and potato foliage are depicted in Tables 1 and 2 below. Because of the similar metabolite profile, most of the identification work was done with potato foliage. The structures of the compounds which were used as standards for the metabolism

work, along with structures of metabolites which were identified by mass spectrometry are shown in Ciba Geigy Figure 1, which is attached. Only 10 of the 34 compounds shown in Figure 1 were identified in this potato metabolism study, some as conjugates or hydrolysis products of the compounds shown.

Less than 32% of the TRR in potato tubers and foliage was identified. An additional 22% of the TRR was unidentified polar metabolites, and 19% was released by strong acid hydrolysis, but not further identified.

Earlier Metabolism Studies

In an earlier potato metabolism study (MRID 40766601) discussed in the Metolachlor Update of 6/14/89 (R. Quick) where potatoes were treated at 1 lb ai/A preplant, two additional compounds were identified in the organo soluble fraction of potato tubers, CGA-42444 at 7% of the TRR, and CGA-37735 at 6.5% of the TRR. The identification was by co-elution of known standards by HPLC. Confirmation of identity was not done. These two compounds would not be detected by the current enforcement method. The compounds might, however, be converted to 2-methyl-6-ethylaniline during the strong acid hydrolysis used in the enforcement method. In this study, the TRR in potato foliage increased from 0.08 ppm 45 days after planting, to 0.29 ppm at maturity. The TRR in potato tubers was 0.04 ppm. In mature tubers, 16% of the TRR was organosoluble, 65% water soluble, and 16% unextractable. The extractability of the TRR in mature foliage was similar. A total of 40% of the TRR was identified or tentatively identified by comparison to TLC analysis of corn stalks. Unidentified components separated by TLC analysis of the aqueous fraction of tubers ranged from <0.01 % TRR to 9.0% TRR with 18.9% TRR at the origin, totaling 41% TRR. Unidentified components separated by TLC analysis of the aqueous fraction of potato foliage ranged from <0.01 % TRR to 4.4% TRR with 3.0% TRR at the origin, totaling 22.5% TRR. We concluded that the study was unacceptable because the identity of the metabolites had not been confirmed and the enforcement method had not been radiovalidated using the metabolism study samples.

In an earlier potato metabolism study discussed in the Metolachlor FRSTR Residue Chemistry Chapter of 6/13/86 (MRID 00074898), we concluded that the study was unacceptable because only 44% of the TRR in potato tubers was characterized. The registrant claimed that the metabolites found in the acidic fraction of aqueous soluble residues in potato foliage and tubers were qualitatively similar to those found in soybeans and corn (8 zones on the TLC plate). Quantitatively, the radioactivity in seven of the eight radioactive zone in potato foliage and tuber extracts was significantly lower that the radioactivity found in each zone for soybeans and corn (except for 2 zones in soybeans which had no radioactivity reported).

This same study included some analysis following an acid reflux similar to that used in the metolachlor enforcement method. Mature potato tubers were refluxed for 16 hours in 6 N HCl, partitioned with dichloromethane, made basic, partitioned with hexane. The extracts were cleaned up and analyzed by HPLC using a PAC-M9 column eluted with 10% ethanol in heptane for the hexane fraction and 60% methanol in water for the dichloromethane fraction. CGA-37913 was reported in the dichloromethane fraction at 2% of the TRR. CGA-49751 was reported in the hexane fraction at 18.1% of the TRR. CGA-25702 (methyl ethyl aniline) was reported in the dichloromethane fraction at 3% of the TRR. Those compounds and other unknown totaled 43.8% of the TRR; 49.6% of the TRR did not partition into either dichloromethane or hexane after acid hydrolysis.

In the first corn metabolism study (MRID 00022874), discussed in the Metolachlor FRSTR Residue Chemistry Chapter of 6/13/86, where corn was treated preemergence at 2 lb ai/A, two sulfur bonded conjugates were found which totaled 80% of the extracted radioactivity. The cleaved conjugates (cleaved with Raney Nickel) were identified by GC and MS as CGA-41507 and CGA-42446. In the most recent corn study included in this submission, 11% of the radioactivity was non-extractable, so 80% of the extracted radioactivity would be about 70% of the TRR.

In a soybean metabolism study (MRID 00022872), discussed in the Metolachlor FRSTR Residue Chemistry Chapter of 6/13/86, the registrant showed that the metabolites in soybeans are similar to those in corn, although in soybeans there were higher concentrations of less polar metabolites than in corn. The rate of application and additional details were not provided in the FRSTR Residue Chemistry Chapter.

In a lettuce metabolism study (MRID 00074900), discussed in the Metolachlor FRSTR Residue Chemistry Chapter of 6/13/86, the registrant showed that the metabolites in lettuce are similar to those in corn, but quantitatively different. Lettuce was treated preplant with metolachlor at 3 lb ai/A. After acid hydrolysis, about 60% of the residue was found to be CGA-37913 or CGA-49751.

Table 1
Separation and Identification of Metolachlor Residues in Potatoes

	% of fraction	% TRR	ppm metolachlor eq.
Starting concentration		100%	0.100
Hexane soluble		3.1	0.003
Chloroform soluble		4.4	0.004
Aqueous soluble		60.8	0.061
CGA-51202	7.8	4.7	0.005
Enzyme hydrolysis[1]			
Anion Exchange			
Neutrals[2]		13.4	0.013
Acid I[3]		28.0	0.028
Acid II[4]		19.3	0.019
Non-extractable		31.8	0.032
Enzyme hydrolysis	43-53	13-17	0.014-0.017
organosoluble	52-75	8-12	0.008-0.012
A,B,C, D = CGA-51202[5]			
Acid hydrolysis	37-60	19-20	0.019-0.020
Organosoluble		12-14	0.012-0.014
A,C,D			
Another component[6]			
Sequential Hydrolyses			
NaCl+EDTA+$NaClO_2$	71.0	19.7	0.020
Organosoluble		10.1	0.010
NaOH I & II	30.5	9.7	0.010
Organosoluble - not analyzed			

[1] Enzyme Hydrolysis released conjugates, which were not further identified.

[2] The neutral fraction contained low levels of polar metabolites, with a large amount of natural materials interfering in the analysis. No further identification was done.

[3] The Acid I fraction had the same three major radioactive regions on the TLC plates as were found in potato foliage. These three regions were similar to three of the four regions found in TLC analysis of corn stalks.

[4] The acid II fraction had the same metabolite profile as potato foliage. Comparison of TLC separations is found in Ciba Geigy Figure 21. Greater quantities of compounds designated A, C, and D were produced. No further identification was done.

[5] The identity of CGA-51202 was based on co-migration by 2D-TLC.

[6] The acid hydrolysis of potato tubers produced 4 TLC spots (Ciba Geigy Figure 38). Only three of the spots were mentioned in the report.

Table 2
Separation and Identification of Metolachlor Residues in Potato Foliage

	% of fraction	% TRR	ppm metolachlor eq.
Starting concentration		100%	1.726
Hexane soluble		2.1	0.036
Chloroform soluble		8.5	0.147
Combined organic phases			
non-polar[7]	32	3.2	0.055
polar[8]	67.8	7.2	0.124
W = CGA-41507		0.24	0.004
X = CGA-37913		0.78	0.013
Y = CGA-46127		0.73	0.013
Z = CGA-46138[9]		0.29	0.005
Aqueous soluble		76.4	1.318
Anion Exchange			
Neutrals[10]		29.4	0.507
16 components			
largest component		4.5	0.077
N6		1.2	0.022

[7] Non-polar organics were purified by preparative TLC than analyzed by GC and 2D-TLC. No further identification was done.

[8] A total of 9 peaks were present. The registrant reports that C-25702 (2-ethyl-6-methyl-aniline) was not found in this fraction. The registrant claims that the aniline (with different substitutions on the phenyl ring) has been reported as a metabolite in other acetanilide herbicides. We note that the aniline is not expected as a metabolite for this or other acetanilide herbicides, but would be expected to be produced during the strong acid or strong base hydrolysis step in the analytical method. In fact, methyl ethyl aniline was reported (identity based on retention time only) in an earlier potato metabolism study discussed in the FRSTR Residue Chemistry Chapter (MRID 00074878) at 3% of the TRR.

[9] Identification confirmed by mass spectrometry (Ciba Geigy Figure 19).

[10] The neutral fraction was analyzed by 2D-TLC, HPLC, and Mass spectrometry. Zone N6 was acetylated, analyzed by Fast Atom Bombardment (FAB) MS, and was shown to be a sugar conjugate. The structure is shown in Figure 1 as N6. When analyzed by FAB-MS, other zones had spectra with the ion m/z 331, which indicates sugar cleavage.

Table 2
Separation and Identification of Metolachlor Residues in Potato Foliage, continued

	% of fraction	% TRR	ppm metolachlor eq.
Aqueous Soluble , continued			
Anion Exchange, continued			
Enzyme hydrolysis			
Organic soluble		5.3	0.091
5 compounds[11]		0.1-1.8	0.002-0.031 ea
Aqueous soluble		24.1	0.416
10 clusters[12]		0.6-4.5	0.011-0.078
CGA-41638		4.1	0.071
CGA-49751		0.75	0.013
Acid I[13]		39.7	0.685
1[14]		9.3	0.161
2[15]		13.2	0.229
3		3.4	0.059
=> CGA-51502 [16]		1.1	0.023

[11] Enzyme hydrolysis with cellulase and glucosidase cleaved sugar conjugates. Ethyl acetate extraction, followed by Normal Phase HPLC separation and additional HPLC cleanup separated 5 compounds which were different than those found before enzyme hydrolysis. Copies of TLC plates were submitted to show the comparison (Ciba Geigy Figure 33). The cleaved metabolites were analyzed by MS, but no identifications were made. The percentage of each cleaved metabolites were provided (Ciba Geigy Table IX).

[12] This fraction was hydrolyzed with glucosidase, cleaned up using a preparative C-18 column, and separated further by HPLC. Two of the 10 cleaved metabolites were identified. B2.2.3 was silylated and analyzed by GC/MS and identified as CGA-41638.

[13] The Acid I fraction had the same three major radioactive regions on the TLC plates as were found in potato tubers. These three regions were similar to three of the four regions found in the TLC analysis of corn stalks. HPLC separation of this fraction yielded 5 zones. Hydrolysis with protease and cellulase did not change the metabolite profile

[14] Hydrolysis with cellulase and glucosidase produced metabolites similar to those in zone 2, which were more polar than the other zones and were not further identified.

[15] These metabolites were more polar than other zones and were not further identified.

[16] This fraction contained multiple components before enzyme hydrolysis. Cellulase and glucosidase hydrolyzed the multiple components to CGA-51502, which then comprised 35% of the zone.

Table 2
Separation and Identification of Metolachlor Residues in Potato Foliage, continued

	% of fraction	% TRR	ppm metolachlor eq.
Aqueous Soluble , continued			
Anion Exchange, continued			
Acid I fraction, continued			
4 => CGA-118243[17]		7.4	0.128
5[18]		3.3	0.057
CGA-46576		0.7	0.009
CGA-118243		0.7	0.011
Acid II[19]		7.3	0.126
Non-extractable[20]		13.0	0.225

[17] CGA-118243 was produced by enzyme hydrolysis. The identity was confirmed by FAB-MS and MS/MS daughter ion spectra. (Ciba Geigy Figures 27 and 28)

[18] The two components of Zone 5 were identified by cochromatography by TLC on silica gel plates and HPLC using a C-18 column, and had been identified in corn before (Ciba Geigy Figure 29).

[19] The acid II fraction had the same metabolite profile as potato tubers. Comparison of TLC separations is found in Ciba Geigy Figure 21. No further attempt was made to identify the components of this fraction.

[20] No attempt was made to identify the components of this fraction. The only work reported was done in potato tubers.

Table 3
Summary of Metabolites identified in Potatoes and Potato Foliage
TRR = 0.100 ppm

Potatoes	% of residue *
CGA-51202	4.7
CGA-51202 Conjugate	1.1
CGA-51202 Conjugate	<10
CGA-41507	0.24
CGA-37913	0.78
CGA-46127	0.73
CGA-41638	0.29
N6	1.2
CGA-41638 Conjugate	4.1
CGA-49751 Conjugate	0.75
CGA-118243 Conjugate	7.4
CGA-46576 Conjugate	0.7
CGA-118243 Conjugate	0.7
Total Identified	< 32%
Unidentified polar metabolites	22.5%
Unidentified non-polar organics	3.2%
Unidentified Acid II fraction	19.3%
Unidentified Non-extractables	> 20
Total Unidentified	> 65%

* Assuming that the relative amounts of metabolites found in potato foliage are the same as the relative amounts in potato tubers.

Analytical Methodology

Ciba Geigy Corporation has submitted a radiovalidation of an update of the metolachlor enforcement method (Ciba Geigy Method AG-338, which updates Method AG-265) using samples from the corn and potato metabolism studies included in this review (1993, MRID 42644301).

Method AG-338, "Analytical Method for the Residues of Metolachlor Plant Metabolites Determined as CGA-37913 and CGA-49751 after Acid Hydrolysis," was modified for this study.

In the original method, metolachlor and its metabolites are converted to a mixture of CGA-37913 and CGA-49751 by refluxing with 6N HCl for 16 hours. An aliquot is partitioned with hexane and made basic. The hexane phase, which contains CGA-37913, is cleaned up on an alumina column and a silica column. Analysis is be GC with a Hall electrolytic conductivity detector specific for nitrogen. A second aliquot is partitioned with dichloromethane. The dichloromethane phase, which contains CGA-49751, is washed with sodium carbonate and cleaned up on an alumina column. The analyte is derivatized with boron trichloride/2-chloroethanol, producing the chloroethyl derivative. The derivative is partitioned with hexane, the hexane phase cleaned up on a silica gel and then alumina columns. Analysis is by GC with a Dohrmann microcoulometric detector specific for chloride or an alkali flame ionization detector in the nitrogen mode.

The current modifications to the method include substitution of a silica Sep-Pak for the silica cleanup column. The GC system used included a capillary DB-Wax column for the analysis of CGA-37913, and a DB-17 column for the analysis of CGA-49751. Both analyses used a Nitrogen Phosphorus Detector (NPD) in the Nitrogen mode. None of these modifications should seriously affect the use of the method. Chromatograms for the analyses were included.

The limits of detection for the method were reported to be 0.03 ppm for CGA-37913 and 0.05 ppm for CGA-49751. Recoveries were determined for CGA-37913 and CGA-49751 at the reported limits of detection and higher levels. The recoveries are tabulated below in Table 4, followed by the extractability and accountability of the analytical method in Table 5.

The extractability is similar to the percent extracted in the metabolism study, not including the additional enzyme, acid, and sequential extractions done on the "non-extractable" residue in potato tubers. The total accountability in mature potatoes and corn grain could not be determined because the residues were at or below the limit of detection of the analytical method. The total accountability in 75% mature potatoes, and corn stalks was about 50%. Accountability was not determined for potato foliage.

Table 4:
Analytical Method Recoveries of CGA-37913 and CGA-49751

Substrate	Fortification Level (ppm)	Recovery CGA-37913	Recovery CGA-49751
Mature tubers	0.05 + 0.05	60	62
	0.20 + 0.20	86	92
Intermediate tubers	0.05 + 0.05	71	59
	0.20 + 0.20	89	100
Corn grain	0.03 + 0.05	73	91
	0.10 + 0.10	63	85
Corn Stalks	0.03 + 0.05	107	97
	5.0 + 5.0	72	62

Table 5: Radiovalidation:
Extractability and Accountability of the Metolachlor Enforcement Method

Substrate	Residue (ppm)	% Extracted	% Accountability CGA-37913	CGA-49751	Total
Mature potatoes	0.100	73%	ND	ND	ND
Intermediate potatoes	0.212	76%	21%	26%	47%
Corn Stalks	8.555	70%	12%	41%	53%
Corn Grain	0.064	42%	ND	ND	ND

Attachment: Ciba Geigy Figure 1: Structures of standards used

cc w/ attachment: addressee, R.F., circu, S.F., S. Hummel, Metolachlor Reg. Std. F.
RDI:FBS:05/10/93:EZ:05/12/93
H7509C:CBII:SVH:svh:RM:804:CM#2:05/12/93

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