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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

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Reviewers: Sherrie L. Kinard, Chemist
Reregistration Branch 2
Health Effects Division, 7509C

Alan Nielsen, Branch Senior Scientist
Reregistration Branch 2
Health Effects Division, 7509C

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Sponsor: Syngenta Crop Protection, Inc., Greensboro, NC

Executive Summary

Representative rotational crops of lettuce, radish, and wheat were planted 30, 120, 174 (wheat only), and 364 days following a single application of [phenyl-U-^{14}C] *S*-metolachlor to a clay loam soil at 1.45 lb ai/A (0.4x the maximum seasonal use rate for corn). Immediately following the application, total radioactive residues (TRR) in the top soil layer (0-10 cm) were 1.535 ppm, and radioactivity in the soil declined steadily over time, reaching 0.324 ppm by 488-DAT. Over the course of the study, the majority of radioactivity in the soil remained in the top 0-10 cm layer, which accounted for >84% of the radioactivity in the soil at up to 1 year following treatment.

The appropriate commodities were harvested from each plant-back interval (PBI). With a few exceptions, TRRs in plant samples were highest at the 30-day PBI and declined steadily at later PBIs. Among the different plant samples, TRRs were highest in wheat fodder (0.157-0.784 ppm) at each PBI and were generally lowest in wheat grain (0.014-0.065 ppm) and radish roots (0.007-0.037 ppm). In lettuce, TRR declined from 0.109 ppm at the 30-day PBI to 0.009 ppm by the 364-day PBI, and in radish roots, TRR declined from 0.037 ppm at the 30-day PBI to 0.007 ppm by the 364-day PBI. Maximum TRR levels were attained in radish tops (0.229 ppm), wheat forage (0.141 ppm), wheat fodder (0.784 ppm), and wheat grain (0.065 ppm) at the 120-day PBI, but TRR levels in each of these commodities declined at later PBIs.

Methanolic extraction released 86.3-104.1% of the TRR from lettuce, radish roots and tops, and wheat forage, and 75.6-86.8% of the TRR from wheat fodder. The extractability of ^{14}C-residues was considerably lower from wheat grain (17.5-25.0% TRR). Solvent extracted ^{14}C-residues were profiled and quantified by 2D-TLC of the initial or purified extract fractions. Specific metabolites were isolated from wheat fodder and identified by TLC analysis with reference standards, LC/MS and NMR.

Radioactivity remaining in post-extraction solids (PES) of wheat fodder were further solubilized and characterized as either being incorporated into natural plant compounds (5.5-7.8% TRR) or as consisting of a variety of minor unknown polar components (14.6-17.3%TRR). Radioactivity in the PES fraction from grain was low (0.012-0.043 ppm) and was adequately solubilized by mild base extraction and acid hydrolysis. The majority of the radioactivity in grain was characterized as being incorporated into starch (~55% TRR) and protein (2.1-14.3% TRR) fractions. The overall recovery of radioactivity from the analysis of all plant samples was 96.8-112% of the TRR, and ^{14}C-residues in plant samples were adequately identified and/or characterized.

The metabolic profile of [^{14}C]*S*-metolachlor in rotational crops was complex, and the majority of the solvent extracted ^{14}C-residues (30.1-70.6% TR) from each commodity were characterized as consisting of multiple minor unknowns. However, detailed analyses identified parent and up to 15 metabolites in rotational crops, although the majority of these metabolites were minor components (<10% TRR and <0.01 ppm). Parent was detected only in lettuce from the 30-day PBI at 0.001 ppm (0.9% TRR). The metabolite profile was generally similar among the different rotational crop and at the different PBIs, although there were both qualitative and quantitative differences.

The following five metabolites were identified in lettuce: CGA 46576, NOA 443819, CGA 443156, NOA 436611, and CGA 351916. Each of these were minor metabolites (≤5% TRR), with the exception of CGA 351916 (10.6% TRR), and each was present at present at ≤0.006 ppm.

A total of nine metabolites were identified in radish roots and tops. The principal metabolites were CGA 380168, CGA 49750, CGA 133275-glucose, CGA 133275-glucose-malonyl, and Metabolite WH-7. These metabolites each accounted for >0.01 ppm in radish tops at one or more PBIs. All of the other metabolites identified in radishes were present at ≤0.003 ppm and included: CGA 46576, NOA 44381, Metabolite I_{17}, and CGA 133275.

The metabolite profile was similar among the wheat forage samples from the different PBIs. A total of six metabolites were identified in wheat forage. The principal metabolites were CGA 133275-glucose, CGA 380168, and Metabolite WH-7, along with minor (<5% TRR) amounts of NOA 443819, CGA 351916, CGA 443156, and CGA 133275.

The metabolite profile was also similar among the wheat fodder samples from each PBI and was qualitatively similar to the profile found in wheat forage. A total of seven metabolites were identified. The principal metabolites were CGA 380168, CGA 133275-glucose, CGA 133275,

and Metabolite WH-7, along with minor amounts (<5% TRR) of Metabolite I_{12}, NOA 436611, and CGA 351916.

Based on the metabolite profile, the metabolism of [^{14}C]*S*-metolachlor in rotational crops is similar to the metabolism observed in the primary crops. Metabolism in rotational crops primarily involves two pathways: (i) conjugation of the parent molecule with glutathione by substitution of the chlorine, followed by the degradation of the glutathione moiety to form a variety of sulfur containing metabolites; and (ii) direct oxidation of parent or secondary metabolites, primarily on the chloroacetyl side chain (Figure 1). Complete degradation of secondary metabolites either in the soil and/or plants also resulted in the incorporation of molecule fragments into natural plant constituents.

Analysis of soil (0-10 cm) identified parent and up to seven metabolites. Levels of parent declined steadily from 65.7% of the TRR (0.898 ppm) at 30 DAT to 1.9% of the TRR (0.006 ppm) by 488 DAT. Seven metabolites were identified in soil, but each accounted for <10% of the TRR. These metabolites included CGA 380168, CGA 351916, CGA 217498, NOA 413173, CGA 368208, NOA 436611, and CGA 46129.

The submitted confined rotational crop study adequately reflects the nature and quantity of ^{14}C-residues in rotational crops following a soil application of [^{14}C]*S*-metolachlor at rates up to 1.45 lb ai/A. However, the maximum seasonal use rate for *S*-metolachlor on any rotated crop is 3.7 lb ai/A on corn. Therefore, the usefulness of this confined study in assessing the need for more extensive rotational crop field trials is equivocal.

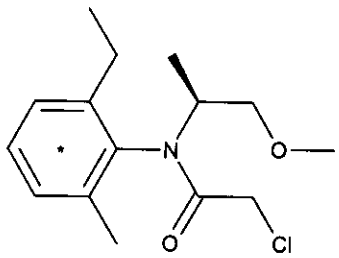
GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. There were no deviations from regulatory requirements that would impact the study results or their interpretation.

1. Materials and Methods

1.1. Substance

Common Name: *S*-Metolachlor
IUPAC Name: (S)-2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-acetamide
CAS Name: (S)-2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-acetamide
CAS Number: 87392-12-9
Company Name/Code: CGA 77102
Other Synonyms: Dual Magnum® Herbicide
Purity of Non-labeled Material: 99.6%
Location of Isotopic Label: Uniformly ^{14}C-labeled in the phenyl ring
Radiochemical Purity: ≥97.8% (as determined by TLC and HPLC)
Specific Activity: Specific activity of the formulated ^{14}C-test material was 23.51 µCi/mg (52,200 dpm/µg equivalents).



* denotes position of ^{14}C-label

1.2. Crop and Cultural Information

Types and Varieties of Crops: No primary crop was planted after the soil application. The representative rotational crops included: lettuce (*var.* Sunny and Libusa), radish (*var.* Selma and Wiela), spring wheat (*var.* Toronit), and winter wheat (*var.* Galaxie).
Test plots: The test consisted of a single 6 m^2 treated plot that was subdivided into 1 m^2 plots for lettuce and radishes or 2 m^2 plots for wheat. Portions of the test plot that were used for the initial planting (30-day PBI) were reused for different crops at the later PBIs.
Soil: Clay loam (22.7% sand, 45.7% silt, 31.6% clay, and 1.8% organic matter; pH 7.18; and CEC of 23.5 meq/100 g)
Growth Environment: The tests was conducted outdoors under ambient conditions, except during the winter, when the plots containing wheat were protected with a plastic cover to prevent freezing of the crop. The in-life phase of the study was conducted at the Syngenta Field Station in Stein (AG), Switzerland.

Conditions: The test crops were planted and maintained in accordance with good agricultural practices. Fertilizers, insecticides, and fungicides were applied as needed. Information on the fertilizer and pesticide applications were provided along with temperature and rainfall data. No unusual environmental conditions were observed during the study.

1.3. Application Information

Type of Application: [^{14}C]-*S*-Metolachlor was applied as a broadcast application to the soil surface.
Application Matrix: [^{14}C]-*S*-Metolachlor was formulated as an EC and diluted with water for application.
Application Rate: 1.63 kg ai/ha (1.45 lb ai/A; 0.4x rate) [note: the current maximum seasonal application rate for *S*-metolachlor on rotational crops is 3.7 lb ai/A/season on corn.]
Number of Applications: one
Plant-back Interval(s): The representative rotational crops of lettuce, radishes, and spring wheat were planted at 30, 120, and 364 days after application. In addition, a crop of winter wheat was planted at 174 days after application. Radish and wheat were directly seeded into the plots, and the lettuce was transplanted as seedlings.

1.4. Harvest/Post-harvest Procedures

For each PBI, samples of lettuce and radishes were harvested at maturity, 48-61 days after planting (DAP). Radish samples were then separated into roots and tops. For the spring wheat, samples of forage were collected at approximately 50% maturity (48-96 DAP), and samples of grain and fodder (straw plus husks) were collected at maturity (111-145 DAP). For the winter wheat crop, samples of forage were collected at in fall at 25% maturity (64 DAP) and in spring at 50% maturity (234 days DAP), and samples of grain and fodder were collected at maturity (286 DAP). After collection, samples of lettuce, radish tops and roots, and wheat forage were immediately frozen, homogenized in liquid nitrogen, and stored at -20° C. Samples of wheat grain and fodder were allowed to air dry in the laboratory for 6-49 days after collection. Fodder samples were then chopped, homogenized in liquid nitrogen, and placed in frozen storage. Grain samples were ground and then placed in frozen storage.

Soil core samples were also collected at 0, 30, 78, 120, 174, 238, 364, and 488 days after treatment (DAT). At the 0-day interval, soil was sampled to a depth of 10 cm. At later intervals, soil was sampled to a depth of 30 cm, and the soil cores were separated into 10 cm segments. The samples were pooled by depth, air dried, homogenized, and placed in frozen storage.

Table 1.3.1. Summary of Storage Conditions		
Matrices	Storage Temperature (°C)	Duration (days)
Lettuce	≤-18	18-75
Radish roots and tops		19-75
Wheat, forage, fodder, and grain		2-149

To demonstrate the stability of ^{14}C-residues during frozen storage, the registrant compared the TLC results from two extractions and analyses of spring wheat fodder from the 120-day PBI. This sample was initially air dried in the lab for 49 days, stored at ≤-18° C for 2 days, and then extracted. The initial extract was then analyze by 2D-TLC 12 days after extraction. A separate subsample was reextracted after 241 days of frozen storage and the extract was analyzed 7 days later by 2D-TLC.

1.4. Analytical Methods

Radioassay. For determination of TRR, triplicate plant and soil samples were radioassayed by combustion and liquid scintillation counting (LSC). The limit of quantitation (LOQ) for the radioassays was 0.002 ppm in lettuce, radish roots and tops, and soil, 0.003 ppm in wheat forage, and 0.004 ppm in wheat grain and fodder. Results from the radioassays of plant and soil samples are reported in Tables 2.1.1 and 2.1.2, respectively.

Extraction and fractionation. Homogenized plant and soil samples were extracted repeatedly by shaking for 4-24 hours in 80% aqueous methanol (MeOH), followed by centrifugation. The methanolic extracts for each sample were pooled, concentrated, and then analyzed by 2D-TLC for the initial identification and quantitation of metabolites. The fractionation and distribution of ^{14}C-residues in the various rotational crops are presented in Tables 2.2.1 through 2.2.7

As the wheat fodder sample from the 120-day PBI contained the highest level of ^{14}C-residues (0.784 ppm), this sample was used for isolation and confirmation of metabolite identities. This fodder sample was extracted as above, and the resulting solvent extract was partitioned with dichloromethane (CH_2Cl_2). The CH_2Cl_2 fraction was analyzed using several different TLC systems, and the aqueous fraction was further fractionated using a Serdolit AD-4 column eluted with a step gradient of water to MeOH. The resulting aqueous and methanolic fractions were further purified and compared to reference standards using multiple TLC systems, C_{18} and silica gel column chromatography, and various HPLC systems.

Post-extraction solids (PES) from lettuce, radish tops and roots, and wheat forage were not further analyzed as ^{14}C-residues in these fractions were low or accounted for <10% of the TRR. Only PES fractions from wheat fodder and grain from the 30-day and 120-day PBIs were further analyzed.

PES fractions from wheat fodder. The PES fraction from the 120-day wheat fodder was soxhlet extracted in MeOH overnight, and the resulting MeOH fraction was analyzed by TLC. The PES fractions from fodder of both the 30- and 120-day PBI samples were then extracted in boiling

water for 16 hours and hot filtered. The filtrate was concentrated, adjusted to pH 1.5, and then diluted with ethanol (EtOH) to precipitate pectins, which were removed the centrifugation. Solids remaining after the hot water extraction were then base hydrolyzed by refluxing in 10% NaOH for 3 hours. The remaining solids were not further analyzed, but the solubilized ^{14}C-residues were filtered hot, acidified to pH 1, concentrated, and then refrigerated to precipitate lignins. The remaining acidic fraction was then partitioned with CH_2Cl_2.

A subsample of the PES fraction from the 120-day PBI fodder was also acid hydrolyzed overnight the refluxing in 6N HCl. The resulting hydrolysate was partitioned with CH_2Cl_2, adjusted to pH 10.5, and then repartitioned with CH_2Cl_2. The resulting CH_2Cl_2 fractions were analyzed by TLC.

PES fractions from wheat grain. The PES fractions from the 30- and 120-day PBI wheat grain samples were extracted overnight at room temperature in 0.05N NaOH and centrifuged. The extract was neutralized to pH 6, and a protein fraction was precipitated by the addition of EtOH. The resulting protein and ethanolic fractions were not further analyzed. Solids remaining after the mild base extraction were then acid hydrolyzed by refluxing overnight in 1N HCl and filtered. The acidic filtrate was adjusted to pH 6.5 and partitioned with CH_2Cl_2. The CH_2Cl_2 fraction was not further analyzed. Glucose in the aqueous hydrolysate was then derivatized to glucosazone by refluxing for three hours in an aqueous solution containing phenylhydrazine-HCl and sodium acetate. The solution was cooled, and the precipitated osazone was recrystallized three time by dissolving in water/MeOH and then drying.

Identification of ^{14}C-Residues.

^{14}C-Residues in the initial solvent fractions were profiled and quantified by 2D-TLC using silica gel plates and two solvent systems consisting of EtOAc:PrOH:formic acid:water (64:24:4:8) and chloroform:MeOH:formic acid:water (75:20:4:2). Reference standards on TLC plates were visualized under UV light (254 nm) and radioactive residues were detected and quantified using a Fuji Bioimaging Analyzer BAS-1000.

For confirmation of metabolite identities, an initial solvent extract from the 120-day PBI, wheat fodder sample was used for isolation of metabolites. The methanolic extract was concentrated and partitioned between CH_2Cl_2 and water. The organic fraction was then analyzed using two different 2D-TLC systems. The aqueous fraction, which contained the majority of the radioactivity, was separated into four fractions using a Serdolit AD-4 column eluted with a step gradient of water to MeOH. The resulting fractions were further purified using preparative TLC, C_{18} and silica gel column chromatography, and HPLC to isolate individual metabolites.

Depending on the fraction, isolated components were further characterized by (i) electrophoresis, (ii) enzymatic treatment overnight at 37° C with cellulase or β-glucosidase in a phosphate buffer (pH 4.7), and/or (iii) acid hydrolysis by refluxing overnight in 6N HCl. Isolated components were identified by co-chromatography with reference standards using 1D- and 2D-TLC. TLC analyses were conducted with either silica gel or reverse-phase plates using one or two of the 12 different solvent systems. A total of 46 reference standards were used in the current study for comparison, including compounds isolated and identified (MS) in an earlier soybean metabolism

study. Metabolites WH-7, WH-9, and WH-10, which did not correspond to any of the available reference standards were positively identified using LC/MS and NMR.

Summaries of the characterization and identification of ^{14}C-residues in crops from the 30-day and 120-day PBIs are presented in Tables 2.3.1 and 2.3.2., and a summary of the compounds identified in soil (0-10 cm layer) over time is presented in Table 2.4.

2. Results

Table 2.1.1. Total Radioactive Residues in Rotational Crops Following a Single Soil Application of [Phenyl-U-^{14}C] *S*-Metolachlor at 1.63 kg ai/ha (1.45 lb ai/A).

Crop	Crop Matrix	PBI (days)	Sampling interval [a]		TRR (ppm) [b]
			DAT	DAP	
Lettuce	leaves	30	78	48	0.109
		120	174	54	0.045
		364	419	55	0.009
Turnip	tops	30	78	48	0.180
		120	174	54	0.229
		364	425	61	0.039
	roots	30	78	48	0.037
		120	174	54	0.028
		364	425	61	0.007
Spring Wheat	forage (50% mature)	30	78	48	0.108
		120	174	54	0.141
		364	460	96	0.044
	fodder	30	141	111	0.340
		120	265	145	0.784
		364	488	124	0.187
	grain	30	141	111	0.016
		120	265	145	0.065
		364	488	124	0.014
Winter Wheat	forage (25% mature)	174	238	64	0.082
	forage (50% mature)		408	234	0.025
	fodder		460	286	0.157
	grain		460	286	0.015

[a] The sampling interval is reported both in terms of days after treatment (DAT) and days after planting (DAP) of the rotational crop.

[b] TRR data are the average of triplicate subsamples and are expressed in [^{14}C]metolachlor equivalents..

Table 2.1.2. Total Radioactive Residues in Soil Following a Single Broadcast Application of [Phenyl-U-^{14}C] *S*-Metolachlor at 1.63 kg ai/ha (1.45 lb ai/A; 0.4x).

Sampling interval (DAT) [a]	Sample depth (cm)	TRR (ppm) [b]	Distribution of radioactivity (%) [c]
0	0-10	1.535	100
30	0-10	1.367	99.3
	10-20	0.004	0.3
	20-30	0.004	0.4
78	0-10	1.174	94.9
	10-20	0.069	4.7
	20-30	0.004	0.4
120	0-10	0.957	99.0
	10-20	0.006	0.7
	20-30	0.003	0.3
174	0-10	1.047	85.3
	10-20	0.180	13.8
	20-30	0.014	0.9
238	0-10	0.522	80.8
	10-20	0.104	11.6
	20-30	0.041	7.5
364	0-10	0.836	84.3
	10-20	0.111	9.5
	20-30	0.045	6.3
488	0-10	0.324	77.5
	10-20	0.053	13.5
	20-30	0.021	9.0

[a] DAT = days after treatment

[b] TRR data are the average of triplicate subsamples and are expressed in [^{14}C]metolachlor equivalents.

[c] Percentage of total soil radioactivity in each soil layer.

Table 2.2.1 Extraction, Characterization, and Identification of ^{14}C-Residues in **Lettuce** harvested from the 30- and 120-day PBIs.

Fraction ID	% TRR	ppm	Characterization/identification
30-day PBI (0.109 ppm) [a]			
MeOH/water extract	95.5	0.104	Concentrated and analyzed by 2D-TLC: CGA 77102 0.9% TRR 0.001 ppm CGA 46576 3.1% TRR 0.003 ppm NOA 443819 5.0% TRR 0.005 ppm NOA 436611 0.9% TRR 0.001 ppm CGA 351916 2.1% TRR 0.002 ppm NOA 443156 5.1% TRR 0.006 ppm unresolved [b] 8.2% TRR 0.009 ppm 21 minor unknown fractions each at ≤7.0% TRR (≤0.008 ppm), totaling 70.2% TRR (0.077 ppm)
PES	5.8	0.006	Not further analyzed
120-Day PBI (0.045 ppm) [a]			
MeOH/water extract	92.8	0.042	Concentrated and analyzed by 2D-TLC: CGA 46576 2.7% TRR 0.001 ppm NOA 436611 2.7% TRR 0.001 ppm CGA 351916 10.6% TRR 0.005 ppm NOA 443156 1.8% TRR 0.001 ppm unresolved [b] 4.3% TRR 0.002 ppm 15 minor unknown fractions each at ≤13.2% TRR (<0.006 ppm), totaling 70.6% TRR (0.032 ppm)
PES	7.0	0.003	Not further analyzed

[a] TRR for each matrix is listed in parentheses.
[b] Unresolved radioactivity was not associated with any specific TLC region.

Table 2.2.2 Extraction, Characterization, and Identification of ^{14}C-Residues in **Radish Tops** harvested from the 30-, 120-, and 364-day PBIs.

Fraction ID	% TRR	ppm	Characterization/identification
30-day PBI (0.180 ppm) [a]			
MeOH/water extract	97.8	0.176	Concentrated and analyzed by 2D-TLC: CGA 380168 8.6% TRR 0.016 ppm Metabolite WH-7 4.4% TRR 0.008 ppm CGA-46576 4.0% TRR 0.007 ppm CGA 133275-glucose 7.7% TRR 0.014 ppm CGA 133275-gluc-malonyl 4.3% TRR 0.008 ppm NOA 443819 4.3% TRR 0.008 ppm Metabolite I_{17} 2.8% TRR 0.005 ppm CGA 49750 8.1% TRR 0.015 ppm unresolved [b] 3.3% TRR 0.006 ppm 16 minor unknown fractions each at ≤8.1% TRR (<0.015 ppm), totaling 50.3% TRR (0.091 ppm)
PES	4.7	0.009	Not further analyzed
120-Day PBI (0.229 ppm) [a]			
MeOH/water extract	104.1	0.238	Concentrated and analyzed by 2D-TLC: CGA 380168 11.4% TRR 0.026 ppm Metabolite WH-7 6.6% TRR 0.015 ppm CGA 133275-glucose 13.6% TRR 0.031 ppm CGA 133275-gluc-malonyl 5.7% TRR 0.013 ppm CGA 49750 9.5% TRR 0.022 ppm unresolved [b] 13.9% TRR 0.032 ppm 8 minor unknown fractions each at ≤8.5% TRR (<0.019 ppm), totaling 43.6% TRR (0.100 ppm)
PES	3.7	0.009	Not further analyzed
364-Day PBI (0.039 ppm) [a]			
MeOH/water extract	96.8	0.038	Concentrated and analyzed by 2D-TLC: Metabolite WH-7 2.6% TRR 0.001 ppm CGA 133275-glucose 8.3% TRR 0.003 ppm CGA 133275-gluc-malonyl 7.7% TRR 0.003 ppm CGA 49750 8.9% TRR 0.003 ppm unresolved [b] 13.2% TRR 0.005 ppm 5 minor unknown fractions each at ≤24.1% TRR (≤0.009 ppm), totaling 56.0% TRR (0.021 ppm)
PES	10.2	0.004	Not further analyzed

[a] TRR for each matrix is listed in parentheses.

[b] Unresolved radioactivity was not associated with any specific TLC region.

Table 2.2.3 Extraction, Characterization, and Identification of ^{14}C-Residues in **Radish Roots** harvested from the 30- and 120-day PBIs.

Fraction ID	% TRR	ppm	Characterization/identification
30-day PBI (0.037 ppm) [a]			
MeOH/water extract	95.4	0.035	Concentrated and analyzed by 2D-TLC: CGA 380168 8.2% TRR 0.003 ppm Metabolite WH-7 0.8% TRR <0.001 ppm CGA-46576 5.9% TRR 0.002 ppm CGA 133275-glucose 1.2% TRR <0.001 ppm CGA 133275-gluc-malonyl 6.8% TRR 0.003 ppm NOA 443819 2.7% TRR 0.001 ppm Metabolite I_{17} 3.0% TRR 0.001 ppm CGA 49750 3.2% TRR 0.001 ppm CGA 133275 1.6% TRR <0.001 ppm unresolved [b] 4.3% TRR 0.002 ppm 17 minor unknown fractions each at ≤7.5% TRR (<0.003 ppm), totaling 57.7% TRR (0.021 ppm)
PES	8.9	0.003	Not further analyzed
120-Day PBI (0.028 ppm) [a]			
MeOH/water extract	91.2	0.026	Concentrated and analyzed by 2D-TLC: CGA 133275-glucose 5.5% TRR 0.002 ppm CGA 133275-gluc-malonyl 1.7% TRR <0.001 ppm CGA 49750 3.3% TRR <0.001 ppm CGA 133275 7.5% TRR 0.002 ppm unresolved [b] 10.9% TRR 0.003 ppm 8 minor unknown fractions each at ≤13.3% TRR (<0.004 ppm), totaling 62.4% TRR (0.017 ppm)
PES	10.0	0.003	Not further analyzed

[a] TRR for each matrix is listed in parentheses.
[b] Unresolved radioactivity was not associated with any specific TLC region.

Table 2.2.4 Extraction, Characterization, and Identification of ^{14}C-Residues in **Spring Wheat Forage** harvested from the 30-, 120-, and 364-day PBIs.

Fraction ID	% TRR	ppm	Characterization/identification
30-day PBI (0.108 ppm) [a]			
MeOH/water extract	95.3	0.103	Concentrated and analyzed by 2D-TLC: CGA 380168 7.7% TRR 0.008 ppm Metabolite WH-7 7.1% TRR 0.008 ppm CGA 133275-glucose 18.5% TRR 0.020 ppm NOA 443819 4.9% TRR 0.005 ppm CGA 351916 0.7% TRR <0.001 ppm NOA 443156 1.3% TRR 0.001 ppm unresolved [b] 10.0% TRR 0.011 ppm 10 unknown fractions each at ≤13.0% TRR (<0.014 ppm), totaling 45.2% TRR (0.049 ppm). The single fraction accounting for >10% TRR was WH-1 (13% TRR), which was the most polar fraction.
PES	7.9	0.009	Not further analyzed
120-Day PBI (0.141 ppm) [a]			
MeOH/water extract	104.0	0.147[14]	Concentrated and analyzed by 2D-TLC: Fraction WH-5/WH-6 [c] 14.0% TRR 0.020 ppm Metabolite WH-7 9.2% TRR 0.013 ppm CGA 133275-glucose 35.0% TRR 0.049 ppm NOA 443819 1.6% TRR 0.002 ppm CGA 133275 4.5% TRR 0.006 ppm unresolved [b] 9.6% TRR 0.014 ppm 3 unknown fractions each at ≤12.2% TRR (≤0.017 ppm), totaling 30.1% TRR (0.042 ppm)
PES	5.2	0.007	Not further analyzed
364-Day PBI (0.044 ppm) [a]			
MeOH/water extract	91.4	0.040	Concentrated and analyzed by 2D-TLC: Fraction WH-5/WH-6 [b] 9.6% TRR 0.004 ppm Metabolite WH-7 8.1% TRR 0.004 ppm CGA 133275-glucose 25.1% TRR 0.011 ppm Fraction WH-26/WH-27[d] 3.8% TRR 0.002 ppm 2 unknown polar fractions accounting for 18.7% TRR (0.008 ppm) and 26.0% TRR (0.011 ppm)
PES	14.7	0.007	Not further analyzed

[a] TRR for each matrix is listed in parentheses.
[b] Unresolved radioactivity was not associated with any specific TLC region.
[c] Includes several fractions, one of which co-chromatographed with CGA-380168.
[d] Contains minor amounts of CGA 217498.

Table 2.2.5 Extraction, Characterization, and Identification of ^{14}C-Residues in **Spring Wheat Fodder** harvested from the 30-, 120-, and 364-day PBIs.

Fraction ID	% TRR	ppm	Characterization/identification
30-day PBI (0.340 ppm) [a]			
MeOH/water extract	75.6	0.257	Concentrated and analyzed by 2D-TLC: Fraction WH-3/WH-4 [b] 3.7% TRR 0.013 ppm Fraction WH-5/WH-6 [c] 8.2% TRR 0.028 ppm Metabolite WH-7 0.6% TRR 0.002 ppm CGA 133275-glucose 5.8% TRR 0.020 ppm Metabolite I_{12} 3.2% TRR 0.011 ppm NOA 436611 1.2% TRR 0.004 ppm CGA 351916 2.2% TRR 0.008 ppm CGA 133275 6.4% TRR 0.022 ppm unresolved [d] 8.2% TRR 0.028 ppm 8 unknown fractions each at ≤9.8% TRR (≤0.033 ppm), totaling 36.1% TRR (0.123 ppm).
PES	24.3	0.083	The residual solids were first soxhlet extracted with MeOH and then extracted in boiling water for 16 hrs.
Aqueous	10.8	0.037	Acidified to pH 1.6 and diluted with EtOH to precipitate pectins.
EtOH	11.5	0.039	Concentrated and analyzed by 2D-TLC. Radioactivity was separated into 7 regions, each accounting for ≤2.7% TRR (≤0.009 ppm).
Precipitate	1.0	0.003	Not further analyzed; radioactivity was characterized as being incorporated into pectins
Solids	NR	--	Refluxed for 3 hours in 10% NaOH and hot filtered.
Base hydrolysate	6.5	0.022	Acidified to pH 1, concentrated, and refrigerated to precipitate lignins
Precipitate	2.0	0.007	Not further analyzed; radioactivity was characterized as being incorporated into lignins
Acidic fraction	6.2	0.021	Partitioned with CH_2Cl_2 and centrifuged.
CH_2Cl_2	1.0	0.003	Not further analyzed.
Aqueous	4.8	0.016	Not further analyzed.
Residual solids	2.5	0.009	Not further analyzed; radioactivity was characterized as being incorporated into cellulose.

(continued; footnotes follow)

Table 2.2.5 Extraction, Characterization, and Identification of ^{14}C-Residues in **Spring Wheat Fodder** harvested from the 30-, 120-, and 364-day PBIs.

Fraction ID	% TRR	ppm	Characterization/identification
120-Day PBI (0.784 ppm) [a]			
MeOH/water extract	86.8	0.681	Concentrated and analyzed by 2D-TLC: Fraction WH-2/-3/-4 [b] 11.8% TRR 0.092 ppm Fraction WH-5/WH-6 [c] 6.3% TRR 0.049 ppm Metabolite WH-7 5.7% TRR 0.044 ppm CGA 133275-glucose 20.6% TRR 0.161 ppm Metabolite I_{12} (partial) 2.8% TRR 0.022 ppm CGA 133275 12.1% TRR 0.095 ppm WH-26/WH-27 [e] 1.6% TRR 0.013 ppm unresolved [d] 8.6% TRR 0.068 ppm 3 unknown fractions totaling 17.5% TRR (0.137 ppm). The major unknown fraction (WH-1, 10.9% TRR, 0.085 ppm) was also the most polar fraction.
PES	16.2 (24.8) [f]	0.127 (0.0194)	Extracted in boiling water for 16 hrs and hot filtered.
MeOH (soxhlet)	7.8	0.061	Concentrated and analyzed by 2D-TLC. Radioactivity was separated into 6 regions, each accounting for ≤2.0% TRR (≤0.015 ppm).
Solids	15.1	0.118	The remaining solids were subsampled and either (**I**) extracted for 16 hours in boiling water; or (**II**) refluxed overnight in 6N HCl.
I - Aqueous	4.7	0.037	Acidified to pH 1.6 and diluted with EtOH to precipitate pectins.
EtOH	4.7	0.037	Concentrated and analyzed by 2D-TLC. Radioactivity was separated into 5 regions, each accounting for ≤1.2% TRR (≤0.009 ppm).
Precipitate	0.2	0.002	Not further analyzed; radioactivity was characterized as being incorporated into pectins
Solids	NR	--	Refluxed for 3 hours in 10% NaOH and hot filtered.
Base hydrolysate	7.4	0.058	Acidified to pH 1, concentrated, and refrigerated to precipitate lignins
Precipitate	5.2	0.040	Not further analyzed; radioactivity was characterized as being incorporated into lignins, and the aqueous fraction was partitioned with CH_2Cl_2 and centrifuged.
CH_2Cl_2	0.6	0.005	Not further analyzed.
Aqueous	1.5	0.012	Not further analyzed.
Residual solids	2.4	0.019	Not further analyzed; radioactivity was characterized as being incorporated into cellulose.
II - Acid hydrolsyate	6.0	0.047	Partitioned with CH_2Cl_2, then adjusted to pH 10.5 and repartitioned with CH_2Cl_2.
Acidic CH_2Cl_2	2.0	0.016	2D-TLC analysis tentatively detected CGA 49751, but confirmation was not possible.
Basic CH_2Cl_2	0.6	0.005	2D-TLC analysis tentatively detected CGA 37913.
Aqueous	3.0	0.034	Not further analyzed
Residual solids	9.1	0.071	

(continued; footnotes follow)

Table 2.2.5 Extraction, Characterization, and Identification of ^{14}C-Residues in **Spring Wheat Fodder** harvested from the 30-, 120-, and 364-day PBIs.

Fraction ID	% TRR	ppm	Characterization/identification
364-Day PBI (0.187 ppm) [a]			
MeOH/water extract	76.2	0.143	Concentrated and analyzed by 2D-TLC: Metabolite WH-7 3.1% TRR 0.006 ppm CGA 133275-glucose 6.8% TRR 0.013 ppm CGA 133275 17.5% TRR 0.033 ppm unresolved [d] 8.8% TRR 0.016 ppm 7 unknown fractions each at ≤11.2% TRR (≤0.021 ppm), totaling 39.9% TRR (0.075 ppm).
PES	25.0	0.047	Not further analyzed.

[a] TRR for each matrix is listed in parentheses.
[b] Fraction WH-3/WH-4 was composed partially of Metabolite I_3 (NOA 413173).
[c] Fraction WH-5/WH-6 was composed primarily of CGA-380168.
[d] Unresolved radioactivity was not associated with any specific TLC region.
[e] Includes several fraction, along with minor amounts of CGA 217498.
[f] The PES fraction analysis from 120-day Fodder that was used for further analysis was obtained from another extraction and accounted for 24.8% of the TRR (0.194 ppm).

Table 2.2.6 Extraction, Characterization, and Identification of ^{14}C-Residues in **Winter Wheat Forage and Fodder** harvested from the 174-day PBI.

Fraction ID	% TRR	ppm	Characterization/identification
Forage 25% maturity (0.082 ppm) [a]			
MeOH/water extract	86.3	0.071	Concentrated and analyzed by 2D-TLC: CGA 380168 9.0% TRR 0.007 ppm Metabolite WH-7 4.7% TRR 0.004 ppm CGA 133275-glucose 23.8% TRR 0.020 ppm unresolved 6.9% TRR 0.006 ppm 2 unknown polar fractions accounting for 23.2% TRR (0.019 ppm) and 18.6% TRR (0.015 ppm).
PES	13.0	0.011	Not further analyzed
Forage 50% maturity (0.025 ppm) [a]			
MeOH/water extract	94.6	0.024	Concentrated and analyzed by 2D-TLC: Metabolite WH-7 6.7% TRR 0.002 ppm CGA 133275-glucose 34.8% TRR 0.009 ppm CGA 133275 3.0% TRR <0.001 ppm unresolved 9.4% TRR 0.002 ppm 3 unknown fractions each at ≤16.5% TRR (≤0.004 ppm), totaling 40.7% TRR (0.010 ppm)
PES	20.9	0.005	Not further analyzed
Fodder (0.157 ppm) [a]			
MeOH/water extract	78.8	0.124	Concentrated and analyzed by 2D-TLC: Metabolite WH-7 3.3% TRR 0.005 ppm CGA 133275-glucose 5.5% TRR 0.009 ppm CGA 133275 15.1% TRR 0.024 ppm unresolved [b] 15.4% TRR 0.024 ppm 7 unknown fractions each at ≤10.3% TRR (≤0.016 ppm), totaling 37.2% TRR (0.058 ppm)
PES	23.3	0.037	Not further analyzed

[a] The 25% and 50% mature forage were harvested 64 and 234 days after planting, respectively. TRR for each matrix is listed in parentheses.

[b] Unresolved radioactivity was not associated with any specific TLC region.

Table 2.2.7 Extraction, Characterization, and Identification of ^{14}C-Residues in **Spring Wheat Grain** harvested from the 30-, 120-, and 364-day PBIs and **Winter Wheat Grain** harvested from the 172-day PBI.

Fraction ID	% TRR	ppm	Characterization/identification
Spring wheat grain 30-day PBI (0.016 ppm) [a]			
MeOH/water extract	20.1	0.003	Not further analyzed.
PES	79.0	0.013	Solids were first extracted with 0.05N NaOH at room temp. overnight and centrifuged. The remaining solids were then refluxed overnight in 1N HCl to hydrolyze the starch.
0.05N NaOH	28.0	0.004	Neutralized to pH 6 and proteins were precipitated by the addition of EtOH.
Filterate	12.4	0.002	Not further analyzed.
Precipitate	14.3	0.002	Characterized as radioactivity incorporated into proteins.
1N HCl reflux	46.6	0.006	Adjusted to pH 6.5 and partitioned with CH_2Cl_2
CH_2Cl_2	3.0	<0.001	Not further analyzed.
Aqueous	43.6	0.006	Solubilized glucose was derivatized to form glucosazone which was recrystalized 3 times.
Filtrates	40.5	0.005	Not further analyzed.
Glucosazone	0.3	<0.001	Characterized as radioactivity incorporated into starch.
Residual solids	6.2	<0.001	Not further analyzed.; radioactivity was characterized as being incorporated into cellulose.
Spring wheat grain 120-Day PBI (0.065ppm) [a]			
MeOH/water extract	25.0	0.016	Partitioned with CH_2Cl_2.
CH_2Cl_2	4.2	0.003	Analyzed by 2D-TLC. Radioactivity was separated into 6 regions, each accounting for ≤1.2% TRR (<0.001 ppm).
Aqueous	19.1	0.012	Fractionated on a XAD-4 column eluted with water and MeOH.
water	13.3	0.009	Not further analyzed.
MeOH	5.4	0.004	
PES	65.8	0.043	Solids were first extracted with 0.05N NaOH at room temp. overnight and centrifuged. The remaining solids were then refluxed overnight in 1N HCl to hydrolyze the starch.
0.05N NaOH	14.1	0.009	Neutralized to pH 6 and proteins were precipitated by the addition of EtOH.
Filterate	15.1	0.010	Not further analyzed.
Precipitate	2.1	0.001	Characterized as radioactivity incorporated into proteins.
1N HCl reflux	45.2	0.029	Adjusted to pH 6.5 and partitioned with CH_2Cl_2
CH_2Cl_2	<0.1	--	Not further analyzed.
Aqueous	48.8	0.032	Solubilized glucose was derivatized to form glucosazone which was recrystalized 3 times.
Filtrates	47.6	0.031	Not further analyzed.
Glucosazone	16.1	0.010	Characterized as radioactivity incorporated into starch.
Residual solids	7.0	0.005	Not further analyzed.; radioactivity was characterized as being incorporated into cellulose.
Spring wheat grain 364-Day PBI (0.014 ppm) [a]			

Table 2.2.7 Extraction, Characterization, and Identification of ^{14}C-Residues in **Spring Wheat Grain** harvested from the 30-, 120-, and 364-day PBIs and **Winter Wheat Grain** harvested from the 172-day PBI.

Fraction ID	% TRR	ppm	Characterization/identification
MeOH/water extract	18.2	0.003	Not further analyzed
PES	85.7	0.012	
Winter wheat grain 174-Day PBI (0.015 ppm) [a]			
MeOH/water extract	17.5	0.003	Not further analyzed
PES	86.1	0.013	

[a] TRR for each matrix is listed in parentheses.

Table 2.3.1. Summary of Characterization and Identification of ^{14}C-Residues in Rotational Crops Planted **30 days** after a Soil application of [^{14}C-Phenyl]*S*-Metolachlor at 1.45 lb ai/A (0.4x the maximum seasonal rate).

Metabolite or Fraction [a]	Lettuce (0.109 ppm)		Radish tops (0.180 ppm)		Radish roots (0.037 ppm)		Wheat forage (0.108 ppm)		Wheat fodder (0.340 ppm)		Wheat grain (0.016 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
CGA 77102 (parent)	0.9	0.001	ND	--	ND	--	ND	--	ND	--	ND	--
CGA 380168	ND	--	8.6	0.016	8.2	0.003	7.7	0.008	8.2 [b]	0.028	ND	--
Metabolite WH-7	ND	--	4.4	0.008	0.8	<0.001	7.1	0.008	0.6	0.002	ND	--
CGA 46576	3.1	0.003	4.0	0.007	5.9	0.002	ND	--	ND	--	ND	--
CGA 133275-glucose	ND	--	7.7	0.014	1.2	<0.001	18.5	0.020	5.8	0.020	ND	--
CGA 133275-glucose-malonyl	ND	--	4.3	0.008	6.8	0.003	ND	--	ND	--	ND	--
Metabolite I_{12}	ND	--	ND	--	ND	--	ND	--	3.2	0.011	ND	--
NOA 443819	5.0	0.005	4.3	0.008	2.7	0.001	4.9	0.005	ND	--	ND	--
Metabolite I_{17}	ND	--	2.8	0.005	3.0	0.001	ND	--	ND	--	ND	--
NOA 436611	0.9	0.001	ND	--	ND	--	ND	--	1.2	0.004	ND	--
CGA 351916	2.1	0.002	ND	--	ND	--	0.7	<0.001	2.2	0.008	ND	--
CGA 443156	5.1	0.006	ND	--	ND	--	1.3	0.001	ND	--	ND	--
CGA 49750	ND	--	8.1	0.015	3.2	0.001	ND	--	ND	--	ND	--
CGA 133275	ND	--	ND	--	1.6	<0.001	ND	--	6.4	0.022	ND	--
Total Identified (TI)	17.1	0.018	44.2	0.081	33.4	0.011	40.2	0.043	27.6	0.100	NA	--
Minor unknowns (<10% TRR)	70.2	0.077	50.3	0.091	57.7	0.021	45.2	0.049	51.3	0.175	NA	--
Unresolved TLC radioactivity	8.2	0.009	3.3	0.006	4.3	0.002	10.0	0.011	8.2	0.028	NA	--
Pectin fraction	NA	--	NA	--	NA	--	NA	--	1.0	0.003	NA	--
Lignin fraction	NA	--	NA	--	NA	--	NA	--	2.0	0.007	NA	--
Protein fraction	NA	--	NA	--	NA	--	NA	--	NA	--	14.3	0.002
Glucose fraction	NA	--	NA	--	NA	--	NA	--	NA	--	0.3 [c]	<0.001
Minor solvent fractions	NA	--	NA	--	NA	--	NA	--	5.8	0.019	76.0	0.012
Total Characterized (TC)	78.4	0.086	53.6	0.097	62.0	0.023	55.2	0.060	68.3	0.232	90.6	0.014
Total Bound (TB)	5.8	0.006	4.7	0.009	8.9	0.003	7.9	0.009	2.5	0.009	6.2	0.001
% Mass Balance	101.3		102.5		104.3		103.3		98.4		96.8	

[a] Metabolite names and structures are presented in Table 2.5; the TRR for each matrix is listed in parentheses.

[b] Includes other minor unknown components.

[c] Based on the specific activity of the isolated glucosazone and the starch content (% weight) of wheat, registrant calculated that starch accounted for ~54% of the TRR in wheat grain.

ND = not detected; NA = not applicable and % Mass Balance = TI (%TRR) + TC (%TRR) + TB (%TRR)

Table 2.3.2. Summary of Characterization and Identification of ^{14}C-Residues in Rotational Crops Planted **120 days** after a Soil Application of [^{14}C-Phenyl] *S*-Metolachlor at 1.45 lb ai/A (0.4x the maximum seasonal rate).

Metabolite or Fraction [a]	Lettuce (0.045 ppm)		Radish tops (0.229 ppm)		Radish roots (0.028 ppm)		Wheat forage (0.141 ppm)		Wheat fodder (0.784 ppm)		Wheat grain (0.065 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
CGA 77102 (parent)	ND	--	ND	--	ND	--	ND	--	ND	--	ND	--
CGA 380168	ND	--	11.4	0.026	ND	--	14.0 [b]	0.020	6.3 [b]	0.049	ND	--
Metabolite WH-7	ND	--	6.6	0.015	ND	--	9.2	0.013	5.7	0.044	ND	--
CGA 46576	2.7	0.001	ND	--	ND	--	ND	--	ND	--	ND	--
CGA 133275-glucose	ND	--	13.6	0.031	5.5	0.002	35.0	0.049	20.6	0.161	ND	--
CGA 133275-glucose-malonyl	ND	--	5.7	0.013	1.7	<0.001	ND	--	ND	--	ND	--
NOA 443819	ND	--	ND	--	ND	--	1.6	0.002	ND	--	ND	--
NOA 436611	2.7	0.001	ND	--	ND	--	ND	--	ND	--	ND	--
CGA 351916	10.6	0.005	ND	--	ND	--	ND	--	ND	--	ND	--
CGA 443156	1.8	<0.001	ND	--	ND	--	ND	--	ND	--	ND	--
CGA 49750	ND	--	9.5	0.022	3.3	<0.001	ND	--	ND	--	ND	--
CGA 133275	ND	--	ND	--	7.5	0.002	4.5	0.006	12.1	0.095	ND	--
Total Identified (TI)	17.8	0.007	46.8	0.107	18.0	0.005	64.3	0.090	44.7	0.349	NA	--
Minor unknowns (<10% TRR)	70.6	0.032	43.6	0.100	62.4	0.017	30.1	0.042	46.2 [c]	0.362	4.2	0.003
Unresolved TLC radioactivity	4.3	0.002	13.9	0.032	10.9	0.003	9.6	0.014	8.6	0.068	NA	--
Pectin fraction	NA	--	NA	--	NA	--	NA	--	0.2	0.002	NA	--
Lignin fraction	NA	--	NA	--	NA	--	NA	--	5.2	0.040	NA	--
Protein fraction	NA	--	NA	--	NA	--	NA	--	NA	--	2.1	0.001
Glucose fraction	NA	--	NA	--	NA	--	NA	--	NA	--	16.1 [d]	0.010
Minor solvent fractions	NA	--	NA	--	NA	--	NA	--	2.1	0.017	82.6	0.054
Total Characterized (TC)	74.9	0.034	57.5	0.132	73.3	0.020	39.7	0.056	62.3	0.488	105.0	0.068
Total Bound (TB)	7.0	0.003	3.7	0.009	10.0	0.003	5.2	0.007	2.4	0.019	7.0	0.005
% Mass Balance	99.7		108.0		101.3		109.2		109.4		112.0	

[a] Metabolite names and structures are presented in Table 2.5; The TRR for each matrix is listed in parentheses.
[b] Includes other minor unknown components.
[c] Unknown fractions included minor amounts of NOA 413173 and CGA 217498.
[d] Based on the specific activity of the isolated glucosazone and the starch content (% weight) of wheat, registrant calculated that starch accounted for ~55% of the TRR in wheat grain.

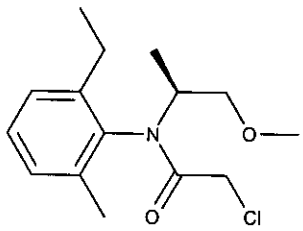
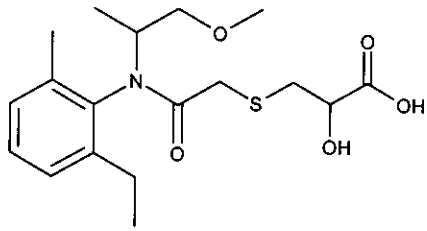
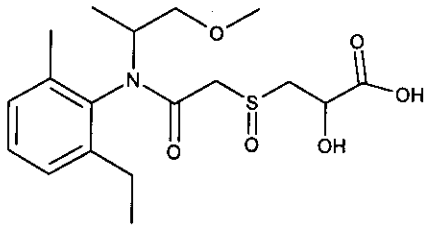
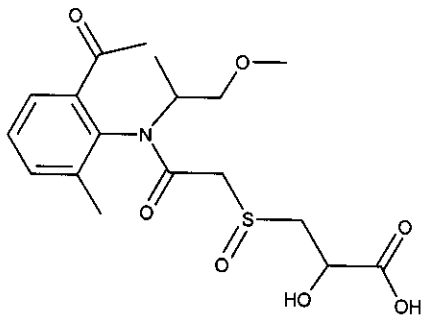
ND = not detected; NA = not applicable
% Mass Balance = TI (%TRR) + TC (%TRR) + TB (%TRR)

Table 2.4. Identification of ^{14}C-Residues in Soil (0-10 cm layer) following a Single Soil Application of [^{14}C-phenyl]*S*-metolachlor at 1.45 lb ai/A (0.4x the maximum seasonal rate).

Metabolite or Fraction [a]	30-DAT (1.367 ppm) [b]		78-DAT (1.174 ppm)		120-DAT (0.957 ppm)		174-DAT (1.047 ppm)		238-DAT (0.522 ppm)		364-DAT (0.836 ppm)		488-DAT (0.324 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
CGA 77102 (parent)	65.7	0.898	21.6	0.254	13.6	0.130	4.7	0.049	3.7	0.019	6.4	0.054	1.9	0.006
NOA 413173	0.6	0.009	1.0	0.012	0.7	0.007	0.6	0.006	ND	--	ND	--	ND	--
CGA368208	ND	--	0.3	0.004	0.5	0.004	0.6	0.007	ND	--	ND	--	ND	--
CGA 380168	1.9	0.026	6.6	0.078	6.0	0.058	5.8	0.061	0.3	0.002	0.3	0.002	0.5	0.002
NOA 436611	0.9	0.012	3.2	0.038	3.0	0.028	2.2	0.023	0.1	<0.001	0.1	0.001	0.2	<0.001
CGA 351916	1.6	0.021	7.4	0.087	8.1	0.077	4.5	0.047	0.2	0.001	0.3	0.002	0.5	0.002
CGA 46129	0.4	0.006	0.5	0.006	0.3	0.003	0.2	0.002	0.1	<0.001	<0.1	<0.001	ND	--
CGA 217498	ND	--	5.6	0.066	3.5	0.034	4.4	0.047	6.1	0.032	5.0	0.041	1.2	0.004
Total Identified	71.1	0.972	46.2	0.545	35.7	0.341	23.0	0.242	10.5	0.058	12.2	0.100	4.3	0.014

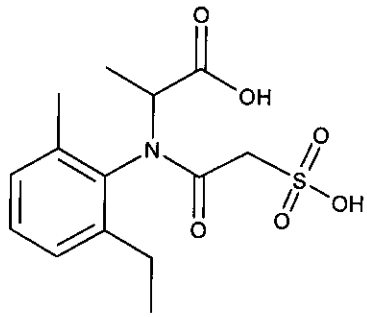
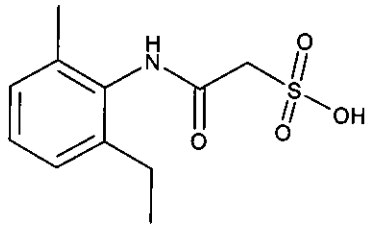
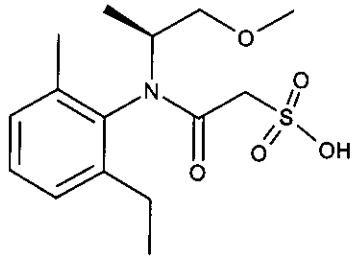
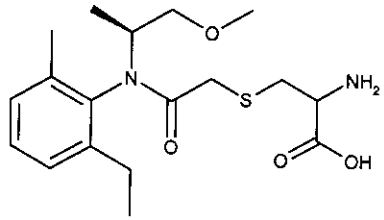
[a] Metabolite names and structures are presented in Table 2.5.
[b] The TRR for each matrix is listed in parentheses.
ND = not detected.

Table 2.5. Metabolites of S-Metolachlor Identified in Rotational Crops and Soil.

Metabolite Identifier	Chemical Name	Structure	Crop/matrix
S-Metolachlor (CGA 77102)	(S)-2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-acetamide		lettuce soil
NOA 443156	3-{[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methylsulfanyl}-2-hydroxy-propionic acid		lettuce wheat
NOA 443819	3-{[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfinyl}-2-hydroxy-propionic acid		lettuce radish wheat
Metabolite WH-7			radish wheat

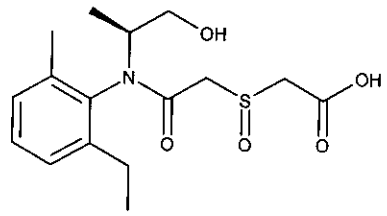
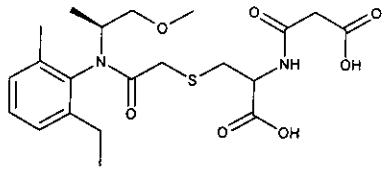
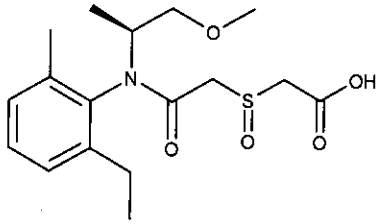
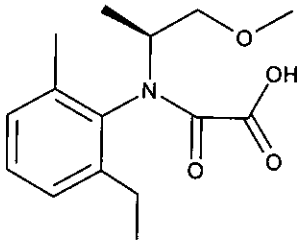
(continued)

Table 2.5. Continued.

Metabolite Identifier	Chemical Name	Structure	Crop/matrix
NOA 413173	2-[(2-ethyl-6-methyl-phenyl)-sulfoacetyl-amino]-propionic acid		wheat soil
CGA 368208	(2-ethyl-6-methyl-phenylcarbamoyl)-methanesulfonic acid		soil
CGA 380168	[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfonic acid		radish wheat soil
CGA 46576 Cysteine conjugate of CGA 77102	2-amino-3-{[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methylsulfanyl}-propionic acid		lettuce radish

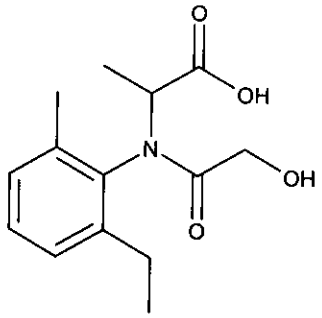
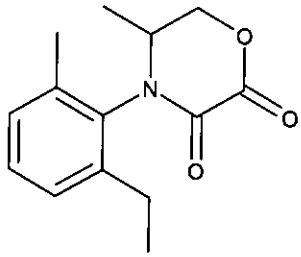
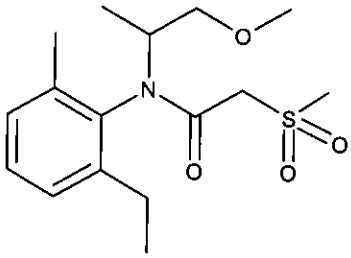
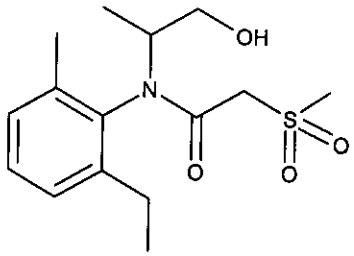
(continued)

Table 2.5. Continued.

Metabolite Identifier	Chemical Name	Structure	Crop/matrix
Metabolite I_{12}	{[(2-ethyl-6-methyl-phenyl)-(2-hydroxy-1-methyl-ethyl)-carbamoyl]-methanesulfinyl}-acetic acid		wheat
Metabolite I_{17} Malonyl-cysteinyl-conjugate of CGA 77102	2-(2-carboxy-acetylamino)-3-{[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methylsulfanyl}-propionic acid		radish
NOA 436611	{[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfinyl}-acetic acid		lettuce wheat soil
CGA 351916	N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-oxalamic acid		lettuce wheat soil

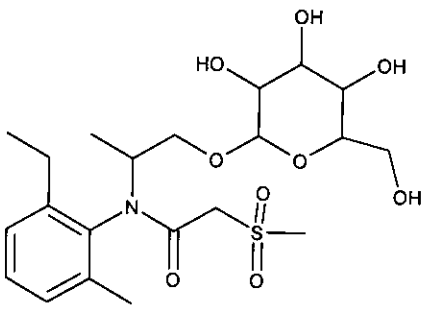
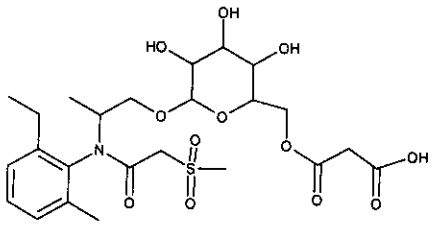
(continued)

Table 2.5. Continued.

Metabolite Identifier	Chemical Name	Structure	Crop/matrix
CGA 46129	2-[(2-ethyl-6-methyl-phenyl)-(2-hydroxy-acetyl)-amino]-propionic acid		soil
CGA 49750	4-(2-ethyl-6-methyl-phenyl)-5-methyl-morpholine-2,3-dione		radish
CGA 217498	N-(2-ethyl-6-methyl-phenyl)-2-methanesulfonyl-N-(2-methoxy-1-methyl-ethyl)-acetamide		wheat soil
CGA 133275 Metabolite I_{28}	N-(2-ethyl-6-methyl-phenyl)-N-(2-hydroxy-1-methyl-ethyl)-2-methanesulfonyl-acetamide		radish wheat

(continued)

Table 2.5. Continued.

Metabolite Identifier	Chemical Name	Structure	Crop/matrix
CGA 133275-glucose Metabolite WH-9			radish wheat
CGA 133275-glucose-malonyl Metabolite WH-10			radish

3. Discussion

3.1. Methods

With the exception of the application rate used, the methods used to treat the soil and grow the rotational crops were adequate. [^{14}C-U-Phenyl]S-Metolachlor was applied to a bare plot of clay loam soil at a rate of 1.45 lb ai/A. The current maximum seasonal application rate for *S*-metolachlor on crops that can be rotated is 3.7 lb ai/A/season on corn. Therefore, the rate used in the current study is 0.4x the maximum seasonal rate. Following application, crops of lettuce, radishes, and spring wheat were each planted at 30, 120, and 364 DAT, and a crop of winter wheat was planted 174 DAT.

The appropriate RACs were collected from each representative rotational crop at the appropriate time. Samples of lettuce and radish roots and tops were harvested 48-61 DAP. Spring wheat forage was harvested at 48-96 DAP, and spring wheat grain and fodder were harvested at 111-145 DAP. For the winter wheat crop, forage was collected twice at 64 and 234 DAP, and grain and fodder were collected at 234 days DAP. In the laboratory, samples were stored at ≤-18° C for 18-149 days (<5 months) prior to the initial analyses. The registrant present data comparing TLC analyses of ^{14}C-residues extracted from wheat fodder after 2 and 241 days of frozen storage. The data indicate that ^{14}C-residues were relatively stable over the course of the study.

Methanolic extractions released 92.8-95.5% of the TRR from lettuce, 91.2-104.1% of the TRR from radish roots and tops, 86.3-104% of the TRR from wheat forage, and 75.6-86.8% of the TRR from wheat fodder. The extractability of ^{14}C-residues was considerably lower from wheat grain (17.5-25.0% TRR). Except for wheat grain, solvent extracted ^{14}C-residues were profiled and quantified by 2D-TLC of the initial or purified extract fractions. Specific metabolites were isolated from wheat fodder and identified by TLC analysis with reference standard, LC/MS and NMR.

In lettuce samples from the 30- and 120-day PBI, only 17-18% of the TRR was identified, but the remaining solubilized ^{14}C-residues were adequately characterized as multiple minor unknowns. The 364-day PBI lettuce sample was not extracted due to low levels of radioactivity. In radishes tops from the 30- and 120-day PBI, 44-47% of the TRR was identified and another 54-58% of the TRR was adequately characterized as multiple minor unknowns. Due to low levels of radioactivity, only 28% of the TRR was identified in radish tops from the 365-day PBI, but another 69% of the TRR was adequately characterized and accounted for only 0.026 ppm. For radish roots from the 30- and 120-day PBIs, 33 and 18% of the TRR was identified and another 62-73% of the TRR was adequately characterized as multiple minor unknowns. The 364-day PBI, radish root sample was not extracted due to low levels of radioactivity.

In spring wheat forage from each PBI,40-64% of the TRR was identified and another 40-55% was adequately characterized as minor unknowns. Similar results were obtained from the two winter wheat forage samples from the 174-day PBI, 38-49% of the TRR was identified and another 45-50% was adequately characterized.

In spring wheat fodder from the 30- and 120-day PBI, 28 and 45% of the TRR was respectively identified and another 45 and 26% of the TRR, which was solvent extracted, was adequately

characterized as minor unknowns. Similar results were obtained from the spring wheat fodder from the 364-day PBI and winter wheat fodder from the 174-day PBI. For these samples, 24-27% of the TRR was identified and another 49-53% was adequately characterized.

No metabolites were identified in the solvent extracts from grain, as solvent extraction released only 18-25% of the TRR from wheat grain and these fractions contained low levels of radioactivity (0.003-0.016 ppm). Although the PES fractions from wheat grain contained low levels of radioactivity (<0.05 ppm), the PES fractions from the 30- and 120-day PBI grain samples were adequately characterized following mild base extraction and acid hydrolysis.

Radioactivity remaining in the PES fractions following solvent extraction was either <10% of the TRR or <0.05 ppm in all samples except fodder from the 30- and 120-day PBIs (24.3 and 24.8% TRR). Further extractions in boiling water and methanol released 10.8-12.5% TRR from these PES fractions and a subsequent base hydrolysis released an additional 6.5-7.4% of the TRR. Solubilized ^{14}C-residues were characterized as either natural plant products base on their fractionation or were characterized by TLC as minor unknowns.

The overall recovery of radioactivity from the analysis of all plant samples was 96.8-112% of the TRR. The methods used to extract, fractionate, and identify ^{14}C-residues in rotation crops were adequate.

For soil (0-10 cm layer), solvent extraction released 83% of the TRR from the 30-DAT sample, and the extractability of the ^{14}C-residues declined steadily at subsequent sampling intervals. Solvent extraction released 59% of the TRR from the 78-DAT sample, 50% of the TRR from the 120-DAT sample, 32% of the TRR from the 174-DAT sample, 17% of the TRR from the 364-DAT sample, and 8% of the TRR from the 488-DAT sample. The same trend was observed in the identification of ^{14}C-residues in soil. For the 30-DAT sample, 71% of the TRR was identified, but in the 364-DAT sample, only 12% of the TRR was identified.

3.2. Results

Immediately (Day 0) following a soil application of [^{14}C]*S*-metolachlor at 1.45 lb ai/A, TRR in the top soil layer (0-10 cm) was 1.535 ppm. Radioactivity in the 0-10 cm soil layer generally declined steadily over time, reaching 0.324 ppm by 488-DAT. Over the course of the study, the majority of radioactivity in the soil remained in the top 0-10 cm layer, which accounted for >84% of the radioactivity at up to 1 year following treatment.

With the exceptions of radish tops and wheat forage, fodder, and grain from the 120-day PBI, radioactive residues in plant samples were highest at the 30-day PBI and declined steadily at later PBIs. Among the different plant samples, TRRs were highest in wheat fodder (0.157-0.784 ppm) at each PBI and were generally lowest in wheat grain (0.014-0.065 ppm) and radish roots (0.007-0.037 ppm). In lettuce, TRR declined from 0.109 ppm at the 30-day PBI to 0.009 ppm by the 364-day PBI, and in radish roots, TRR declined from 0.037 ppm at the 30-day PBI to 0.007 ppm by the 364-day PBI. Maximum TRR levels were attained in radish tops (0.229 ppm), wheat forage (0.141 ppm), wheat fodder (0.784 ppm), and wheat grain (0.065 ppm) at the 120-day PBI, but TRR levels in each of these commodities declined at later PBIs.

A total of six compounds were identified in lettuce from the 30-day PBI, none of which accounted for ≥10% of the TRR. Trace amounts of parent (0.9% TRR, 0.001 ppm) were detected, but the two most prominent metabolites were NOA 443819 (5.0% TRR) and CGA 443156 (5.1% TRR). In lettuce from the 120-day PBI, parent was not detected, but four metabolites were identified. These were all minor metabolites (<3% TRR), with the exception of CGA 351916, which accounted for 10.6% of the TRR (0.005 ppm). The majority of ^{14}C-residues in lettuce (75-78% TRR) were characterized as minor unknown components.

Parent was not detected in radish tops or roots from any PBI, but up to 9 metabolites were identified in radishes. In radish tops from the 30-day PBI, the principal metabolites were CGA 380168 (8.6% TRR), CGA 49750 (8.1% TRR), and a glucose conjugate of CGA 133275 (7.7% TRR). The remaining metabolites each accounted for <5% of the TRR and included: Metabolite WH-7, CGA 46576, CGA 133275-glucose-malonyl, NOA 443819, and Metabolite I_{17}. Five of these metabolites were also identified in radish tops from the 120-day PBI. The principal metabolites were again CGA 380168 (11.4% TRR), CGA 49750 (9.5% TRR), and the glucose conjugate of CGA 133275 (13.6% TRR), along with minor amounts of Metabolite WH-7 (6.6% TRR) and CGA 133275-glucose-malonyl (5.7% TRR). Although data from the extraction of the 364-day PBI, radish tops were not included in the summary tables, the same components were identified in this sample as in the earlier samples (see Table 2.2.2).

The metabolite profile in radish roots was similar, although each of the metabolites were present at <0.005 ppm. For the 30-day PBI sample, the principal metabolites were CGA 380168 (8.2% TRR), CGA 46576 (5.9% TRR), and CGA 133275-glucose-malonyl (6.8% TRR). The remaining metabolites each accounted for <4% of the TRR and included: Metabolite WH-7, CGA 133275-glucose, NOA 443819, Metabolite I_{17}, CGA 49750, and CGA 133275. Four of these metabolites were also identified in radish roots from the 120-day PBI. The principle metabolites were CGA 133275-glucose (5.5% TRR) and CGA 133275 (7.5% TRR), along with minor amounts of CGA 133275-glucose-malonyl and CGA 49750. In both radish roots and tops, a major portion (54-73% TRR) of the extracted ^{14}C-residues were characterized as minor unknown components.

The metabolite profile was similar among the wheat forage samples from the different PBIs. In spring wheat forage from the 30-day PBI, a total of 6 metabolites were identified. The principal metabolites were CGA 133275-glucose (18.5% TRR), CGA 380168 (7.7% TRR), and Metabolite WH-7 (7.1% TRR). The other three metabolites (NOA 443819, CGA 351916, and CGA 443156) each accounted for <5% of the TRR. In spring wheat forage from the 120-day PBI, the principal metabolites were again CGA 133275-glucose (35.0% TRR), CGA 380168 (14.0% TRR), and Metabolite WH-7 (9.2% TRR), along with minor amounts of NOA 443819 and CGA 133275. The same relative distribution of metabolites was also observed in wheat forage from the 174- and 364-day PBIs (see Tables 2.2.4 and 2.2.6), although the actual levels of the metabolites were lower (≤0.020 ppm).

The metabolite profile was also similar among the wheat fodder samples from each PBI and was qualitatively similar to the profile found in wheat forage. In spring wheat fodder from the 30-day PBI, a total of 6 metabolites were identified. The principal metabolites were CGA 380168 (~8.2% TRR), CGA 133275-glucose (5.8% TRR), and CGA 133275 (6.4% TRR). The other three metabolites (Metabolite I_{12}, NOA 436611, and CGA 351916) each accounted for <5% of

the TRR. In spring wheat forage from the 120-day PBI, the major metabolites were CGA 133275 (12.1% TRR) and its glucose conjugate (20.6% TRR), along with minor amounts of Metabolite WH-7 (5.7% TRR) and CGA 380168 (~6.3% TRR). The same relative distribution of metabolites was also observed in wheat fodder from the 174- and 364-day PBIs (see Tables 2.2.5 and 2.2.6). ^{14}C-Residues remaining in the PES fraction (16-25% TRR) of fodder following solvent extraction were characterized as being either incorporated into pectin, lignin, or cellulose fractions or consisting of a variety of minor polar residues.

Levels of radioactivity released from wheat grain by solvent extraction were low (18-25% TRR; 0.003-0.016 ppm), and no metabolites were identified in these extracts. Following mild base extraction of the PES fraction to solubilize proteins, 2.1-14.3% of the TRR in grain from the 30- and 120-day PBI samples were recovered in the protein fraction. Subsequent acid hydrolysis released an additional 45-47% of the TRR, of which only a minor portion (≤3% TRR) was organosoluble. The remaining aqueous soluble ^{14}C-residues were derivatized to form glucosazone. Based on the specific activity of the isolated glucosazone and the typical starch content of wheat grain, the registrant calculated that ~55% of the TRR in grain was incorporated into starch.

Based on the metabolite profile observed in plants, the metabolism of [^{14}C]*S*-metolachlor in rotational crops is similar to the metabolism observed in the primary crops. Metabolism in rotational crops primarily involves two pathways: (i) conjugation of the parent molecule with glutathione by substitution of the chlorine, followed by the degradation of the glutathione moiety to form a variety of sulfur containing metabolites; and (ii) direct oxidation of parent or secondary metabolites, primarily on the chloroacetyl side chain (Figure 1). Complete degradation of secondary metabolites either in the soil and/or plants also resulted in the incorporation of molecule fragments into natural plant constituents.

Analysis of soil (0-10 cm) over time, identified parent and up to 7 metabolites. Levels of parent declined steadily from 65.7% of the TRR (0.898 ppm) at 30 DAT to 1.9% of the TRR (0.006 ppm) by 488 DAT. At 30-DAT, five metabolites were identified in soil, but the relative levels of these metabolites were low (<2% TRR). At the next three sampling intervals (78-, 120-, and 174-DAT), seven metabolites were identified. The principle metabolites were CGA 380168 (5.8-6.6% TRR), CGA 351916 (4.5-8.1% TRR), and CGA 217498 (3.5-5.6% TRR). Other metabolites, which each accounted for ≤3.2% of the TRR, included: NOA 413173, CGA 368208, NOA 436611, and CGA 46129. By 364-DAT, the only isolated ^{14}C-residues present at >0.01 ppm were parent (0.054 pm) and CGA 217498 (0.041 ppm).

The submitted confined rotational crop study adequately reflects the nature and quantity of ^{14}C-residues in rotational crops following a soil application of *S*-metolachlor at rates up to 1.45 lb ai/A.

4. Deficiencies

Although the submitted study adequately depicts the general metabolism of [^{14}C]*S*-metolachlor in rotational crops, it does not indicate what the actual levels of the various metabolites would be in rotational crops following application at the maximum seasonal rate for any rotated crop in the U.S. According to the available U.S. labels, the current maximum seasonal application rate for

S-metolachlor on crops that can be rotated is 3.7 lb ai/A/season on corn. The rate used in the current study is 1.45 lb ai/A, 0.4x the maximum seasonal rate. Therefore, the usefulness of this confined study in assessing the need for more extensive rotational crop field trials is equivocal.

5. References

None

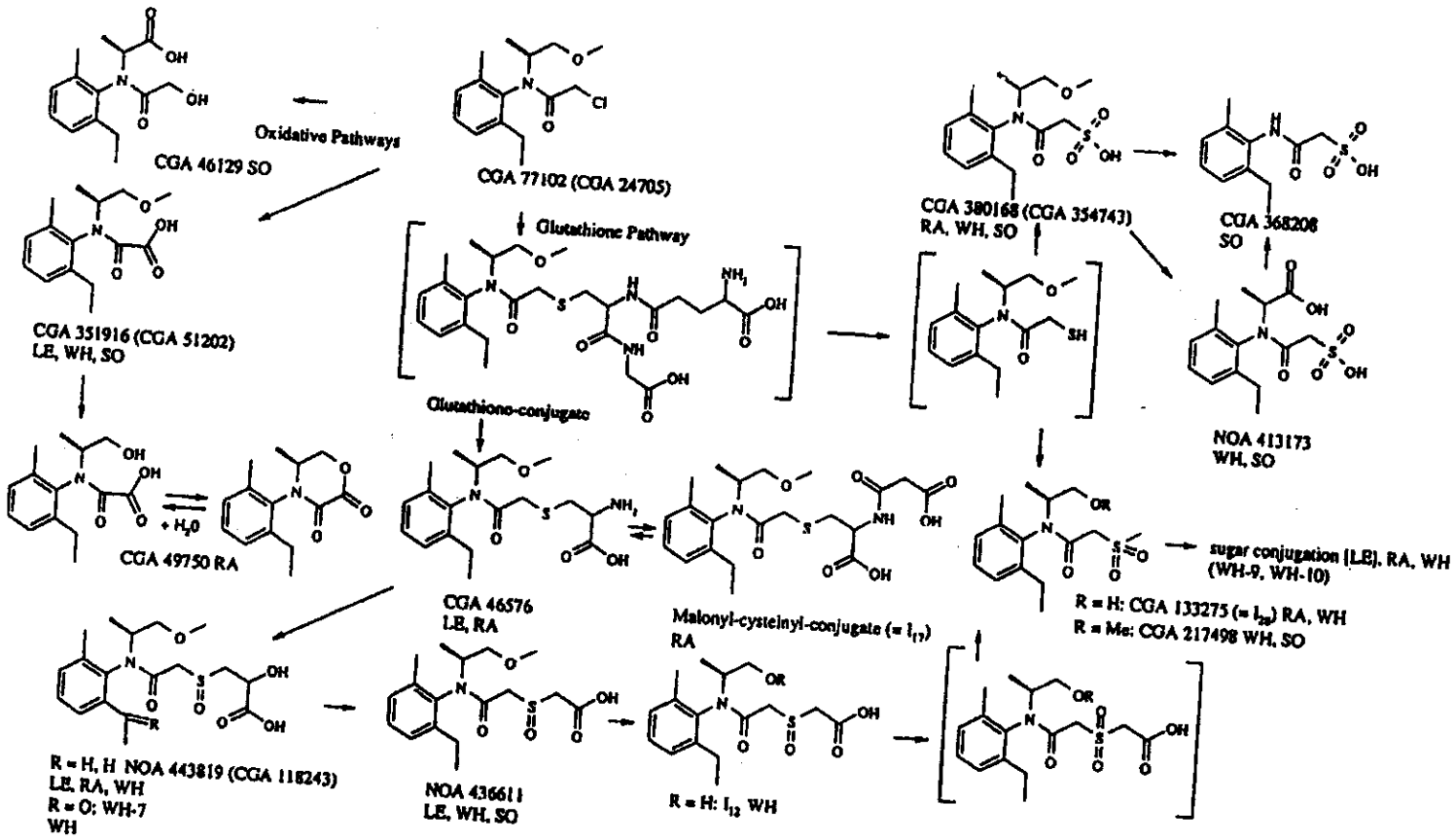
6. Document Tracking

PC Code: 108800
DP Barcode(s): D283235

cc: Sherrie L. Kinard (RRB2), Metolachlor Reg. Std. File, Metolachlor Subject File, RF, LAN. RD/I: Metolachlor Team Review (08/11/03), A. Nielson (08/15/03).

7509C: RRB2: S. Kinard: CM#2:Rm 712M: 703-305-0563: 08/15/03.

Figure 1. Proposed Pathway for the Metabolism of *S*-Metolachor in Rotational Crops.



[M] for all structures the same stereochemistry is shown as for CGA 77102, though this was not confirmed for the metabolites. If a code number is available for the (*S*)-isomer as well as for the (S/R)-mixture, the code for the mixture is given in parenthesis