UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

DATE: August 15, 2003

SUBJECT: **S-Metolachlor.** Information Supporting the Assertion That the Metabolism of *S*-metolachlor Is Essentially the Same as for the Racemic Mixture.

DP Barcode:	D278742 and D279110	Case No.:	0001
PC Code:	108801	Submission:	
40 CFR 180.	368	MRID Nos.:	45499603, 45499604, 45499605, 45533103

FROM: Sherrie L. Kinard, Chemist
Reregistration Branch II
Health Effects Division (7509C)

THROUGH: Alan Nielsen, Branch Senior Scientist 8/15/03
Reregistration Branch II
Health Effects Division (7509C)

TO: Sherrie L. Kinard, Chemist
Reregistration Branch II
Health Effects Division (7509C)

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

S-METOLACHLOR

PC Code No. 108801

DP Barcodes D278742 and D279110

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

Metolachlor is a racemic mixture of four diastereomers, two *R*-enantiomers and two *S*-enantiomers. The *S*-enantiomers, which account for 50% of racemic metolachlor, are the more biologically active enantiomers for weed control, and are the active ingredients in *S*-metolachlor [*S*-2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide]. Syngenta has registered *S*-metolachlor for use on corn, cotton, legume vegetables, peanuts, potatoes, safflower, and sorghum, with the intent of replacing the racemic mixture of metolachlor. To support the registration of *S*-metolachlor, Syngenta Crop Protection, Inc. has submitted plant metabolism studies for *S*-metolachlor on soybeans (1999, MRID 45499610) and field corn (1997, MRID 454499609). As these submissions consist of essentially complete plant metabolism studies, they are reviewed in detail in separate DERs, which are attached. Summaries and conclusions from these DERs are also included in this report.

In addition to the above studies, Syngenta has submitted supplemental information supporting their assertion that the metabolism of *S*-metolachlor is essentially the same as for the racemic mixture. These studies include a published article on the metabolism in soybeans of propachlor, a related chloroacetanilide herbicide (1989, MRID 45499603); an unpublished report comparing the *in vitro* activity of glutathione S-transferases (GST) following treatment with different

metolachlor isomers (1994, MRID 45499604); an unpublished report examining the uptake and disappearance of metolachlor isomers from seedling corn (1994, MRID 45499605); and an unpublished report examining the uptake and disappearance of *S*-metolachlor and metolachlor from seedlings of corn, sorghum, soybeans, and peanuts (2001, MRID 45533103). This information is reviewed and summarized in this report. The Conclusions and Recommendations stated in this review pertain only to plant metabolism.

The Agency has previously determined that the qualitative nature of metolachlor (racemic mixture) residues in plants is adequately understood based upon the adequate corn, potato, and soybean metabolism studies. The metabolism of metolachlor involves conjugation with glutathione, breakage of this bond to form the mercaptan, conjugation of the mercaptan with glucuronic acid, hydrolysis of the methyl ether, and conjugation of the resultant alcohol with a neutral sugar. A minor pathway may involve sugar conjugation of metolachlor directly to the corresponding oxo-compounds. Residues of concern in plants include metolachlor and its metabolites, determined as the derivatives 2-[(2-ethyl-6-methylphenyl)amino]-1-propanol (CGA-37913) and 4-(2-ethyl-6-methylphenyl)-2-hydroxy-5-methyl-3-morpholinone (CGA-49751). Currently the residues of concern for *S*-metolachlor are the same as for metolachlor (DP Barcode D226780, L. Kutney, 11/12/96).

Tolerances for residues of metolachlor in or on plant and animal commodities are currently expressed in terms of the combined residues (free and bound) of the herbicide metolachlor and its metabolites, determined as the derivatives, CGA-37913 and CGA-49751, each expressed as the parent compound. Permanent tolerances for residues in/on plant commodities range from 0.1 ppm in/on a variety of plant commodities to 30 ppm in/on peanut forage and hay [40 CFR §180.368(a)]. Permanent tolerances for residues in/on animal commodities range from 0.02 ppm in milk, egg, fat, and meat and meat-byproducts (except liver and kidney) to 0.2 ppm in kidney [40 CFR §180.368(a)]. Temporary tolerances have been established on grass forage and hay, spinach, tomatoes, and tomato puree and paste [40 CFR §180.368(b)], and tolerances with regional registrations have also been established for residues in/on dry bulb onions and non-bell peppers [40 CFR §180.368(c)]. Tolerances for metolachlor presently cover residues resulting from the use of *S*-metolachlor. Adequate enforcement methods are available for the determination of these residues.

CONCLUSIONS

1. To support their position that the metabolism of *S*-metolachlor in plants is the same as that of the racemic mixture, Syngenta has provided the following data/information:

    - An adequate soybean metabolism study indicating that the metabolism of [^{14}C]*S*-metolachlor in field-grown soybeans primarily involves two pathways: (i) conjugation of the parent molecule with homoglutathione by substitution of the chlorine, followed by the degradation of the homoglutathione moiety to form a

variety of sulfur containing metabolites; and (ii) direct oxidation of parent or secondary metabolites, primarily on the chloroacetyl side chain.

- A corn metabolism study comparing the gross fractionation and TLC profile of ^{14}C-residues in corn plants treated with either [^{14}C]metolachlor or [^{14}C]*S*-metolachlor as a post-emergence application. Levels of total radioactive residues (TRR) were similar of the two test substances and the distribution and fractionation of ^{14}C-residues within each matrix were similar. Although only limited TLC characterizations were provided, these showed a similar metabolic profile for the two compounds.

- Data from *in vitro* tests using extracts from corn seedlings that indicate that metolachlor isomers are enzymatically conjugated with glutathione in corn and that the rate of conjugation is higher for *S*-metolachlor than for *R*-metolachlor.

- Data from tests on corn seedlings indicating that the uptake and metabolism of *S*-metolachlor is higher than for R-metolachlor.

- Data from tests on seedlings of corn, sorghum, soybeans and peanuts that indicate that the uptake of *S*-metolachlor was similar to metolachlor, but that *S*-metolachlor was more rapidly metabolized than metolachlor.

- A published report on the metabolism of a related chloroacetanilide herbicide, propachlor, in soybeans, which illustrates the similarities in the metabolism of propachlor and *S*-metolachlor in soybeans.

DETAILED CONSIDERATIONS

GLN 860.1300. Qualitative Nature of the Residue in Plants

To support the registration of *S*-metolachlor, Syngenta Crop Protection, Inc. has submitted information supporting their assertion that the metabolism of *S*-metolachlor is essentially the same as for the racemic mixture. These studies include a published article on the metabolism in soybeans of [^{14}C]propachlor, a related chloroacetanilide herbicide (1989, MRID 45499603); an unpublished report comparing the *in vitro* activity of glutathione S-transferases (GST) following treatment with different metolachlor isomers (1994, MRID 45499604); an unpublished report examining the uptake and disappearance of metolachlor isomers from seedling corn (1994, MRID 45499605); and an unpublished report examining the uptake and disappearance of *S*-metolachlor and metolachlor from seedlings of corn, sorghum, soybeans, and peanuts (2001, MRID 45533103). The information provided in these submissions is summarized below. However, none of these submissions are guideline studies, and complete details of the experiments and raw data were not included in the reports.

MRID 45499603

In a report published in *Pesticide Biochemistry and Physiology* (1989, Vol 34, pp 187-204), the registrant presented information on the metabolism of the chloroacetanilide herbicide [^{14}C]propachlor in excised soybean leaves and roots, in hydroponically grown soybean plants, in soil-grown soybeans plants, and in soil.

For the *in vitro* tests, trifoliate leaves were excised from 6-week old plants and incubated at 30 C in a growth chamber for various intervals in aqueous solutions containing [^{14}C-carbonyl] propachlor (97% radiochemical purity) at concentrations of 6.88 or 13.3 µM. Root segments were also excised from 5-week old plants and were vacuum infiltrated for 30 seconds in 40 µM [^{14}C]propachlor. After infiltration, the root segments incubated in a water bath at 30 C. Treated leaves and roots were extracted with aqueous acetone, and ^{14}C-residues were purified by partitioning with dichloromethane or using a C_{18} SPE cartridge, and were analyzed by HPLC, along with reference standards.

In another set of tests, hydroponically grown, 2-week old soybean plants were pulse-treated by adding [^{14}C]propachlor to the nutrient solution at a concentration of 11.1 µM. After 6 days, the plants were transferred to fresh nutrient solution and grown until sampling at 31, 48, and 72 days post-treatment. At sampling, plants were separated into shoots and roots and were ground in liquid nitrogen. ^{14}C-Residues were then extracted with 70% acetone, purified using a C_{18} SPE cartridge, and analyzed by HPLC.

In another set of tests, soybean seed were planted in pots containing a sandy loam soil and were covered with a layer of [^{14}C]propachlor-treated soil. The application rate was equivalent to ~2.1 kg ai/ha. Plants were sampled 7 weeks after treatment, and were separated into roots and shoots.

^{14}C-Residues were extracted and analyzed in the same manner as for the hydroponically-gown plants.

The authors also examined metabolism in soil. [^{14}C]Propachlor was applied and mixed into a sample of sandy loam soil at a rate equivalent to 1.7 kg ai/ha and placed in a fretted glass funnel. The treated soil was incubated at 25 C in the laboratory and soil moisture was maintained at ~85% of field capacity. After 28 days, ^{14}C-residues were extracted from soil using aqueous acetone, purified using a C_{18} SPE cartridge, and analyzed by HPLC.

Metabolites were identified by co-chromatography with reference standards using HPLC and TLC, and the identities of isolated metabolites were confirmed by Fast atom bombardment and direct chemical ionization mass spectrometry.

Up to 7 metabolites were identified in extracts of excised soybean leaves and hydroponically grown soybeans. In excised leaves, the initial (0.1 day) metabolite was identified as the homoglutathione conjugate of propachlor [Metabolite I, S-(Prop)HomoGSH], and by 1 and 2 days post-treatment, the major metabolite was identified as the cysteine conjugate of propachlor [Metabolite II, *S*-(Prop)Cys], along with small amounts of the cysteine S-oxide conjugate of propachlor [Metabolite III, *SO*-(Prop)Cys]. At later intervals (31-72 days), ^{14}C-residues in shoots of hydroponically grown plants consisted primarily of four metabolites including: the malonylcysteine conjugate of propachlor [Metabolite IV, *S*-(Prop)MalCys]; the S-oxide malonylcysteine conjugate of propachlor [Metabolite V, *SO*-(Prop)MalCys]; 2-(3-sulfinyllactic acid)-N-isopropylacetanilide [Metabolite VI, *SO*-(Prop)Lact]; and the 6-*O*-malonyl-β-1-*O*-D-glucoside of 2-hydroxy-N-isopropylacetanilide [Metabolite VIII, *O*-(Prop)MalGlc]. The authors proposed pathway for metabolism of [^{14}C]propachlor in soybeans is shown in Figure 1.

Analysis of treated soil extracts identified 3 major and 3 minor metabolites. The major soil metabolites included: N-isopropyl-oxanilic acid [Metabolite IX, Prop-COOH]; 2-sulfo-N-isopropylacetanilide [Metabolite X, Prop-SO_3H]; and 2-sulfinylmethyl-enecarboxy-N-isopropylacetanilide [Metabolite XI, *SO*-(Prop)Acet]. The three minor soil metabolites included: 2-methylsulfinyl-N-isopropylacetanilide [Metabolite XII, Prop-$SOCH_3$]; 2-hydroxy-N-isopropylacetanilide [Metabolite XIII, Prop-OH]; and 2-methylsulfonyl-N-isopropylacetanilide [Metabolite XIV, Prop-SO_2CH_3].

The major extractable ^{14}C-residues derived from [^{14}C]propachlor in hydroponically-grown and soil-grown soybeans are summarized in Table 1. At all three sampling intervals, the major ^{14}C-residue in shoots of hydroponically grown plants was *S*-(Prop)MalCys (13.0-22.2% TRR). The other three major metabolites were found in approximately the same amounts: *SO*-(Prop)MalCys (4.3-11.9% TRR); *SO*-(Prop)Lact (9.6-10.2% TRR); and *O*-(Prop)MalGlc (9.1-10.7% TRR). However, these four metabolites together only accounted for 6.0% of the TRR in shoots of soil grown (49-DAT) soybeans. The major ^{14}C-residues in shoots of soil-grown soybeans consisted of Prop-COOH (42.4% TRR) and Prop-SO_3H (15.5% TRR).

Based on these data, the study authors concluded that the major metabolites present in soybeans grown in propachlor-treated soil are those that are produced in the soil and are then taken up by the plants.

Figure 1. Proposed metabolism of [^{14}C]propachlor in soybean plants.

FIG. 4. *Probable route of propachlor metabolism in soybean plants. The percentage of ^{14}C in the form of a terminal metabolite 72 days after treatment (IV, V, VI, and VIII) is indicated in parentheses.*

Table 1. Metabolites identified in hydroponically grown and soil grown soybeans following an application of [^{14}C]propachlor.

Metabolite/fraction	% Distribution of Metabolites (% TRR)							
	Hydroponic-grown						Soil-grown	
	31-day		48-day		72-day		49-day	
	Leaves	Root	Leaves	Root	Leaves	Root	Leaves	Roots
S-(Prop)MalCys	22.2	22.9	16.1	17.9	13.0	21.4	6.0 [a]	4.1 [a]
SO-(Prop)MalCys	4.3	7.6	11.9	8.1	7.1	8.6		
SO-(Prop)Lact	9.6	ND	10.0	ND	10.2	ND		
O-(Prop)MalGlc	9.1	ND	10.6	ND	10.7	ND		
Prop-COOH	ND	ND	ND	ND	ND	ND	42.4	1.2
Prop-SO_3H	ND	ND	ND	ND	ND	ND	15.5	3.9
Total identified	45.2	30.5	48.6	26.0	41.0	30.0	63.9	9.2
Other soluble ^{14}C-residues	NR	NR	NR	NR	NR	NR	14.6	3.2
Non-extractable	4.3	6.2	6.0	4.9	6.4	7.1	2.4	6.0

[a] Combined Levels of four metabolites.

ND = not detected.

NR = not reported; other soluble ^{14}C-residues for the hydroponically grown plants were reported as an average of all three sampling intervals in Table 4 of the article (leaves - 6.0% TRR; roots - 5.7% TRR).

MRID 45499604

To examine induction of GST, two varieties of Corn (Blizzard and Pioneer 3737) were planted in vermiculite and watered with solutions containing either metolachlor (CGA 24705, racemic mixture), S-metolachlor (CGA 77102), *R*-metolachlor (CGA 77101), or benoxacor (CGA 1542810). Each herbicide was tested at concentrations of 3 and 8 mg/mL, but the concentration of benoxacor was not specified. Benoxacor is a herbicide safener that is formulated with metolachlor to protect corn from herbicide damage by inducing GST. After growing for 3 days in the dark, plants were harvested and levels of GST in roots and shoots were determined by immunoblotting and ELISA, using antibodies that recognize four different GST isoenzymes.

In separate tests, the level of GST enzyme activity was also determined in response to the various metolachlor isomers. Corn seedling were grown in the dark with or without benoxacor treatment. Seedlings were harvested and extracts of roots and shoots were obtained for enzyme assays. GST activity was determined by using ^{14}C-labeled CGA 24705, CGA 77101, and CGA 77102 as the substrates.

The relative levels of GST in corn seedlings following treatment with metolachlor or benoxacor are shown in Table 2. Treatment with benoxacor resulted in substantial increases (5-12x) in the levels of GST in roots and shoots of both varieties. However, treatment with metolachlor had little or no effect on GST levels (0.2-1.5x). The report also stated that treatment with S- or R-metolachlor did not increase levels of GST; however, no data were provided.

Table 2. Relative concentration of GST enzyme in metolachlor and benoxacor treated corn seedlings [a].

Corn Variety	matrix	Chemical	Concentration (mg/mL)	GST content (as % of GST in control shoots)
Blizzard	Shoots	Metolachlor (CGA 24705)	3	100
			8	120
		Benoxacor	NS	500
	Roots	Metolachlor (CGA 24705)	3	20
			8	40
		Benoxacor	NS	880
Pioneer 3737	Shoots	Metolachlor (CGA 24705)	3	100
			8	150
		Benoxacor	NS	780
	Roots	Metolachlor (CGA 24705)	3	100
			8	90
		Benoxacor	NS	1170

[a] Values were estimated by reviewer from bar graphs in Figure 1 of MRID; data for separate the R- and S-isomers of metolachlor were not provided.

NS = not specified.

The activity of GST in extracts of corn shoots and roots using [^{14}C]metolachlor isomers (0.1 mM) as the substrates is shown in Table 3. As expected GST activity was higher in corn plants treated with benoxacor. Using any of the metolachlor isomers as substrates, GST activity was 0.21-0.50 nmol/h/mg in control shoots and 0.50-2.23 nmol/h/mg in shoots from benoxacor-treated seedlings. The difference was even more pronounced in root extracts, with GST activities of 0.66-1.45 nmol/h/mg in control roots and 3.95-6.78 nmol/h/mg in roots from benoxacor-treated seedlings. With the exception of shoots from the variety Blizzard, GST activities in shoots and roots of benoxacor-treated seedlings were highest (0.71-6.78 nmol/h/mg) when *S*-metolachlor was used as the substrate and were lowest (0.50-4.00 nmol/h/mg) with *R*-metolachlor; the values for the racemic mixture were intermediate (1.07-5.66 nmol/h/mg). The same trend was observed in extracts from control shoots and roots, although the differences in GST activities were less pronounced.

GST enzyme activity curves generated using *S*- and *R*-metolachlor at various concentrations also showed that GST activity was consistently higher for the *S*-isomer than for the *R*-isomer (Table 4). In extracts from shoots of control corn seedlings, V_{max} was 0.48-0.54 nmol/h/mg for the *S*-isomer and 0.25-0.45 nmol/h/mg for the *R*-isomer. In extracts from shoots of benoxacor-treated corn seedlings, V_{max} was 2.86-3.03 nmol/h/mg for the *S*-isomer and 1.33-2.33 nmol/h/mg for the *R*-isomer.

Based on these findings, the registrant concluded that metolachlor is enzymatically conjugated with glutathione in corn and that the rate of conjugation is higher for *S*-metolachlor than for *R*-metolachlor.

Table 3. GST enzyme activity in shoot and root extracts from control or benoxacor-treated corn seedlings [a].

Corn Variety	Matrix	Treatment [b]	Chemical Substrate [c]	GST activity (nmol/h/mg protein)
Blizzard	Shoots	Untreated	Metolachlor	0.28
			R-Metolachlor	0.21
			S-metolachlor	0.50
		Benoxacor- treated	Metolachlor	1.07
			R-Metolachlor	0.50
			S-metolachlor	0.71
Pioneer 3737	Shoots	Untreated	Metolachlor	0.44
			R-Metolachlor	0.30
			S-metolachlor	0.50
		Benoxacor- treated	Metolachlor	1.85
			R-Metolachlor	1.21
			S-metolachlor	2.23
Blizzard	Roots	Untreated	Metolachlor	1.18
			R-Metolachlor	1.00
			S-metolachlor	1.45
		Benoxacor- treated	Metolachlor	5.53
			R-Metolachlor	4.00
			S-metolachlor	6.78
Pioneer 3737	Roots	Untreated	Metolachlor	1.00
			R-Metolachlor	0.66
			S-metolachlor	1.05
		Benoxacor- treated	Metolachlor	5.66
			R-Metolachlor	3.95
			S-metolachlor	5.80

[a] Values were estimated by reviewer from bar graphs in Figure 2 of MRID.

[b] The concentration of benoxacor used to treated the corn seedlings was not specified.

[c] Enzyme assays were conducted using ^{14}C-labeled herbicides as the substrates, each at a concentration of 0.1mM.

12

Table 4. GST enzyme activity in shoot extracts from control or benoxacor-treated corn seedlings [a].

Corn Variety	Treatment	GST activity	
		V_{max} (nmol/h/mg)	K_m (μmol/L)
Blizzard	*R*-metolachlor	0.25	34
	S-metolachlor	0.54	38
	Benoxacor + *R*-metolachlor	2.33	37
	Benoxacor + *S*-metolachlor	3.03	29
Pioneer 3737	*R*-metolachlor	0.45	53
	S-metolachlor	0.48	16
	Benoxacor + *R*-metolachlor	1.33	25
	Benoxacor + *S*-metolachlor	2.86	51

[a] Enzyme assays were conducted using ^{14}C-labeled herbicides as the substrates at various concentrations, using corn seedling extracts from plant treated with or without benoxacor.

MRID 45499605

In this study, 3-day old corn seedlings, which were grown in the dark, were immersed for 2 hours in a solution containing either [^{14}C]metolachlor (CGA 24705) at 4.0 mg/L, [^{14}C]*S*-metolachlor (CGA 77102) at 2.6 mg/L, or [^{14}C]*R*-metolachlor (CGA 77101) at 2.7 mg/L. After treatment, seedling were sampled at 0, 1, and 2 hours post-treatment and were separated into roots, seeds, and shoots. The plant samples were lyophilized, ground, and extracted with acetone, and the extractable radioactivity radioassayed by LSC and non-extractable radioactivity was determined by combustion/LSC. Extractable ^{14}C-residues from shoots and roots were analyzed by radio-TLC to determine the amount of parent remaining.

The uptake of radioactivity expressed in parent equivalents is shown in Table 5. For all three test substances, the majority of the radioactivity was recovered in the shoots (66.6-90.6%) at each interval. Translocation of radioactivity was limited, with the roots accounting for 23.4-28.5% of the total plant radioactivity by the 2-hour interval. Seeds accounted for <10% of the total plant radioactivity at each interval. When corrected for the difference in the amount applied, the uptake of radioactivity was lowest with *R*-metolachlor (141-164 ng/plant) and highest with *S*-metolachlor (219-259 ng/plant), with the racemic mixture having an intermediate value (184-216 ng/plant).

At the 0-hour interval, the percentage of extractable radioactivity remaining in shoots as parent was highest for the R-isomer (17.8%) and lowest for the S-isomer (7.9%), with the racemic mixture having an intermediate value (12.5%). This trend continued at the subsequent sampling interval (Table 6).

Considering that the uptake of *S*-metolachlor was higher than for R-metolachlor, and that levels of parent were lower for *S*-metolachlor, the registrant concluded that *S*-metolachlor is more rapidly metabolized in corn than the R-metolachlor.

Table 5. Uptake and distribution of radioactivity in corn seedlings following a 2-hour treatment with either [^{14}C]metolachlor, [^{14}C]R-metolachlor, or [^{14}C]S-Metolachlor [a].

[^{14}C]compound (rate - mg/L)	Sampling interval (hour)	Shoots			Roots			Seeds			Total plant	
		ng/shoot		% total plant	ng/root		% total plant	ng/seed		% total plant	ng/plant	corrected [b] ng/plant
		Extractable	non-Extr.		Extractable	non-Extr.		Extractable	non-Extr.			
Metolachlor (4.0 mg/L)	0	563.6	1.9	74.7	124.6	9.0	16.9	67.8	4.8	8.5	772	193
	1	538.1	2.0	72.6	143.5	13.2	21.8	38.6	2.2	5.4	737	184
	2	575.6	2.3	66.6	211.9	23.4	27.8	48.8	3.1	5.7	865	216
R-Metolachlor (2.7 mg/L)	0	330.2	0.9	87.7	27.1	1.8	7.4	18.9	0.7	4.9	379	141
	1	364.6	1.2	83.5	53.0	5.1	12.7	15.6	1.2	3.9	442	164
	2	270.2	0.8	65.1	122.1	9.6	28.5	22.9	3.0	6.5	419	155
S-metolachlor (2.6 mg/L)	0	517.5	1.5	90.6	19.5	1.5	3.9	29.3	0.7	5.6	570	219
	1	449.2	1.8	77.5	102.8	9.1	19.9	13.9	1.2	2.7	580	222
	2	454.0	1.9	67.8	144.1	14.0	23.4	59.2	0.5	8.8	673	259

[a] Data are the average of two plant samples per compound at each interval.

[b] Adjusted to reflect the different application rates; expressed as ng/plant per mg/L applied.

Table 6. Percent of extractable radioactivity remaining as parent compound remaining in seedling shoots at 0, 1 and 2 hours post-treatment.

Chemical	Percent Parent Remaining [a]		
	0 hour	1 hour	2 hours
CGA 24705	12.5	5.1	3.6
CGA 77101	17.8	8.6	4.3
CGA 77102	7.9	1.9	1.3

MRID 45533103

In this study, the shoots of corn, sorghum, soybean, and peanut seedlings (0.5-0.75 leaf stage) were immersed for 2 hours in a solution containing either [^{14}C]metolachlor at 4.0 mg/L or [^{14}C]*S*-metolachlor at 2.6 mg/L. The treatment solution also contained the surfactant Tween 20 at 2 g/L. After treatment, seedling were sampled at 0, 1, and 2 hours post-treatment and were separated into roots, seeds, and shoots. The plant samples were lyophilized, ground, and extracted with acetone, and the extractable radioactivity was analyzed by radio-TLC to determine the amount of parent remaining.

The uptake of radioactivity expressed in parent equivalents is shown in Table 7. When corrected for the difference in the amount applied, the uptake of radioactivity was similar for both compounds in each crop. Although supporting data were not provided, the registrant also reported that <1% of the radioactivity was translocated to the roots in any crop.

In both corn and sorghum, the percentage of *S*-metolachlor remaining in the shoots at each sampling interval was significantly ($p<0.05$) lower than for metolachlor (Table 8). The percent of *S*-metolachlor remaining in soybean shoots was also significantly lower than for metolachlor, although the difference was not as great as observed for corn and sorghum. In peanuts, the percentage of *S*-metolachlor remaining the shoots was again lower than for metolachlor, but difference was not substantial.

Based on these findings, the registrant suggested that *S*-metolachlor is more rapidly metabolized in plants than the racemic mixture of metolachlor.

Table 7. Uptake of radioactivity by seedlings following a 2-hour exposure of shoots to [^{14}C]*S*-metolachlor at 2.6 mg/L or [^{14}C]metolachlor at 4.0 mg/L.

Crop	*S*-metolachlor (CGA 77102) [a]		Metolachlor (CGA-24705) [a]	
	µg/plant	ng/plant per µg/mL applied	µg/plant	ng/plant per µg/mL applied
Corn	1.664	640	2.436	609
Sorghum	0.138	53	0.240	60
Soybean	0.400	154	0.740	185
Peanut	1.607	618	2.212	553

[a] Expressed in parent equivalents.

Table 8. Percent of parent compound remaining in seedling shoots at 0, 1 and 2 hours post-treatment.

Crop	Chemical	Percent Parent Remaining [a]		
		0 hour	1 hour	2 hours
Corn	CGA-77102	75	41	39
	CGA 24705	87	71	73
Sorghum	CGA-77102	49	28	29
	CGA 24705	78	68	34
Soybean	CGA-77102	81	67	65
	CGA 24705	90	80	76
Peanut	CGA-77102	81	50	58
	CGA 24705	94	65	62

[a] Values were estimated by reviewer from bar graphs in Figures 1-4 of MRID.

In addition, to the above informational submissions, Syngenta Crop Protection, Inc. has submitted plant metabolism studies for *S*-metolachlor on soybeans (1999, MRID 45499610) and field corn (1997, MRID 454499609). These submissions consisted of essentially complete plant metabolism studies and are reviewed in detail in attached DERs. Summaries of these submissions are presented below.

MRID 45499609

Field grown corn plants (3-leaf stage) were treated with either [^{14}C-U-phenyl]-labeled S-metolachlor or metolachlor (racemic mix) as a single post-emergence application at a target rate of 1.28 lb ai/A. Whole plants (forage) were collected at 1 hour and 30 and 82 days after treatment (DAT), and whole plants were harvested at maturity (153 DAT) and separated into stover, cobs, and grain. Radioassays of the soil and forage at 1 hour post-treatment indicated that the actual application rate of [^{14}C]*S*-metolachlor was somewhat higher than for [^{14}C]metolachlor (forage, 184 vs. 118 ppm; soil, 1.47 vs. 1.15 ppm). Total radioactive residues (TRR) were also initially higher in forage samples from the *S*-metolachlor treatment, but TRR levels were similar for the two compounds (*S*-metolachlor vs. metolachlor) at later sampling intervals: 30-day forage, 2.741 vs. 0.774 ppm; 82-day forage, 0.074 vs 0.058 ppm; stover, 0.164 vs 0.133 ppm; cobs, 0.016 ppm; and grain, 0.019 vs 0.020 ppm.

The extraction and fractionation of crop samples from [^{14}C]*S*-metolachlor and [^{14}C]metolachlor treated corn showed a similar pattern of distribution. Methanolic extractions released 82.7-92.9% of the TRR from 30-day forage, and the extractability of ^{14}C-residues declined to 70.8-77.5% of the TRR in the 82-Day forage and 53.2-55.4% of the TRR in the 153-Day stover samples. The solvent extractability of ^{14}C-residues was considerably lower in both cobs (7.6-9.1% TRR) and grain (5.4-6.3% TRR). Base hydrolysis of the PES fraction from stover released 29.2-29.3% of the TRR from both samples, and in each case, the lignin fraction accounted for 4.0-5.5% of the

TRR. For grain, pronase treatment released 38.6% of the TRR from the PES, and mazeroym/cellulase released an additional 3.0-5.3% of the TRR.

Of the solvent-extracted radioactivity from each ^{14}C-compound, organosoluble ^{14}C-residues accounted for 10.7-11.1% of the TRR in 30-Day forage, 17.4-21.6% of the TRR in 82-Day forage, 10.6-12.7% of the TRR in stover, and $<$2% of the TRR in cobs and grain.

TLC analyses of selected fractions from forage and stover treated with either ^{14}C-compound showed a similar metabolite profile, although there were quantitative differences between corresponding regions of radioactivity. The metabolite profile consisted primarily of unidentified polar components each accounting for $\leq$14.8% of the TRR. Parent compound was only detected (by TLC) in the 1-hour forage at 42-49 ppm for each compound, and the metabolite CGA 40172, [N-(2-ethyl-6-methyl-phenyl)-(2-hydroxy-1-methyl-ethyl) acetamide], was tentatively detected in forage samples, accounting for 1.4-5.2% TRR. For both ^{14}C-test substances, treatment of aqueous soluble ^{14}C-residues from forage and stover with Raney-Nickel resulted in a more simplified TLC profile that was also more non-polar in nature. One of these reduced components co-chromatographed with CGA 41507 (3.4-13.1% TRR; N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl) acetamide). The registrant stated that the conversion of polar ^{14}C-residues by Raney-Nickel treatment indicates the presence of glutathione conjugates or their derivatives.

Based upon (i) the TRR levels in each commodity, (ii) the similar distribution and fractionation of ^{14}C-residues within each matrix, and (iii) the limited TLC characterizations, the metabolism of [^{14}C]*S*-metolachlor and [^{14}C]metolachlor appears to be similar in corn treated post-emergence, and may initially involve the conjugation of parent with glutathione.

MRID 45499610

The registrant submitted an adequate metabolism study using *S*-metolachlor on soybeans. Field-grown soybeans were treated with a single, pre-emergence application of [^{14}C-U-phenyl] *S*-metolachlor at 1.53 or 4.60 ai/A, which were reported to represent 1x and 3x rates, respectively. For the 1x treatment, samples of forage were collected 19, 57, and 75 days after treatment (DAT) and hay was collected at 75 DAT. Mature samples of seeds and stalks were collected at 156 DAT from both the 1x and 3x treatments. To provide metabolites for identification, young plants (3rd trifoliate) were also stem-injected with [^{14}C]*S*-metolachlor at 0.6 mg ai/plant, and were harvested at 1, 7, and 99 DAT.

For the 1x treatment, total radioactive residues (TRR) in forage were similar (1.146-1.462 ppm) at all three sampling intervals (19-75 DAT); TRR were 7.555 ppm in hay and 0.227 and 2.042 ppm in mature beans and stalks, respectively. Increasing the application rate by 3x resulted in a proportional increase in the TRR levels in beans (0.688 ppm) and stalks (7.296 ppm).

Solvent extraction released 92.1-98.8% of the TRR from forage and hay, 63.2-65.3% of the TRR from stalks, and 34.9-54.4% of the TRR from seeds. Extracted ^{14}C-residues were profiled and quantified by 2D-TLC, and isolated metabolites were characterized and identified by TLC analysis with reference standards, HPLC, enzymatic treatments, Raney-Nickel reduction, electrophoresis, and LC/MS. Radioactivity remaining in post-extraction solids (PES) of stalks (22.9-26.0% TRR) and beans (42.4-49.9% TRR) were further released by mild base extraction and acid hydrolysis, and the solubilized ^{14}C-residues were characterized by TLC.

For the forage, hay, and stalk samples, 39.0-56.9% of the TRR was identified and another 26.2-57% of the TRR was adequately characterized. For beans, 10.0-25.5% of the TRR was identified and another 75.3% of the TRR was adequately characterized. The overall recovery of radioactivity from the analysis of forage, hay, stalks, and beans was 91.5-103.5%.

The metabolic profile of [^{14}C]*S*-metolachlor in soybeans was complex. Detailed analyses of forage, hay, stalks, and beans identified parent and up to 16 metabolites, along with numerous minor unknown components. In the earliest forage sample (19-DAT), the major extractable ^{14}C-residues were identified as the cysteine conjugate of *S*-metolachlor (CGA 46576; 19.9% TRR) and the malonyl-cysteinyl-conjugate of *S*-metolachlor (Metabolite I_{17}, 5.7% TRR), along with 15.6% of the TRR that the authors attributed to the homoglutathione conjugate of *S*-metolachlor, which was identified in the stem injection experiment. Parent was detected at 1.8% of the TRR, and all other metabolites each accounted for <4.0% of the TRR. In the later forage and hay samples (57- or 75-DAT), parent was not detected. The major ^{14}C-residues consisted of the sulfonate metabolites, NOA 413173 (3.9-7.3% TRR) and CGA 380168 (~15% TRR) and the oxalamic acid metabolites, CGA 351916 (2.8-6.2% TRR) and Metabolite I_8 (5.0-5.4% TRR). All other identified metabolites each accounted for <5% of the TRR.

In mature stalks, none of the identified metabolites accounted for ≥10% of the TRR, and only the two sulfonate metabolites, NOA 413173 (6.6-7.3%% TRR) and CGA 380168 (8.9-9.9% TRR) accounted for more than 5% of the TRR. ^{14}C-Residues remaining in the PES fraction of stalks were characterized as being incorporated into the pectin, lignin, or cellulose fractions or consisting of a variety of minor polar residues.

In seeds, a total of 12 metabolites were identified; however, NOA 436611 (4.5-9.8% TRR) was the only metabolite that accounted for more than 5% of the TRR. The hexane-extractable radioactivity (~10% TRR) was characterized as incorporated into fatty acids, and ^{14}C-residues remaining in the PES fraction were characterized as being incorporated into the protein fraction or consisting of a variety of minor polar residues.

Based on the metabolite profile, the metabolism of [^{14}C]*S*-metolachlor in soybeans primarily involves two pathways: (i) conjugation of the parent molecule with homoglutathione by substitution of the chlorine, followed by the degradation of the homoglutathione moiety to form a variety of sulfur containing metabolites; and (ii) direct oxidation of parent or secondary metabolites, primarily on the chloroacetyl side chain.

AGENCY MEMORANDA CITED IN THIS DOCUMENT

DP Barcode:	D226780
Subject:	Replacement of Metolachlor Technical (Racemic Metolachlor) with Alpha-Metolachlor (formerly called Chiral Metolachlor) Technical; Review of Bridging Data.
From:	L. Kutney
To:	R. Giffin
Dated:	11/12/96
MRID(s):	43928901-43928903 and 43928939-43928942

MRID CITATIONS

45499603 Lamoureux, G.; Rusness, D. (1989) Propachlor Metabolism in Soybean Plants, Excised Soybean Tissues, and Soil. Study published in Pesticide Biochemistry and Physiology, Vol. 34. pp. 187-204 (OPPTS 860.1300}

45499604 Kreuz, K. (1994) Isomer-specific Properties of CGA-77101 and CGA-77102: Results of *in vitro* studies. Unpublished study prepared by Syngenta Crop Protection, Inc. 8 p. (OPPTS 860.1300}

45499605 Kreuz, K. (1994) Isomer-specific Properties of CGA-77101 and CGA-77102: Results of metabolism studies. Unpublished study prepared by Syngenta Crop Protection, Inc. 6 p. (OPPTS 860.1300}

45499609 Gentile, B. (1997) Behaviour and Metabolism of CGA 77102 and CGA 24705 in Field Grown Corn after Post-Emergence Treatment of [Phenyl-(U)-^{14}C] Labelled Materials: Laboratory Project Numbers: 9/97: 853-95. Unpublished study prepared by Syngenta Crop Protection, Inc. 91 p. (OPPTS 860.1300}

45499610 Sandmeier, P. (1999) Behaviour and Metabolism of CGA 77102 in Field Grown Soybean after Pre-Emergent Treatment of [Phenyl-(U)-^{14}C] Labelled Material: Laboratory Project Nos.: 97PSA48: 1266-99. Unpublished study prepared by Syngenta Crop Protection, Inc. 527 p. (OPPTS 860.1300}

45533103 Hall, G. (2001) Comparative Metabolism of S Isomer (CGA-77102) and Isomer Mixture (CGA-24705) in Different Crop Species. Unpublished study prepared by Syngenta Crop Protection, Inc. 11 p. (OPPTS 860.1300}

S-Metolachlor/PC Code: 108800/IR-4
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Green Onion

Primary Evaluator Sherrie L. Kinard, Chemist Date: 8/15/03 Sherrie Kinard
Reregistration Branch 2
Health Effects Division, 7509C

Reviewer Alan Nielsen, Branch Senior Scientist Date: 8/15 Alan Nielsen
Reregistration Branch 2
Health Effects Division, 7509C

STUDY REPORT:

45544701 Arsenovic, M. (2001) Metolachlor: Magnitude of the Residue on Onion (Green). Lab Project Number: 06717.99-BER02. Unpublished study prepared by IR-4 Project, Rutgers, State Univ. of New Jersey, North Brunswick, NJ. 216 p.

EXECUTIVE SUMMARY:

In a total of 3 field trials conducted in CA, MI, and NY during 1999, *S*-metolachlor (7.62 lb/gal EC) was applied as a single broadcast application at 0.133-1.40 lb ai/A to green onions at the 2nd leaf stage. Duplicate green onion samples were collected from each test at 43-45 days post-treatment. The number of crop field trials and geographic representation of the residue data on green onions are adequate.

Green onion samples were stored frozen for a maximum of 637 days prior to analysis, an interval supported by the concurrent storage stability data. These storage stability data indicate that CGA-37913 and CGA-49751 are stable in green onions stored at -20°C for up to 660 days (21.7 months).

S-metolachlor residues in/on green onions were determined by the adequate GC/NPD Method AG-612, which is similar to Method I in PAM, Vol. II. Following acid hydrolysis, residues of CGA-39713 and CGA-49751 are purified by solvent partitioning, cleanup using alumina and silica gel columns, and derivatization of CGA-49751. Residues are then determined by GC/NPD. The validated limit of quantitation (LOQ) for CGA-37913 and CGA-49751, expressed in parent equivalents, is each 0.05 ppm in/on green onions. The method limit of detection (LOD) was not reported.

Following a single broadcast application of *S*-metolachlor (EC) at 1.33-1.40 lb ai/A, combined residues of CGA-37913 and CGA-49751 were 0.057-0.168 ppm in/on 6 green onion samples harvested 43-45 days post-treatment.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The green onion field trial residue data are classified as scientifically acceptable under the conditions and parameters used in the study The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D292881].

COMPLIANCE:

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

A. BACKGROUND INFORMATION

S-Metolachlor is a selective, preemergence, chloroacetanilide herbicide that is applied to a wide variety of crops as a preplant, preplant-incorporated (PPI), preemergence, or postemergence-directed application, primarily for the control of grass weeds. S-Metolachlor products currently registered to Syngenta include emulsifiable concentrate (EC), granular (G), flowable concentrate (FlC) and ready-to-use (RTU) formulations. IR-4 has submitted a petition proposing the use for S-metolachlor (Dual Magnum ®, 7.62 lb/gal EC) on green onions.

TABLE A.1.	Nomenclature of Test Compound and Metabolite
Compound	
Common name	*S*-Metolachlor
Company experimental names	CGA-77102
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-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-acetamide
CAS #	87392-12-9
End-use products/EP	7.62 lb/gal EC [EPA Reg. No. 100-816]

TABLE A.2. Physicochemical Properties		
Parameter	Value	Reference (MRID)
Melting point/range	Not applicable (NA), liquid at room temperature	NA
pH	7-9 @ 25 C (1% aqueous suspension)	43928903
Density	1.117 g/cm^3	43928903
Water solubility (25°C)	0.48 g/l	43928903
Solvent solubility (g/l at 25°C)	completely soluble in n-hexane, methanol, acetone, toluene and n-octanol	43928903
Vapor pressure at 25°C	2.8 x 10^{-5} mm Hg	43928903
Dissociation constant (pK$_a$)	NA	43928903
Octanol/water partition coefficient Log(K_{OW})	3.05 @ 25°C	43928903
UV/visible absorption spectrum (λmax, nm)	NA	

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Temperature and rainfall data were collected at each site, along with information on the pesticides and fertilizers applied to the plots during the year of the tests.

TABLE B.1.1 Soil Characterization.	
Study Location (City, State, Year)	Soil Type
Freeville, NY, 1999	Silt Loam
Langsburg, MI, 1999	Loam
Salinas, CA, 1999	Silt Loam

TABLE B.1.2. Study Use Pattern on Green Onion									
Location (City, State, Year)	Application								Tank Mix Adjuvants
	Timing [1]	Formulation	Single Rate (lb a.i./A)	RTI [2] (days)	No. of Appl.	Method [3]	Volume (gal/A)	Total Rate (lb a.i./A)	
Freeville, NY, 1999	postemergence	7.62 lb/gal EC	1.328	NA	1	broadcast	29	1.328	None
Langsburg, MI, 1999	postemergence	7.62 lb/gal EC	1.342	NA	1	broadcast	36	1.342	None
Salinas, CA, 1999	postemergence	7.62 lb/gal EC	1.398	NA	1	broadcast	21	1.398	None

1 All applications were made to plants at the 2nd true leaf stage.
2 RTI = Retreatment Interval
3 All applications were made using ground equipment.

S-Metolachlor/PC Code: 108800/IR-4
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Green Onion

TABLE B.1.3. Trial Numbers and Geographical Locations

NAFTA Growing Region [1]	Total Green Onion Trials		
	Submitted	Requested [2]	
		Canada	US
1	1	NA	NA
2	--	NA	NA
3	-	NA	NA
4	--	NA	NA
5	1	NA	NA
6	-	NA	NA
7	--	NA	NA
8	--	NA	NA
9	--	NA	NA
10	1	NA	NA
11	--	NA	NA
12	--	NA	NA
Total	3	NA	3

1 Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the proposed use is for the US only.
2 Current Agency guidance does not specify in which regions the 3 requested green onion trials should be conducted.
NA = not applicable.

B.2. Analytical Methodology

Residues in/on green onions were determined using a gas chromatographic/nitrogen-phosphorus detection (GC/NPD) method (Method AG-612), which determines residues of S-metolachlor and its metabolites as CGA-37913 and CGA-49751, following acid hydrolysis. The combined residues of CGA-37913 and CGA-49751 are expressed in parent equivalents. Method AG-612 is similar to Method I in PAM, Vol. II. A brief description and procedural recovery data were submitted in conjunction with the subject residue field trial data. Samples were analyzed by the USDA/ARS Environmental Chemistry Laboratory, Beltsville, MD, using minor modifications.

Samples are initially refluxed in 6 N HCl for 16 hours, cooled, and filtered. For analysis of CGA-37913, an aliquot of the acid extract is made basic. Residues are partitioned into hexane, and cleaned up using an alumina column followed by a silica Sep Pak. Residues of CGA-37913 are then analyzed by GC/NPD. For analysis of CGA-49751, residues in an aliquot of the acidic hydrolysate are partitioned directly into dichloromethane, washed with a 5% sodium carbonate solution, and cleaned up using a silica Sep Pak. Residues of CGA-49751 are then derivatized with boron trichloride/2-trichloroethanol at ~100°C for 30 mintues, partitioned into hexane, and cleaned up on an alumina column. The derivatized residues are then determined by GC/NPD. The validated LOQ for CGA-37913 and CGA-49751 in/on green onions are each 0.05 ppm, expressed in parent equivalents. The method limit of detection was not reported.

C. RESULTS AND DISCUSSION

The number of crop field trials and geographic representation of the residue data on green onions is adequate according to the latest EPA Guidance. The test plots received maintenance applications of fertilizer and pesticides according to standard agricultural practices. No unusual weather conditions were noted at the MI test site, but the petitioner noted that cooler spring soil temperatures at the CA test site delayed development of the onions and subsequently the test substance application. In addition, temperature and rainfall were above normal at the NY test site during the field trial. However, these conditions are not expected to have a notable impact on the residue data.

Duplicate control and treated samples of green onions were collected from each test at 43-45 days post-treatment and were placed in frozen storage within 2 hours of sampling. Samples were shipped by freezer truck to the analytical laboratory (USDA/ARS, Beltsville, MD), where samples were stored at -20°C until analysis. The total frozen (-20 ± 5 C) storage intervals were 553-637 days (Table C.2.1).

To support the stability of residues in frozen storage, three separate control samples of green onions were fortified by the analytical laboratory with either CGA-39713 or CGA-49751 at 0.5 ppm and placed in storage at -20°C for up to 660 days prior to analysis. The recovery of CGA-37913 was 79.6-85% from the three frozen stored samples and 79.8% from a fresh fortification (Table C.2.2), and the recovery of CGA-49751 was 85.7-87.8% from the three frozen stored samples and 83.2% from a fresh fortification. Although no zero-day sample of the stored samples was analyzed, these data support the frozen stability of residues for the storage intervals in the current trials.

Samples were analyzed using GC/NPD Method AG-612, which determines residues of S-metolachlor and its metabolites as CGA-37913 and CGA-49751 following acid hydrolysis. This method was validated in conjunction with the field trial data. Concurrent method recoveries were 80 ± 8% for CGA-37913 and 103 ± 15% for CGA-49751 from 5 or 6 green onion control samples fortified separately with each analyte at 0.05 or 0.5 ppm (Table C.1). Apparent residues of each analyte were <0.05 ppm in/on all 6 control samples. The LOQ for both analytes is 0.05 ppm in/on green onions. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

In a total of 3 field trials conducted in CA, MI, and NY during 1999, *S*-metolachlor (7.62 lb/gal EC) was applied as a single broadcast application at 0.133-1.40 lb ai/A to green onions at the 2nd leaf stage. Duplicate green onion samples were collected from each test at 43-45 days post-treatment. Residues of CGA-37913 were 0.038-0.061 ppm and residues of CGA-49751 were 0.019-0.107 ppm in/on 6 treated samples of green onions (Table C.3), and combined residues were 0.057-0.168 ppm. The average combined residues were 0.095 ± 0.43 ppm (Table C.4).

TABLE C.1 Concurrent Recovery Results from Green Onions using GC/NPD Method AG-612.

Grapefruit Matrix	Spiking Level (mg/kg)	Sample size	CGA-37913		CGA-49751	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Whole plant	0.05, 0.50	5-6	80, 80, 92, 70.3, 79.8, 61.3 [1]	80.4 ± 8	106, 108, 126, 108, 89.6, 83.2	103 ± 15

[1] Sample went dry during evaporation; therefore, losses were expected. Value was not included in average.

TABLE C.2.1 Summary of Freezer Storage Conditions.

Grapefruit Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days)
Fruit	-20	553-637	660

TABLE C.2.2 Stability of CGA-37913 and CGA-49751 in Green Onions Following Storage at -20 °C.

Commodity	Analyte	Spike level (mg/kg)	Storage interval (days)	Recovered residues (mg/kg)		Corrected % recovery [1]
				Fresh fort.	Stored Fort.	
Green Onions, whole plant	CGA-37913	0.5	660	0.400	0.420, 0.410, 0.393 (0.408) [2]	102
	CGA-49751	0.5	659	0.416	0.435, 0.438, 0.427 (0.433) [2]	104

[1] Corrected for concurrent recoveries.
[2] Average of three stored fortified samples is listed in parentheses.

TABLE C.3. Residue Data from Green Onion Field Trials with *S*-Metolachlor (7.6 lb/gal EC).

Location (City, State), Year	EPA Region	Variety	Total Rate (lbs ai/A)	PHI (days)	Residues (ppm) [1]		
					CGA-37913	CGA-49751	Combined
Freeville, NY, 1999	1	Kincho	1.328	45	<0.05, <0.05	0.051, 0.070	<0.101, <0.120
Langsburg, MI, 1999	5	Long White	1.342	43	*0.038*, <0.05	*0.019, 0.029*	*0.057*, <0.079
Salinas, CA, 1999	10	White Spear	1.398	44	0.061, *0.026*	0.107, 0.094	0.168, 0.120

[1] Residues are expressed in parent equivalents and the LOQ for each analyte is 0.05 ppm. Residue values >LOD and <LOQ are listed in italics.

TABLE C.4. Summary of Residue Data for Green Onion from Crop Field Trials with *S*-Metolachlor (7.6 lb/gal EC).

Matrix	Total Rate (lb a.i./A)	PHI [1] (days)	Analyte	No of samples	Residue Levels (ppm) [2]				
					Mean [3]	Std. Dev.	HAFT [4]	Min	Max
Green onion, whole plant	1.33-1.40	43-45	CGA-37913	6	0.033	0.014	0.050	0.038	0.061
	1.33-1.40	43-45	CGA-49751	6	0.062	0.035	0.101	0.019	0.107
	1.33-1.40	43-45	Total [5]	6	0.095	0.043	0.144	0.057	0.168

1 The proposed PHI is 45 days.
2 The LOQ for both analytes is 0.05 ppm in/on green onions; the LOD was not provided.
3 For calculating the mean, a value of ½ the LOQ was used for residues reported to be <LOQ, unless an actual value below the LOQ was reported.
4 HAFT = Highest Average Field Trial.

D. CONCLUSION

The green onion field trial data are adequate and reflect the use of *S*-metolachlor (EC) as a single post-emergence broadcast application to green onions at the 2nd true leaf stage at a maximum rate of 1.33 lb ai/A /season.

E. REFERENCES

None

F. DOCUMENT TRACKING

PC Code: 108800
Petition Number(s): 2E06374
DP Barcode(s): D288570

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.

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

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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: August 15, 2003

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

Alan Nielsen, Branch Senior Scientist *AL Nielsen 8/15/03*
Reregistration Branch 2
Health Effects Division, 7509C

DP Barcode: D278742

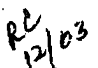
Citation: 45499607 Muller, T., Loffler, A. (1997) Metabolism of [Phenyl-(U)-^{14}C] CGA 77102 after Multiple Oral Administration to Lactating Goats: Lab Project Number: 2/97: 564-96. Unpublished study prepared by Syngenta Crop Protection, Inc. 140 p. {OPPTS 860.1300}

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

Executive Summary

Two lactating goats were dosed orally via gelatin capsules with [Phenyl-U-^{14}C] *S*-metolachlor for 4 consecutive days at 3.85 mg ai/kg body weight/day, equivalent to 121.3 ppm in the diet. Urine, feces, and milk were collected daily and tissue samples were collected at sacrifice, which occurred within 6 hours of administering the final dose. A total of 99.96% of the dosed radioactivity was recovered. The majority of the dose was excreted in the urine (77.8% dose) and feces (11.1% dose), and 0.07% of the dose was contained in the milk. At sacrifice concentrations of radioactivity were highest in the bile (15.5 ppm), liver (3.56 ppm), and kidney (3.76 ppm). Concentrations were considerably lower in muscle (0.122-0.125 ppm) and fat (0.051-0.056 ppm). Radioactivity in milk plateaued at 0.117 ppm by Day 2 (pooled).

Solvent extraction and microwave extraction released 90.4-98.8% of the total radioactive residues (TRR) from milk and tissue samples, and 2D-TLC and HPLC analyses identified a total of 9 metabolites, including parent. Metabolite identities were confirmed by LC/MS, and identified components accounted for 80.7% of the TRR in milk and 34.6-67.1% of the TRR in tissues. The major metabolites included 1L, 3L, 1aN, and 1bN, which were detected in milk and all tissues. In milk, the major ^{14}C-residues were 1L (32.5% TRR) and 3L (42.5% TRR), along with minor amounts (each ≤2.7% TRR) of 5L, 1aN, and 1bN. In muscle, the major ^{14}C-residues were 1L (7.3% TRR), 3L (10.2% TRR), and 1aN (11.4% TRR), along with minor amounts of 1bN (5.7%



27

TRR). In fat, the major ^{14}C-residues were parent (8.9% TRR), 3L (9.7% TRR), and 1aN (11.7% TRR), along with minor amounts (each ≤5.8% TRR) of Metabolites CGA 46129, 1L, 5L, and 1bN. In liver, the major ^{14}C-residues were 1L (12.9% TRR) and 3L (26.3% TRR), along with minor amounts (each ≤5.9% TRR) of 4L, 5L, 6L, 1aN, and 1bN. In kidneys, the major ^{14}C-residues were 3L (10.8% TRR), 6L (11.4% TRR), 1aN (22.3% TRR), and 1bN (11.2% TRR), along with minor amounts of CGA 46129 (4.8% TRR) and 1L (6.6% TRR).

S-Metolachlor was readily absorbed and extensively metabolized by goats via glutathione conjugation and/or oxidation reactions. Substitution of the chlorine atom with glutathione and subsequent enzymatic cleavage of the glutathione moiety yields metabolites 4L and 3L. O-Demethylation of the Metabolite 3L yields Metabolite 1L, which is conjugated with glucuronic acid to form Metabolite 6L or is oxidize at the methyl-phenyl group to form the minor Metabolite 5L. Direct O-demethylation of the parent compound followed by glucuronic acid conjugation forms Metabolite 1aN, and O-demethylation and oxidation of the primary alcohol to the carboxylic acid, followed by glucuronic acid conjugation forms the Metabolite 1bN. Metabolites 1aN and 1bN were also identified in poultry. The submitted ruminant metabolism study is adequate.

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

Active Ingredient

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-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-acetamide
CAS Number: 87392-12-9
Company Name: CGA 77102
Other Synonyms: none
Purity of Non-labeled Material: >99%
Location of Isotopic Label: Uniformly ^{14}C-labeled in the phenyl ring
Radiochemical Purity: >96% (unspecified method)
Specific Activity: 2.04 MBq/mg (55.1 µCi/mg). For dosing the test substance was isotopically diluted with non-radiolabeled *S*-metolachlor to a final specific activity of 557 kBq/mg (33420 dpm/µg)

1.2. Test Animals and Site

Species/breed: Goat, Gemsfarbige Gebirgsziege
Age: 14-15 months
Gender: Females
Number: 2 goats
Test location: Ciba-Geigy Ltd., Agricultural Research Center, St. Abin, CH
Housing: Goats housed individually metabolism cages suitable for the separate collection of urine and feces.
Diet and Water: tape water and feed (UFA No. 867, Kliba Muhlen, Kaiseraugst), maize, and hay were provided *ad libitum*. Feed intake was measured daily.
Acclimation period: 7 days
Environmental conditions: The test area was maintained at 19-20 C with a relative humidity of 37-72% and a 12/12 hour light/dark cycle
Predosing: Animals were not predosed.
Study Dates: The in-life phase of the study was conducted from 4/15/96 to 4/25/96, with dosing occurring from 4/22/96 to 4/25/96. The analytical phase of the study was conducted from 4/26/96 to 3/5/97 (10.3 months).

1.3. Dosing

Type(s) of Dosing: Intraruminally with a capsule
Dosing Vehicle: Gelatin capsules containing D-(+)-lactose
Dosing Rate: Average dose level was 3.85 mg ai/kg body weight/day, which was equivalent to 121.3 ppm in the diet based upon the daily feed consumption
Number of Doses: once a day in the morning
Duration of Dosing: 4 days

1.4. Sample Collection Procedures

Beginning during the acclimation period, urine and feces were collected daily, and milk was collected twice a day in the morning and evenings and stored separately at 4° C. Animals were sacrificed by exsanguination within 6 hours of receiving the final dose on Day 4, and the following samples were collected from each goat: blood, muscle (leg and tenderloin), fat (omental and perirenal), kidney, liver, bile, and G.I. tract (with contents). A cage wash (ethanol:water, 1:1) was also collected prior to dosing and at the end of the study, and cage debris was collected at the end of the study. Muscle, fat, and G.I. tract samples were homogenized and the remaining samples were left intact. All samples were refrigerated (1-4 days) and shipped the day after sacrifice to the analytical laboratory (Animal Metabolism Laboratories, Basel, CH). At the analytical laboratory, tissue samples were chopped. Samples were frozen in liquid nitrogen, homogenized, and placed in storage at -18° C for up to 4.8 months prior to extraction for analysis.

Table 1.3.1. Summary of Storage Conditions

Matrix	Storage Temperature (°C)	Duration (months) [a]
Feces	-18	1
Urine [b]	-18	5.7
Liver	-18	3
Kidney	-18	3
Milk	-18	2.9
Fat	-18	4.8
Muscle	-18	3.8

[a] Interval from collection to extraction; sample extracts were analyzed within one week of extraction.
[b] Urine was analyzed directly by HPLC and TLC without extraction.

To demonstrate the stability of ^{14}C-residues during frozen storage, the analytical laboratory extracted samples of milk and liver after 14 days of frozen storage and again after 8.5 months of frozen storage. The initial 14-day extracts were immediately analyzed by 2D-TLC. The 14-day extracts were also stored at -18° C and were reanalyzed with the extracts obtained after 8.5 months of frozen storage.

1.4. Analytical Methods

Total Radioactive Residues (TRR). For determination of TRR, homogenized samples were radioassayed in triplicate either directly by liquid scintillation counting (milk, bile, urine, and cage wash), by combustion/LSC (excreta, rumen contents, and blood), or treatment with a tissue solubilizer and LSC (all tissues). The limit of detection (LOD) for the radioassays ranged from 0.0002 ppm in milk to 0.0021 ppm in bile; the limit of quantitation (LOQ) ranged from 0.0007 ppm in milk to 0.0064 ppm in bile.

For extraction and analysis of ^{14}C-residues, proportional aliquots from each animal of the following samples were separately pooled: urine from each sampling interval; feces from each sampling interval, leg muscle and tenderloin; omental and perirenal fat; milk from each sampling interval; liver; and kidney. The registrant presented flow charts for each analysis, including the %TRR for each fraction. Although the reported percentages were corrected for procedural recoveries, the actual recoveries through each step were typically reported and were reasonable.

Urine and Feces. Samples of urine were analyzed directly by HPLC and 2D-TLC. Samples of feces were extracted sequentially with acetonitrile (ACN) and ACN:water (4:1, v/v), methanol (MeOH), and MeOH:water (4:1, v/v). The extracts were combined, concentrated and analyzed by TLC and HPLC.

Milk. The pooled milk samples was extracted with ACN and centrifuged. The resulting pellet was then extracted with ACN:water (4:1, v/v). The two extracts were combined, concentrated, diluted with ACN, and partitioned with n-hexane. The resulting polar fraction was analyzed by HPLC and 2D-TLC.

Fat. The pooled fat sample was extracted sequentially with ACN, ACN containing 5% formic acid, and MeOH. Each extraction used ultrasonication at 37° C (1 hour) followed by 1 hour of shaking and 15 minutes of homogenization. The extracts were combined, concentrated, filtered, and partitioned with hexane. The resulting polar fraction was concentrated, redissolved in tetrahydrofuran (THF) and purified by preparative TLC. The radioactive areas from the preparative TLC were eluted and analyzed by TLC and HPLC.

Muscle. The pooled muscle sample was extracted sequentially with ACN, ACN:water (4:1, v/v), and MeOH, centrifuging and collecting the supernatant after each extraction. The ACN extracts were combined, concentrated, and partitioned with n-hexane after the addition of NaCl. The resulting polar fraction (ACN/water) was concentrated, filtered, diluted with water, adjusted to pH 3.0 with formic acid, and loaded onto a C_{18} column. The column was washed with water (pH 3 and pH 7), and ^{14}C-residues were then eluted with ACN, THF, and MeOH. The eluates were combined, concentrated, analyzed by HPLC. The 20-81 minute fraction was collected, concentrated, and further analyzed by 2D-TLC.

The muscle PES fraction was microwave extracted in 2-propanol (PrOH):water (4:1, v/v) at 140° C and the remaining solids were then microwave extracted in PrOH:water at 190° C. The remaining solids were not further analyzed. The 140° C extract was concentrated and purified using a C_{18} SPE column eluted with MeOH, and the resulting MeOH eluate was analyzed by

2D-TLC. The 190° C extract was partitioned with ethyl acetate (EtOAc) resulting in an EtOAc fraction, a PrOH/water fraction, and a precipitate. An aliquot of the EtOAc fraction was analyzed by 2D-TLC, and another aliquot was concentrated, redissolved in MeOH and partitioned with petroleum benzene (PB). The MeOH fraction was then combined with the PrOH/water fraction, and the combined fraction was dissolved in 0.01 M ammonium formate buffer (AFB, pH 3) and cleaned up using a C_{18} cartridge eluted with MeOH. The MeOH fraction was analyzed by 2D-TLC and was further purified using a silica gel cartridge eluted with a step-wise gradient of EtOAc to MeOH. The final EtOAc and EtAOc/MeOH fractions were combined, concentrated, and analyzed 2D-TLC.

Liver. Pooled liver was extracted sequentially with ACN, ACN:water (4:1, v/v), MeOH, and MeOH:water (4:1, v/v). The solvent extracts were combined, concentrated, redissolved in THF:water (3:2, v/v), and analyzed by 2D-TLC and HPLC. This fraction was also used for the isolation and identification of individual metabolites.

Radioactivity remaining in the liver PES fraction was microwave extracted in PrOH:water (4:1, v/v) at 190° C. The resulting extract was concentrated, redissolved in PrOH, and purified using a C_{18} column eluted with a step gradient of water:MeOH (9:1, v/v) to 100% MeOH. The MeOH eluate was concentrated, redissolved in PrOH, and analyzed by 1D- and 2D-TLC and HPLC using a C_{18} and a Diol column. The fraction was also purified by preparative TLC, and the resulting fraction was analyzed by HPLC.

Kidney. Pooled kidney was extracted sequentially with ACN, ACN:water (4:1, v/v), and MeOH. The extracts were combined, concentrated, and redissolved in THF. The solvent extracted ^{14}C-residues were then analyzed by TLC and HPLC. Radioactivity remaining in the kidney PES fraction was microwave extracted in PrOH:water (4:1, v/v) at 140° C and the remaining solids were then re-extracted at 190° C. The remaining solids were not further analyzed.

The 140° C extract was analyzed by 2D-TLC and also purified by C_{18} SPE eluted with MeOH. The MeOH fraction was further separated by HPLC into three fractions with retention times of 0-44 min., 44-47 min, and >47 min. The 0-44 minute fraction was analyzed by HPLC and 2D-TLC and the 44-47 minute fraction was analyzed by 2D-TLC and LC/MS.

The 190° C extract was concentrated, redissolved in water and purified using a C_{18} column eluted with water and MeOH. The water fraction was not further analyzed. The MeOH eluate was analyzed by HPLC and 2D-TLC. An aliquot MeOH fraction was also concentrated and extracted with EtOAc resulting in an EtOAc fraction and a precipitate. The EtOAc fraction was analyzed by 2D-TLC and by HPLC using C_{18}, diol, and aspartamide columns.

Identification of ^{14}C-residues. ^{14}C-Residues in fractions from excreta, milk and tissues were separated and quantified by 2D-TLC using a silica gel plate developed first with EtOAc:PrOH:formic acid:water (64:24:6:6, SSI) followed by chloroform:MeOH:formic acid:water (75:25:4:2, SSII). Non-radioactive spots were detected by UV light (254 nm) and radioactivity regions were detected and quantified using a Bio-Imaging Analyzer. Several other solvent systems were also used with 1D- or 2D-TLC for further characterization of specific fractions in specific tissues. The presence of specific metabolites was confirmed by HPLC,

typically using a C_{18} column eluted with a gradient of 0.01M AFB (pH 3) to ACN. The system was equipped with a UV detector (254 nm) and a radioactivity monitor.

The liver and kidney solvent extracts were used for isolation and identification of specific metabolites. The extracts were separated into major fractions by flash chromatography (C_{18}) and semi-preparative HPLC. Isolated fractions were then analyzed by LC/MS for conformation of metabolite identities.

Several of the metabolites were shown to degrade to one or two unknowns during 2D-TLC. TLC regions attributable to a specific degradate were assigned to the respective metabolite.

2. Results

Table 2.1. Total Radioactive Residues in milk, tissues, and excreta from two goats following oral dosing of [^{14}C-phenyl]*S*-metolachlor for 4 days at 3.85 mg/kg body weight/day, equivalent to 121.3 ppm in the diet [a].

Matrix	Sampling Interval (hours)		% Dose	TRR (ppm)
Milk	Day 1	0-7	0.012	0.150
		7-24	0.007	0.055
	Day 2	24-31	0.013	0.204
		31-48	0.009	0.072
	Day 3	48-55	0.013	0.170
		55-72	0.007	0.055
	Day 4	72-78	0.008	0.159
	Total		0.069	0.106
Leg muscle	78		0.176	0.122
Tenderloin muscle	78		0.005	0.125
Total muscle	78		0.181	0.122
Omental fat	78		0.009	0.051
Perirenal fat	78		0.007	0.056
Total fat	78		0.017	0.053
Kidney	78		0.071	3.761
Liver	78		0.404	3.564
Blood	78		0.162	0.503
Bile	78		0.034	15.536
G.I. Tract w/ contents	78		9.95	NA
Urine	0-78		71.85	NA
Feces	0-78		11.13	NA
Cage wash	0-78		5.99	NA
Cage debris	0-78		0.10	NA
Total Recovery			99.96	NA

[a] Data are the average of two goats.
NA = Not applicable.

Table 2.2.1. Extraction, Characterization, and Identification of ^{14}C-Residues in **Milk** from goats dosed orally for 4 days with [^{14}C-phenyl]-S-metolachlor at 3.85 mg ai/kg body weight/day, equivalent to 121.3 ppm in the diet.

Fraction ID	% TRR [a]	ppm	Characterization/Identification
ACN/water	95.5	0.101	Partitioned with n-hexane
hexane	1.3	0.001	Not further analyzed.
ACN/water	94.2	0.100	Analyzed by 2D-TLC [b]: Metabolite 1L 32.5% TRR 0.034 ppm Metabolite 3L 42.5% TRR 0.045 ppm Metabolite 5L 1.6% TRR 0.002 ppm Metabolite 1aN [c] 4.1% TRR 0.004 ppm 3 unknown regions accounting for 0.5-4.3% of the TRR, totaling 6.3% TRR. Presence of metabolites was confirmed by HPLC.
PES	4.5	0.005	Not further analyzed.

[a] %TRR were corrected for recoveries.

[b] Several of the metabolites (1L, 3L, and 1N) were shown to degrade to one or two unknowns during TLC analysis. TLC regions attributable to a specific degradate were assigned to the respective metabolite.

[c] Metabolite 1N was shown to consist of two glucuronide conjugates present at a ration of 2:1, 1aN (2.7% TRR) and 1bN (1.4% TRR).

Table 2.2.2. Extraction, Characterization, and Identification of ^{14}C-Residues in **Fat** from goats dosed orally for 4 days with [^{14}C-phenyl]-S-metolachlor at 3.85 mg ai/kg body weight/day, equivalent to 121.3 ppm in the diet.

Fraction ID	% TRR [a]	ppm	Characterization/Identification
ACN/acidic ACN/MeOH	90.4	0.048	Partitioned with n-hexane
hexane	6.0	0.003	Not further analyzed.
ACN/water	84.4	0.045	Analyzed by 1D-TLC and/or 2D-TLC: CGA 77102 8.9% TRR 0.005 ppm CGA 46129 3.5% TRR 0.002 ppm Metabolite 1L 3.5% TRR 0.002 ppm Metabolite 3L 9.7% TRR 0.005 ppm Metabolite 5L 1.3% TRR <0.001 ppm Metabolite 6L 1.1% TRR <0.001 ppm Metabolite 1N [b] 17.5% TRR 0.009 ppm Radioactivity remaining at the origin accounted for 1% of the TRR, and a diffuse region (L12) accounted for 20.1% of the TRR. Presence of metabolites was confirmed by HPLC analysis.
PES	9.6	0.005	Not further analyzed.

[a] %TRR were corrected for recoveries.

[b] Metabolite 1N was shown to consist of two glucuronide conjugates present at a ratio of 2:1, 1aN (11.7% TRR) and 1bN (5.8% TRR).

Table 2.2.3. Extraction, Characterization, and Identification of ^{14}C-Residues in **Muscle** from goats dosed orally for 4 days with [^{14}C-phenyl]-*S*-metolachlor at 3.85 mg ai/kg body weight/day, equivalent to 121.3 ppm in the diet.

Fraction ID	% TRR [a]	ppm	Characterization/Identification
ACN/water extracts	81.2	0.099	Partitioned with n-hexane
hexane	1.7	0.002	Not further analyzed.
ACN/water	79.5	0.097	Purified on a C_{18} column washed with water (pH 3 and 7) and eluted with ACN, THF, and MeOH
water	2.4	0.002	Not further analyzed.
ACN/THF/MeOH	77.1	0.094	Further purified by HPLC.
20-81min Rt	64.4	0.079	Analyzed by 2D-TLC: Metabolite 1L 7.3% TRR 0.009 ppm Metabolite 3L 10.2% TRR 0.012 ppm Metabolite 1N [b] 17.1% TRR 0.021 ppm Radioactivity remaining at the origin accounted for 0.5% TRR; a diffuse region (L12) accounted for 7.4% TRR; and unresolved radioactivity accounted for 22.0% TRR.
other	12.7	0.015	Not further analyzed.
MeOH extract	3.2	0.004	Not further analyzed.
PES	15.6	0.019	Solids were sequentially microwave extracted in PrOH:water at 140° C and then at 190° C.
PrOH:H_2O at 140° C	1.9	0.002	Purified by C_{18} SPE and analyzed by 2D-TLC. Five unknown fractions were isolated with each accounting for <0.5% TRR. Unresolved radioactivity accounted for 0.6% TRR.
PrOH:H_2O at 190° C	12.1	0.015	Partitioned with EtOAc, resulting in EtOAc, PrOH/water, and precipitate fractions.
EtOAc	9.8	0.012	Partitioned with petroleum benzene (PB) and MeOH.
PB	4.4	0.005	Not further analyzed.
MeOH	5.4	0.007	Fractions were pooled and purified using a C_{18} SPE column rinsed with water and eluted with MeOH.
PrOH/water	2.1	0.003	
MeOH	7.0	0.009	Purified by silica gel SPE eluted with a step gradient of EtOAc to MeOH.
EtOAc/MeOH	5.8	0.007	Analysis by 2D-TLC detected on one region of radioactivity remaining at the origin.
MeOH	1.2	0.001	Not further analyzed.
Aqueous	0.5	<0.001	Not further analyzed.
precipitate	0.2	<0.001	Not further analyzed.
Residual solids	1.6	0.002	Not further analyzed.

[a] %TRR were corrected for recoveries.

[b] Includes degrade attributable to Metabolite 1N; 1N was shown to consist of two glucuronide conjugates present at a ratio of 2:1, 1aN (11.4% TRR) and 1bN (5.7% TRR).

Table 2.2.4. Extraction, Characterization, and Identification of ^{14}C-Residues in **Liver** from goats dosed orally for 4 days with [^{14}C-phenyl]-S-metolachlor at 3.85 mg ai/kg body weight/day, equivalent to 121.3 ppm in the diet.

Fraction ID	% TRR [a]	ppm	Characterization/Identification
ACN/ACN:water/MeOH/ MeOH:water	69.1	2.463	Concentrated, redissolved in THF:water and analyzed by 2D-TLC [b]: Metabolite 6L 3.6% TRR 0.128 ppm Metabolite 5L 4.7% TRR 0.168 ppm Metabolite 1L 12.9% TRR 0.460 ppm Metabolite 3L 26.3% TRR 0.937 ppm Metabolite 4L 5.9% TRR 0.210 ppm Metabolite 1N [c] 1.1% TRR 0.039 ppm Unknown L11 1.1% TRR 0.039 ppm Unknown L12 4.3% TRR 0.153 ppm Radioactivity remaining at the origin accounted for 1.0% TRR and unresolved radioactivity accounted for 8.2% TRR. Presence of metabolites was confirmed by HPLC. Fraction was used for the isolation and identification of metabolites by LC/MS.
PES	30.9	1.101	Solids were microwave extracted in PrOH:water at 190° C.
PrOH:H_2O at 190° C	26.3	0.937	Purified using C_{18} column and a step gradient of MeOH:water to MeOH.
MeOH	25.5	0.909	Analyzed by 2D-TLC: Origin 1.1% TRR 0.039 ppm Fraction 2 0.3% TRR 0.011 ppm Fraction 3 3.4% TRR 0.121 ppm Fraction 4 13.9% TRR 0.495 ppm Unresolved 6.8% TRR 0.242 ppm Fraction was also analyzed by 1D-TLC using several solvent systems and by HPLC with a C_{18} and a diol column. However, no metabolite pattern was resolved.
TLC eluate	21.8	0.777	Analyzed by HPLC with at C_{18} column. No metabolite profile was resolved.
MeOH/water	0.8	0.029	Not further analyzed.
Residual solids	4.6	0.164	Not further analyzed.

[a] %TRR were corrected for recoveries.

[b] Several of the metabolites (1L, 3L, and 1N) were shown to degrade to one or two unknowns during TLC analysis. TLC regions attributable to a specific degradate were assigned to the respective metabolite.

[c] Metabolite 1N was shown to consist of two glucuronide conjugates present at a ratio of 2:1, 1aN (0.7% TRR) and 1bN (0.4% TRR).

Table 2.2.5. Extraction, Characterization, and Identification of ^{14}C-Residues in **Kidney** from goats dosed orally for 4 days with [^{14}C-phenyl]-*S*-metolachlor at 3.85 mg ai/kg body weight/day, equivalent to 121.3 ppm in the diet.

Fraction ID	% TRR [a]	ppm	Characterization/Identification
ACN/ACN:water/MeOH	90.2	3.392	Concentrated and analyzed by 2D-TLC [b]: CGA 46129 4.8% TRR 0.181 ppm Metabolite 6L 10.8% TRR 0.406 ppm Metabolite 1L 6.6% TRR 0.248 ppm Metabolite 3L 10.8% TRR 0.406 ppm Metabolite 1N [c] 33.5% TRR 1.260 ppm Unknown L11 1.4% TRR 0.053 ppm Unknown L12 3.6% TRR 0.135 ppm Radioactivity remaining at the origin accounted for 3.0% TRR and unresolved radioactivity accounted for 15.8% TRR. Presence of metabolites was confirmed by HPLC. Fraction was used for the isolation and identification of metabolites by LC/MS.
PES	9.8	0.369	Solids were sequentially microwave extracted in PrOH:water at 140° C and then at 190° C.
PrOH:H_2O at 140° C	1.3	0.048	Purified by C_{18} SPE and analyzed by 2D-TLC. Five unknown fractions were isolated with each accounting for ≤0.4% TRR, along with Metabolite 2N (0.2% TRR). Fraction was also purified using a C18 SPE eluted with MeOH.
MeOH	1.3	0.048	HPLC analysis separated fraction into Metabolite 2N (=6L, 0.6% TRR), an unknown (0.6% TRR) and the remaining unresolved radioactivity (0.1% TRR).
PrOH:H_2O at 190° C	7.3	0.275	Purified using a C_{18} SPE eluted with water and MeOH.
water	0.1	0.004	Not further analyzed.
MeOH	7.2	0.271	Concentrated and extracted with EtOAc.
EtOAc	6.2	0.233	Analyzed by 2D-TLC: Fraction 1 0.1% TRR 0.004 ppm Fraction 2 0.2% TRR 0.008 ppm Fraction 3 0.8% TRR 0.030 ppm Fraction 4 2.2% TRR 0.083 ppm Unresolved 2.8% TRR 0.105 ppm Fraction was also analyzed by HPLC using C_{18}, diol, and aspartamide columns. However, no metabolite pattern was resolved.
Precipitate	1.0	0.038	Not further analyzed.
Residual solids	1.2	0.045	Not further analyzed.

[a] %TRR were corrected for recoveries.

[b] Several of the metabolites (1L, 3L, and 1N) were shown to degrade to one or two unknowns during TLC analysis. TLC regions attributable to a specific degradate were assigned to the respective metabolite.

[c] Metabolite 1N was shown to consist of two glucuronide conjugates present at a ratio of 2:1, 1aN (22.3% TRR) and 1bN (11.2% TRR).

Table 2.3. Summary of Characterization and Identification of ^{14}C-Residues in goats following oral dosing of [^{14}C-phenyl]S-metolachlor for 4 days at 3.85 mg/kg body weight/day, equivalent to 121.3 ppm in the diet.

Metabolite or Fraction	Milk (0.106 ppm)		Muscle (0.122 ppm)		Fat (0.053 ppm)		Liver (3.564 ppm)		Kidney (3.761 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
CGA 77102	ND	--	ND	--	8.9	0.005	ND	--	ND	--
CGA 46129	ND	--	ND	--	3.5	0.002	Trace	--	4.8	0.181
Metabolite 1L	32.5	0.034	7.3	0009	3.5	0.002	12.9	0.460	6.6	0.248
Metabolite 3L	42.5	0.045	10.2	0.012	9.7	0.005	26.3	0.937	10.8	0.406
Metabolite 4L	ND	--	ND	--	ND	--	5.9	0.210	ND	--
Metabolite 5L	1.6	0.002	ND	--	1.3	<0.001	4.7	0.168	ND	--
Metabolite 6L	ND	--	ND	--	1.1	<0.001	3.6	0.128	11.4	0.429
Metabolite 1aN [a]	2.7	0.003	11.4	0.014	11.7	0.006	0.7	0.025	22.3	0.839
Metabolite 1bN [a]	1.4	0.001	5.7	0.007	5.8	0.003	0.4	0.014	11.2	0.421
Total Identified (TI)	80.7	0.085	34.6	0.042	45.5	0.024	54.5	1.942	67.1	2.524
Unknown L12	4.0	0.004	7.4	0.009	20.1	0.011	4.3	0.153	3.6	0.135
Minor unknowns [b]	1.9	0.002	7.6	0.009	0.8	<0.001	20.8	0.741	8.3	0.312
Unresolved TLC radioactivity [c]	7.7	0.008	35.3 [d]	0.043	18.1	0.010	15.0	0.535	18.7	0.703
Unanalyzed solvent fractions	1.3	0.001	13.4	0.016	6.0	0.003	0.8	0.029	0.1	0.004
Precipitates [e]	NA	--	0.2	<0.001	NA	--	NA	--	1.0	0.038
Total Characterized (TC)	14.9	0.015	63.9	0.078	45.0	0.024	40.9	1.458	31.7	1.192
Total Bound (TB)	4.5	0.005	1.6	0.002	9.6	0.005	4.6	0.164	1.2	0.045
% Mass Balance	100.1		100.1		100.1		100.0		100.0	

[a] Glucuronide metabolites present in a ratio of 2:1 (1aN:1bN).
[b] Comprised of minor unknown TLC regions or HPLC peaks each accounting ≤3.0% TRR, with the exception of one TLC region (13.9% TRR) from the analysis of the liver microwave extract. However, repeated analyses TLC and HPLC analyses of the liver microwave extract failed to produce a distinct metabolite profile.
[c] Radioactivity from TLC analyses that was not associated with a specific region.
[d] Composed primarily of 22% of the TRR that was unresolved in the analysis of the main solvent fraction (64.4% TRR), and of the 12.7% of the TRR from an HPLC purification step that was not further analyzed.
[e] Includes radioactivity that precipitated out with solids during work-up of an extract fraction.

ND = not detected; NA = not applicable
TC = Sum of all unidentified, extractable residues
% Mass Balance = TI (%TRR) + TC (%TRR) + TB (%TRR)

Figure 1. Proposed Metabolic Fate of S-Metolachlor in Goats.

F: Faeces
FE: Fat
L: Liver
M: Milk
MU: Muscle
N: Kidney
U: Urine

CGA 77102 (F, FE)

Glutathione Pathway

Oxidative Reactions

Metabolite 1aN* (FE, L, M, MU, N, U)
Metabolite 1aNGl (F)

Metabolite 1bN* (FE, L, M, MU, N, U)

CGA 46129 (F, FE, N, U)

Metabolite 4L (L)

Metabolite 3L (FE, L, M, MU, N, U)

Metabolite 1L (FE, L, M, MU, N, U)
Metabolite 6L/2N* (FE, L, N)

Metabolite 5L* (FE, L, M)

*conjugated with glucuronic acid

Table 2.4. Metabolites of S-Metolachlor Identified in Poultry Tissues and Eggs.

Metabolite Identifier	Chemical Name	Structure	Comments
S-Metolachlor (CGA 77102)	(S)-2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-acetamide	N, O, O, Cl	Found only in fat
CGA 46129	2-[(2-ethyl-6-methyl-phenyl)-(2-hydroxy-acetyl)-amino]-propionic acid	O, N, OH, CH_3, O, OH	found in fat and kidney
Metabolite 1L	2-amino-3-{[(2-ethyl-6-methyl-phenyl)-(2-hydroxy-1-methyl-ethyl)-carbamoyl]-methylsulfanyl}-propionic acid	OH, NH_2, N, S, OH, O, O, CH_3	found in milk and all tissues
Metabolite 3L	2-amino-3-{[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methylsulfanyl}-propionic acid	O, CH_3, NH_2, N, S, OH, O, O, CH_3	found in milk and all tissues
Metabolite 4L	2-amino-3-{[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methylsulfanyl}-propionylamino)-acetic acid	O, CH_3, NH_2, H, N, S, N, O, O, O, OH, CH_3	found only in liver

41

Table 2.4. Continued.

Metabolite Identifier	Chemical Name	Structure	Comments
Metabolite 5L	2-amino-3-{[(2-ethyl-6-hydroxymethyl-phenyl)-(2-hydroxy-1-methyl-ethyl)-carbamoyl]-methylsulfanyl}-propionic acid	OH, NH_2, N, S, OH, O, O, OH	found in milk, fat, and liver
Metabolite 6L (= Metabolite 2N)	β-glucuronic acid conjugate of metabolite 1L	O-gluc, NH_2, N, S, OH, O, O, CH_3	found in fat, liver, and kidney
Metabolite 1aN	Glucuronic acid conjugate of 2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-hydroxy-1-methyl-ethyl)-acetamide	N, O-Gluc, O, Cl	Found in milk and all tissues, also found in poultry (Metabolite 1aEX)
Metabolite 1bN	Glucuronic acid conjugate of 2-[chloroacetyl-(2-ethyl-6-methyl-phenyl)-amino]-propionic acid	O, N, O-Gluc, O, Cl	Found in milk and all tissues, also found in poultry (Metabolite 1bEX)

3. Discussion

3.1. Methods

Two lactating goats were dosed orally once a day via gelatin capsules containing [^{14}C-phenyl]S-metolachlor at 3.85 mg ai/kg body weight/day for 4 consecutive days. Based on feed consumption, this dose level was equivalent to 121.3 ppm in the diet. Milk and excreta were collected daily from each goat and tissue samples were collected within 6 hours of administering the final dose. Radioactivity in collected samples was determined in triplicate either directly by LSC or by LSC following combustion or treatment with a tissue solubilizer.

Radioactivity was adequately extracted from milk and tissue samples. The initial solvent extractions released 84.4-95.5% of the TRR from milk, muscle, fat, and kidneys, and 69.1% of the TRR from liver. Subsequent microwave extractions released an additional 8.6% TRR from kidneys, 14.0% TRR from muscle, and 26.3% TRR from liver. Radioactivity remaining in the final residual solids accounted for 1.2-9.6% of the TRR. Extracted ^{14}C-residues were generally fractionated into polar and non-polar fractions and were then analyzed by 2D-TLC for metabolite quantitation, and the presence of metabolites was confirmed by HPLC. Specific metabolites isolated from liver and kidney were used for positive identification by LC/MS.

Identified components accounted for 80.7% of the TRR in milk and 34.6-67.1% of the TRR in tissues. With only a few exceptions, isolated unknowns, unresolved radioactivity, and unanalyzed solvent fractions each accounted for either <10% of the TRR or contained <0.01 ppm of radioactivity. Unknown fractions containing >10% of the TRR and >0.01 ppm were characterized as consisting of multiple components. The mass balance for the analysis of milk and tissues was 100.0-101.1%, but was corrected for method recoveries.

To demonstrate the stability of ^{14}C-residues in frozen milk and tissues over the course of the study, the analytical laboratory extracted stored samples of milk and liver after 14 days and again after 8.6 months of frozen storage and analyzed the solvent extracts by 2D-TLC. The 14-day extracts were also stored at -18° C and were reanalyzed with the extracts obtained after 8.6 months of frozen storage. Example chromatograms for each analysis were presented along with data quantifying the %TRR associated with each TLC region. Extractability of the ^{14}C-residues remained unchanged in milk (99.8 and 109.3% TRR) and liver (36.2 and 38.0% TRR) during storage, and the metabolite profile was qualitatively similar at each storage interval. Some quantitative changes were noted over time in the % TRR associated with certain TLC zones. However, these changes were minor and did not adversely affect the integrity of the study. No additional storage stability data are require to support this ruminant metabolism study.

The methods used to extract, fractionate, and identify ^{14}C-residues in milk and tissues of goats were adequate.

3.2. Results

Following four days of dosing with [^{14}C]*S*-metolachlor at a level equivalent to 121.3 ppm in the diet, 99.96% of the dosed radioactivity was recovered from the two goats. The majority of the dosed radioactivity was eliminated in the urine (77.84% dose, including cage wash) and feces (11.13% dose), and 0.07% of the dose was contained in the milk. Radioactivity remaining in the tissues at sacrifice accounted for 0.67% of the dose. At sacrifice, concentrations of radioactivity were highest in the bile (15.54 ppm), kidneys (3.76 ppm), and liver (3.56 ppm), and concentrations were 0.122-0.125 ppm in muscle and 0.051-0.056 ppm in fat. Radioactivity in milk plateaued at 0.117 ppm on Day 2 (pooled).

Solvent extraction and microwave extraction released 90.4-98.8% of the TRR from milk and tissue samples, and 2D-TLC and HPLC analyses identified a total of 9 metabolites, including parent. Identified components accounted for 80.7% of the TRR in milk and 34.6-67.1% of the TRR in tissues.

Parent compound was detected only in fat at 8.9% of the TRR. The major metabolites were 1L, 3L, 1aN, and 1bN, which were detected in milk and all tissues. In milk, the major ^{14}C-residues were 1L (32.5% TRR) and 3L (42.5% TRR), along with minor amounts (≤2.7% TRR) of 5L, 1aN, and 1bN. In muscle, the major ^{14}C-residues were 1L (7.3% TRR), 3L (10.2% TRR), and 1aN (11.4% TRR), along with minor amounts of 1bN (5.7% TRR). In fat, the major ^{14}C-residues were parent (8.9% TRR), 3L (9.7% TRR), and 1aN (11.7% TRR), along with minor amounts (≤5.8% TRR) of Metabolites CGA 46129, 1L, 5L, and 1bN. In liver, the major ^{14}C-residues were 1L (12.9% TRR) and 3L (26.3% TRR), along with minor amounts (≤5.9% TRR) of 4L, 5L, 6L, 1aN, and 1bN. In kidneys, the major ^{14}C-residues were 3L (10.8% TRR), 6L (11.4% TRR), 1aN (22.3% TRR), and 1bN (11.2% TRR), along with minor amounts of CGA 46129 (4.8% TRR) and 1L (6.6% TRR).

Considering the high levels of radioactivity excreted in the urine, *S*-metolachlor was readily absorbed by the goats. The metabolite profile indicates that the metabolism of *S*-metolachlor in goats proceeds via glutathione conjugation and/or oxidation reactions. Substitution of the chlorine atom with glutathione and subsequent enzymatic cleavage of the glutathione moiety yields metabolites 4L and 3L. O-Demethylation of the Metabolite 3L yields Metabolite 1L, which is conjugated with glucuronic acid to form Metabolite 6L or is oxidize at the methyl-phenyl group to form the minor Metabolite 5L. Direct O-demethylation of the parent compound and oxidation of the primary alcohol to the carboxylic acid along with glucuronic acid conjugation forms the Metabolites 1aN and 1bN, which were also identified in poultry.

The submitted goat metabolism study is adequate.

4. Deficiencies

The only deficiency noted in this study was that several unknown fractions (>10% TRR and >0.01 ppm) isolated from tissues could have been more completely characterized. These fractions were generally characterized as consisting of multiple components with indistinct metabolite profiles, but no enzymatic or chemical hydrolyses were performed on these fractions. However, considering the level of identification achieved in milk and tissues, this deficiency will not

adversely affect the adequacy of the goat metabolism study. No additional data are required for this goat metabolism study.

5. References

None

6. Document Tracking

PC Code: 108800
DP Barcode(s): D278742

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.

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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: August 15, 2003

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

Alan Nielsen, Branch Senior Scientist Al Nielsen 8/15/03
Reregistration Branch 2
Health Effects Division, 7509C

DP Barcode: D278742

Citation: 45499608 Muller, T. (1997) Metabolism of [Phenyl-(U)-^{14}C] CGA 77102 after Multiple Oral Administration to Laying Hens: Lab Project Number: 3/97: 565-96. Unpublished study prepared by Syngenta Crop Protection, Inc. 239 p. {OPPTS 860.1300}

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

Executive Summary

A group of five laying hens were dosed orally via gelatin capsules with [Phenyl-U-^{14}C] *S*-metolachlor for 4 consecutive days at 7.2 mg ai/kg body weight/day, equivalent to 100.3 ppm in the diet. Excreta and eggs were collected daily and tissue samples were collected at sacrifice, which occurred within 6 hours of administering the final dose. A total of 86.8% of the dosed radioactivity was recovered, with 81.5% of the dose being recovered in the excreta. Less than 0.03% of the dose was contained in the eggs. At sacrifice concentrations of radioactivity were highest in the bile (110.5 ppm), blood (8.3 ppm), liver (4.3 ppm), and kidney (4.5 ppm). Concentrations in meat, fat, and skin with attached fat were similar (0.294-0.389 ppm). Radioactivity in egg whites plateaued at 0.082 ppm by Day 3, but radioactivity in egg yolks continued to increase throughout the dosing period to 0.288 ppm by Day 4.

Solvent extraction and microwave extraction released 76.4-91.0% of the total radioactive residues (TRR) from egg and tissue samples, and HPLC analysis was used to identify individual components. LC/MS analysis metabolites isolated from excreta were used to confirm metabolite identities. Only 2.3-10.4% of the TRR was identified in eggs and tissues. Parent compound was detected only in egg whites (1.4% TRR), egg yolks (1.8% TRR), and skin/fat (5.6% TRR), and the O-demethylated metabolite, 7EX, was detected in liver (0.2% TRR) and skin/fat (0.6% TRR). Trace amounts of the dicarboxylic acid metabolite 2EX (0.3-2.3% TRR) and the dihydroxy

metabolite 3EX (0.9-3.9% TRR) were detected in all tissues, and the other dihydroxy metabolite 9EX was detected at 0.2% of the TRR only in egg yolks and muscle. Glucuronic acid conjugated metabolites (1aEX, 1bEX, and 5EX) were detected at low levels in eggs (0.5-1.9% TRR), liver (2.4% TRR), and skin/fat (0.5% TRR). In all tissues and excreta, the majority of radioactivity (66.8-79.0% TRR) was characterized as consisting of numerous (+20) minor metabolites, each of which accounted for ≤8.3% of the TRR. The majority of these metabolites were more polar than the parent compound.

S-Metolachlor is readily absorbed and extensively metabolized by poultry. The study author proposes that metabolism of *S*-metolachlor in poultry proceeds via O-demethylation to produce the primary metabolite 7EX. This metabolite is then either conjugated with glucuronic acid or undergoes further oxidation to produce dihydroxy metabolites (e.g. 3EX) or carboxylic acid metabolites that may also be conjugated with glucuronic acid (1bEX and 5EX). Further oxidation and loss of the chloro-acetamide moiety yields Metabolites 2EX and 9EX.

The submitted poultry metabolism study is adequate.

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

Active Ingredient

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-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-acetamide
CAS Number: 87392-12-9
Company Name: CGA 77102
Other Synonyms: none
Purity of Non-labeled Material: 99.8% (88.4% s-enantiomer)
Location of Isotopic Label: Uniformly ^{14}C-labeled in the phenyl ring
Radiochemical Purity: >96% (as determined by HPLC and TLC)
Specific Activity: 2.04 MBq/mg (55.1 μCi/mg). For dosing the test substance was isotopically diluted with non-radiolabeled *S*-metolachlor to a final specific activity of 15.1 μCi/mg (33420 dpm/μg)

1.2. Test Animals and Site

Species/breed: *Gallus gallus*, Leghorn White
Age: 23 weeks
Gender: Females
Number: 5 hens per dose group
Test location: Ciba-Geigy Ltd., Agricultural Research Center, St. Abin, CH
Housing: Hens were kept in individual metabolism cages suitable for the separate collection of eggs and excreta.
Diet and Water: water and feed (UFA No. 526, Syndicat Agricole, CH-1564) were provided *ad libitum*. Feed intake was measured daily.
Acclimation period: 8 days
Environmental conditions: The test area was maintained at 19-23 C with a relative humidity of 21-56% and a light cycle of 16 hours of light and 8 hours of dark
Predosing: Animals were not predosed.
Study Dates: The in-life phase of the study was conducted from 4/14/96 to 4/24/96, with dosing occurring from 4/21/96 to 4/24/96. The analytical phase of the study was conducted from 4/26/96 to 4/22/97.

1.3. Dosing

Type(s) of Dosing: Oral dosing via capsule
Dosing Vehicle: Gelatin capsules containing D-(+)-lactose
Dosing Rate: Average dose level was 7.2 ± 0.3 mg ai/kg body weight/day, which is equivalent to 100.3 ± 6.1 ppm in the diet based upon the daily feed consumption
Number of Doses: once a day in the morning
Duration of Dosing: 4 days

1.4. Sample Collection Procedures

Eggs were collected twice a day from each hen, prior to and throughout the dosing period, and excreta was collected daily. Animals were sacrificed by exsanguination within 6 hours of receiving the final dose on Day 4, and the following samples were collected from each hen: blood, muscle (breast and thigh), peritoneal fat, skin with attached fat, liver, kidney, G.I. tract (intestines and gizzard) with contents, and bile. A cage wash (ethanol:water, 1:1) was also collected prior to dosing and at the end of the study, and cage debris was collected at the end of the study. All samples were refrigerated (1-4 days) and shipped the day after sacrifice to the analytical laboratory (Animal Metabolism Laboratories, Basel, CH). At the analytical laboratory, eggs were separated into whites and yolks samples, and tissue samples were chopped. Samples were frozen in liquid nitrogen, homogenized, and placed in storage at -18° C for up to 4.5 months prior to analysis.

Table 1.3.1. Summary of Storage Conditions

Matrix	Storage Temperature (°C)	Duration (months)
Excreta	-18	1
Egg white	-18	3
Egg yolk	-18	3
Liver	-18	1
Lean meat	-18	4.5
Fat/skin	-18	4.5

To demonstrate the stability of ^{14}C-residues during frozen storage, the analytical laboratory extracted stored samples of egg whites and liver after 1, 5, and 11 months of frozen storage and analyzed the solvent extracts by 2D-TLC.

1.4. Analytical Methods

Total Radioactive Residues (TRR). For determination of TRR, homogenized samples were radioassayed in triplicate either directly by liquid scintillation counting (egg white, egg yolks, and cage wash), by combustion/LSC (excreta, gizzard contents, and blood), or treatment with a tissue solubilizer and LSC (all tissues). The limit of detection (LOD) for the radioassays ranged from 0.0002 ppm in egg whites to 0.0014 ppm in liver; the limit of quantitation (LOQ) ranged from 0.0006 ppm in egg whites to 0.0042 ppm in liver.

For extraction and analysis of ^{14}C-residues, several samples were pooled. Proportional aliquots from each animal of the following samples were separately pooled: excreta from each day; skin with attached fat and peritoneal fat; breast and thigh muscle; 24-78 hour egg whites; and 24-78 hour egg yolks. The registrant presented flow charts for each analysis, including the %TRR for each fraction. Although the reported percentages were corrected for procedural recoveries, the actual recoveries through each step were typically reported and were reasonable.

Excreta. The pooled excreta sample was extracted sequentially with acetonitrile (ACN) and ACN:water (4:1, v/v). The extracts were combined, concentrated and analyzed by HPLC.

Egg whites. Pooled egg whites were extracted sequentially with ACN, ACN:water (4:1, v/v), and methanol (MeOH), centrifuging and collecting the supernatant after each extraction. The MeOH extract and post-extraction solids (PES) were not further analyzed. The ACN fractions were combined, concentrated, and centrifuged to remove a precipitate, and were then partitioned with n-hexane. The resulting hexane (nonpolar) and aqueous (polar) fractions were concentrated and analyzed by reverse-phase HPLC.

Egg yolks. Pooled egg yolks were extracted sequentially with ACN, ACN:water (4:1, v/v), and MeOH, centrifuging and collecting the supernatant after each extraction. The extracts were combined, concentrated, and partitioned with n-hexane. The resulting polar fraction was concentrated and analyzed by reverse-phase HPLC. The nonpolar fraction was further purified using a silica gel SPE cartridge eluted with methanol, hexane/toluene/MeOH, hexane/toluene, and toluene/MeOH/water. The last two eluates were not further analyzed. The hexane/toluene/MeOH fraction was repurified on a silica gel SPE eluted with MeOH, hexane, and toluene/MeOH/water. The resulting MeOH fraction was combined with the MeOH fraction from the initial purification, and the resulting fraction was concentrated and analyzed by HPLC.

Radioactivity remaining in the yolk PES fraction was microwave extracted in 2-propanol (PrOH):water (4:1, v/v) for 10 minutes at 140° C and the remaining solids were then re-extracted at 190° C. The remaining solids were not further analyzed. The 140° C extract was mixed with ACN and centrifuged, and the resulting ACN fraction analyzed by HPLC. The 190° C extract was also mixed with ACN and centrifuged. The resulting pellet was re-extracted with ACN, and this fraction was combined with the initial ACN fraction from the 190° C extraction and analyzed by HPLC.

Muscle. Muscle was homogenized with ACN (1:1), loaded into a glass column and eluted sequentially with ACN:water (4:1, v/v) and MeOH. The solvent extracts were combined,

concentrated, and centrifuged, yielding a solvent fraction and a pellet. The supernatant was partitioned with hexane, and the pellet extracted with 0.01 M ammonium formate buffer (AFB) at pH 3. The AFB extract was combined with the aqueous phase from the partitioning of the supernatant, and the combined aqueous fraction and the hexane fraction were analyzed by HPLC.

Radioactivity remaining in the muscle PES fraction was microwave extracted in PrOH:water (4:1, v/v) for 10 minutes at 140° C and then the remaining solids were re-extracted at 190° C. Both microwave extract fractions were separately diluted with ACN and centrifuged, resulting in pellets and extraction fractions. The ACN fractions were analyzed by HPLC.

Skin/fat. The pooled skin/fat sample was extracted sequentially with hexane (3x), tetrahydrofuran (THF), MeOH, MeOH/THF (1:1), and MeOH/THF/AFB (5:4:1). The hexane extracts were combined into one fraction (HF-1) and the other solvent extracts were pooled into another fraction (HF-2). The hexane fraction was purified using a silica gel SPE column eluted sequentially with hexane, toluene, toluene/MeOH, and MeOH. The two methanolic eluates were combined, concentrated, and extracted with ACN. This ACN fraction was analyzed by HPLC both before and after base hydrolysis with 1 N or 0.1 N KOH at 70° C for 16 hours. The extract fraction HF-2 was concentrated, diluted with AFB and analyzed by HLPC.

Radioactivity in fat PES was microwave extracted in PrOH:water (4:1, v/v) for 10 minutes at 140° C and the remaining solids were then re-extracted at 190° C. The remaining solids were not further analyzed. The 140° C and 190° C extracts were concentrated and separately analyzed by HPLC.

Liver. Pooled liver was extracted sequentially with ACN, ACN:water (4:1, v/v), and MeOH, centrifuging and collecting the supernatant after each extraction. The ACN extracts were combined, concentrated, and partitioned with n-hexane. The resulting polar fraction was concentrated and analyzed by reverse-phase HPLC. The MeOH extract was also concentrated and partitioned with hexane. The resulting MeOH/water fraction was purified using a C_{18} SPE cartridge eluted with MeOH and AFB. The MeOH fraction was analyzed by HPLC, but the AFB fraction was not further analyzed.

Radioactivity remaining in the liver PES fraction was microwave extracted in PrOH:water (4:1, v/v) for 10 minutes at 140° C and the remaining solids were then re-extracted at 190° C. The remaining solids were not further analyzed. The 140° C extract was analyzed by HPLC. The 190° C extract was purified using a C_{18} SPE eluted with MeOH and water. The MeOH eluate was analyzed by HPLC, and the water eluate was not further analyzed.

Identification of ^{14}C-residues. ^{14}C-Residues in tissues, eggs, and excreta were separated and quantified by reverse-phase HPLC. The HPLC system used a C_{18} column with a mobile phase gradient of 0.01 M ammonium formate buffer (pH 3) to MeOH, and had a UV detector (245 nm) and an in-line radioactivity monitor.

The excreta extract was used for identification of specific ^{14}C-residues. The extract was separated into major fractions by flash chromatography (C_{18}) and semi-preparative HPLC. Isolated fractions were than analyzed by LC/MS for conformation of metabolite identities.

2. Results

Table 2.1. Total Radioactive Residues in excreta and tissues from hens following oral dosing of [^{14}C-phenyl]*S*-metolachlor for 4 days at 7.2 mg/kg body weight/day, equivalent to 100.3 ppm in the diet.

Matrix	Sampling Interval (hours)	% Dose	TRR (ppm)
Egg whites	0-24	0.001 ± 0.001	0.019 ± 0.010
	24-48	0.005 ± 0.001	0.062 ± 0.017
	48-72	0.006± 0.002	0.082 ± 0.029
	72-78	0.006 ± 0.001	0.073 ± 0.010
	Total	0.019 ± 0.004	0.059 ± 0.013
Egg yolks	0-24	0.000	0.002 ± 0.001
	24-48	0.001	0.035 ± 0.015
	48-72	0.005 ± 0.001	0.146 ± 0.038
	72-78	0.009 ± 0.002	0.288 ± 0.074
	Total	0.015 ± 0.003	0.117 ± 0.030
Lean Meat	78	0.218 ± 0.032	0.294 ± 0.042
Skin w/ fat	78	0.099 ± 0.019	0.389 ± 0.095
Peritoneal fat	78	0.028 ± 0.021	0.310 ± 0.138
Pooled skin/fat	78	NA	0.383 ± 0.054
Kidney	78	0.115 ± 0.023	4.488 ± 0.778
Liver	78	0.333 ± 0.048	4.267 ± 0.762
Blood	78	0.640 ± 0.190	8.326 ± 1.055
Bile	78	0.257 ± 0.112	110.5 ± 27.0
Gizzard w/ contents	78	2.81 ± 1.30	NA
Excreta	0-78	81.47 ± 3.85	NA
Cage wash	0-78	0.88	NA
Cage debris	0-78	0.13	NA
Total Recovery		86.78 ± 3.37	NA

Table 2.2.1. Extraction, Characterization, and Identification of ^{14}C-Residues in **Egg Whites** from hens dosed orally for 4 days with [^{14}C-phenyl]-*S*-metolachlor at 7.2 mg ai/kg body weight/day, equivalent to 100.3 ppm in the diet.

Fraction ID	% TRR	ppm	Characterization/Identification
ACN/water	77.0	0.045	Partitioned with n-hexane
hexane	12.0	0.007	Analyzed by HPLC: CGA 77102 1.3% TRR <0.001 ppm Metabolite 4EX 0.4% TRR <0.001 ppm Metabolite 8EX 1.3% TRR <0.001 ppm Metabolite 1EX 1.1% TRR <0.001 ppm 19 minor unknown components totaling 7.9% TRR, with each component accounting for ≤1.2% TRR.
ACN/water	65.0	0.038	Analyzed by HPLC: CGA 77102 0.1% TRR <0.001 ppm Metabolite 2EX 1.9% TRR 0.001 ppm Metabolite 4EX 3.4% TRR 0.002 ppm Metabolite 1EX 0.8% TRR <0.001 ppm 25 minor unknown components totaling 58.9% TRR, with each component accounting for ≤5.8% TRR.
MeOH	0.9	<0.001	Not further analyzed.
PES	22.1	0.013	Not further analyzed.

Table 2.2.2. Extraction, Characterization, and Identification of ^{14}C-Residues in **Egg Yolks** from hens dosed orally for 4 days with [^{14}C-phenyl]-*S*-metolachlor at 7.2 mg ai/kg body weight/day, equivalent to 100.3 ppm in the diet.

Fraction ID	% TRR	ppm	Characterization/Identification
ACN/water/MeOH	35.9	0.042	Partitioned with n-hexane
ACN/water/MeOH	15.3	0.018	Analyzed by HPLC: Metabolite 2EX 0.3% TRR <0.001 ppm Metabolite 4EX 0.9% TRR 0.001 ppm Metabolite 8EX 1.5% TRR 0.002 ppm Metabolite 1EX 0.5% TRR <0.001 ppm 22 minor unknown components totaling 13.6% TRR, with each component accounting for ≤2.0% TRR.
hexane	20.6	0.024	Purified using silica gel SPE eluted with several solvents
MeOH	9.0	0.011	Combined with MeOH fraction below, and the combined fraction (19.2% TRR) was analyzed by HPLC: CGA 77102 1.8% TRR 0.002 ppm Metabolite 8EX 0.6% TRR <0.001 ppm 21 minor unknown components totaling 16.8% TRR, with each component accounting for ≤2.8% TRR.
hexane/toluene/MeOH	10.4	0.012	Further purified using silica gel SPE eluted with several solvents
MeOH	10.2	0.012	Combined with above MeOH fraction
other eluates	0.2	<0.001	Not further analyzed.
hexane/toluene	0.3	<0.001	Not further analyzed.
toluene/MeOH/water	0.9	<0.001	Not further analyzed.
PES	64.1	0.075	Solids were sequentially microwave extracted in PrOH:water at 140° C and then 190° C.
PrOH:H_2O at 140° C	12.1	0.014	Extracted with ACN and centrifuged.
ACN	8.5	0.010	Analyzed by HPLC: Metabolite 9EX 0.2% TRR <0.001 ppm 22 minor unknown components totaling 8.3% TRR, with each component accounting for ≤1.2% TRR.
precipitate	3.6	0.004	Not further analyzed.
PrOH:H_2O at 190° C	30.6	0.036	Centrifuged and both the supernatant and pellet were extracted with ACN. The ACN extracts were then combined.
ACN	26.2	0.031	HPLC analysis isolated 22 unknown fractions totaling 26.2% TRR, with each component accounting for ≤2.9% TRR.
precipitate	4.4	0.005	Not further analyzed.
Residual solids	21.4	0.025	Not further analyzed.

Table 2.2.3. Extraction, Characterization, and Identification of ^{14}C-Residues in **Muscle** from hens dosed orally for 4 days with [^{14}C-phenyl]-*S*-metolachlor at 7.2 mg ai/kg body weight/day, equivalent to 100.3 ppm in the diet.

Fraction ID	% TRR	ppm	Characterization/Identification
ACN/water/MeOH	35.3	0.104	Concentrated, centrifuged and partitioned with hexane
hexane	15.5	0.046	Concentrated and analyzed by HPLC: Metabolite 4EX 0.5% TRR <0.001 ppm Metabolite 3EX 0.4% TRR <0.001 ppm Metabolite 9EX 0.2% TRR <0.001 ppm 26 minor unknown components totaling 14.4% TRR, with each component accounting for ≤2.4% TRR.
Aqueous	14.2	0.042	Combined with AFB fraction below. Combined fraction (18.5% TRR) was analyzed by HPLC: Metabolite 2EX 1.0% TRR 0.003 ppm Metabolite 4EX 0.2% TRR <0.001 ppm 25 minor unknown components totaling 17.3% TRR, with each component accounting for ≤2.5% TRR.
pellet	NR	--	Partitioned with n-hexane
AFB	4.3	0.013	Combined with aqueous fraction above.
solids	0.8	0.002	Not further analyzed.
PES	64.7	0.190	Solids were sequentially microwave extracted in PrOH:water at 140° C and then 190° C.
PrOH:H_2O at 140° C	11.8	0.035	Extracted with ACN and centrifuged.
ACN	10.4	0.031	HPLC analysis isolated numerous minor unknowns (26) each accounting for ≤1.1% TRR
pellet	1.4	0.004	Not further analyzed.
PrOH:H_2O at 190° C	29.8	0.088	Extracted with ACN and centrifuged.
ACN	27.8	0.082	HPLC analysis isolated numerous minor unknowns (25) each accounting for ≤3.4% TRR
pellet	2.0	0.006	Not further analyzed.
Residual solids	23.1	0.068	Not further analyzed.

Table 2.2.4. Extraction, Characterization, and Identification of ^{14}C-Residues in **Skin/fat** from hens dosed orally for 4 days with [^{14}C-phenyl]-S-metolachlor at 7.2 mg ai/kg body weight/day, equivalent to 100.3 ppm in the diet.

Fraction ID	% TRR	ppm	Characterization/Identification
hexane	24.0	0.092	Purified using C_{18} SPE column
hexane	0.2	<0.001	Not further analyzed.
toluene/MeOH	0.1	<0.001	Not further analyzed.
MeOH	23.7	0.091	Mixed with ACN
ACN/MeOH	21.0	0.080	Analyzed by HPLC: CGA 77102 5.6% TRR 0.022 ppm 5 other minor unknown fractions were isolated totaling 15.4% TRR, with each component accounting for ≤8.3% TRR. This fraction was also base hydrolyzed with 1N or 0.1N KOH at 70° C for 16 hours, followed by HPLC analysis. Following hydrolysis, the largest fraction (8.3% TRR), which was more non-polar than parent, was broken down into numerous minor unknown fractions.
precipitate	2.7	0.010	Not further analyzed.
MeOH/THF/AFB	19.6	0.075	Concentrated and analyzed by HPLC: Metabolite 4EX 0.4% TRR 0.001 ppm 30 minor unknown components totaling 19.2% TRR, with each component accounting for ≤1.6% TRR.
PES	56.4	0.216	Solids were sequentially microwave extracted in PrOH:water at 140° C and then 190° C.
PrOH:H_2O at 140° C	13.1	0.050	Analyzed by HPLC: Metabolite 5EX 0.5% TRR 0.002 ppm 28 minor unknown components totaling 12.6% TRR, with each component accounting for ≤1.1% TRR.
PrOH:H_2O at 190° C	30.8	0.118	Analyzed by HPLC: Metabolite 2EX 0.3% TRR 0.001 ppm Metabolite 3EX 0.8% TRR 0.003 ppm Metabolite 7EX 0.6% TRR 0.002 ppm 28 minor unknown components totaling 29.1% TRR, with each component accounting for ≤2.5% TRR.
Residual solids	12.5	0.048	Not further analyzed.

Table 2.2.5. Extraction, Characterization, and Identification of ^{14}C-Residues in **Liver** from hens dosed orally for 4 days with [^{14}C-phenyl]-S-metolachlor at 7.2 mg ai/kg body weight/day, equivalent to 100.3 ppm in the diet.

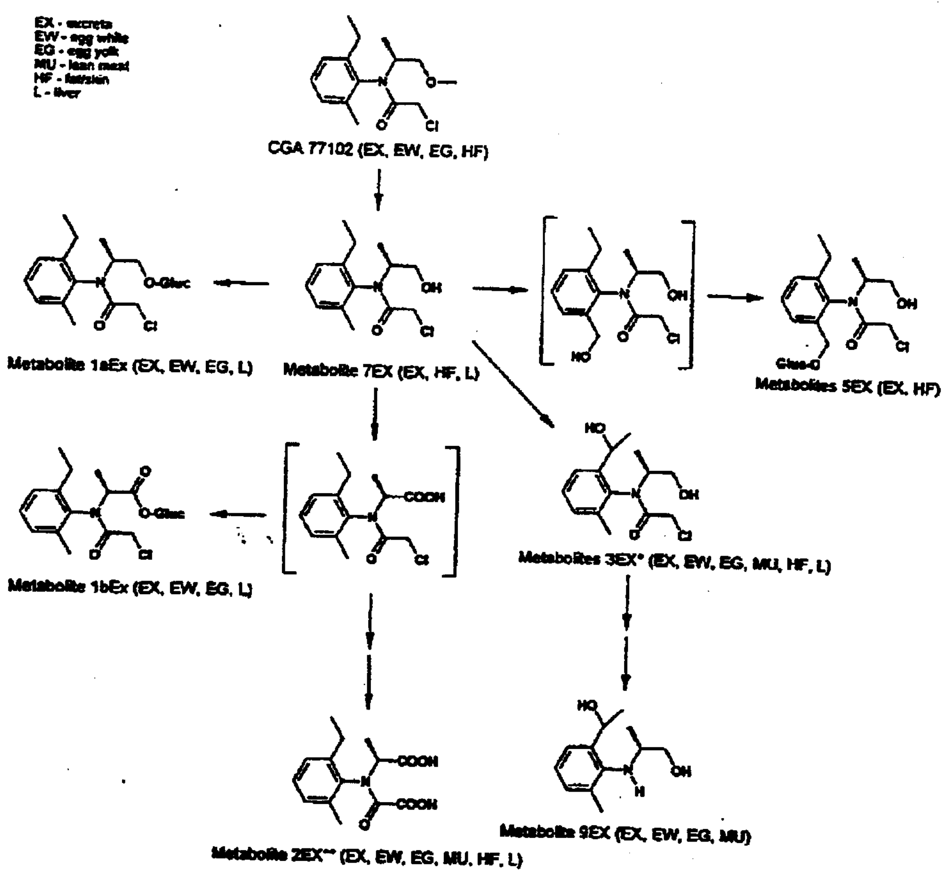
Fraction ID	% TRR	ppm	Characterization/Identification
ACN/water	41.4	1.767	Partitioned with n-hexane
hexane	1.7	0.073	Not further analyzed.
ACN/water	39.7	1.694	Concentrated and analyzed by HPLC: Metabolite 2EX 1.6% TRR 0.068 ppm Metabolite 4EX 1.0% TRR 0.040 ppm Metabolite 1EX 2.4% TRR 0.101 ppm 22 minor unknown components totaling 34.7% TRR, with each component accounting for ≤3.7% TRR.
MeOH	9.8	0.418	Partitioned with n-hexane
hexane	0.5	0.021	Not further analyzed.
MeOH/Water	9.3	0.398	Purified using a C_{18} SPE column eluted with MeOH and AFB
MeOH	9.1	0.388	Concentrated and analyzed by HPLC: Metabolite 2EX 0.5% TRR 0.020 ppm 23 minor unknown components totaling 8.6% TRR, with each component accounting for ≤1.0% TRR.
AFB	0.2	0.009	Not further analyzed.
PES	48.8	2.082	Solids were sequentially microwave extracted in PrOH:water at 140° C and then 190° C.
PrOH:H_2O at 140° C	9.3	0.397	Analyzed by HPLC: Metabolite 2EX 0.2% TRR 0.011 ppm Metabolite 4EX 0.2% TRR 0.007 ppm Metabolite 7EX 0.2% TRR 0.007 ppm 24 minor unknown components totaling 8.7% TRR, with each component accounting for ≤0.8% TRR.
PrOH:H_2O at 190° C	30.5	1.301	Purified using a C_{18} SPE Column eluted with MeOH and water.
MeOH	28.6	1.220	Analyzed by HPLC: Metabolite 3EX 1.6% TRR 0.068 ppm 25 minor unknown components totaling 27.0% TRR, with each component accounting for ≤2.7% TRR.
water	1.9	0.081	Not further analyzed.
Residual solids	9.0	0.384	Not further analyzed.

Table 2.3. Summary of Characterization and Identification of ^{14}C-Residues in hens following oral dosing of [^{14}C-phenyl]*S*-metolachlor for 4 days at 7.2 mg/kg body weight/day, equivalent to 100.3 ppm in the diet.

Metabolite or Fraction	Egg whites (0.059 ppm)		Egg yolks (0.117 ppm)		Muscle (0.294 ppm)		Liver (4.267 ppm)		Skin/fat (0.383 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
CGA 77102	1.4	0.001	1.8	0.002	ND	--	ND	--	5.6	0.022
Metabolite 1EX [a]	1.9	0.001	0.5	<0.001	ND	--	2.4	0.101	ND	--
Metabolite 2EX	1.9	0.001	0.3	<0.001	1.0	0.003	2.3	0.099	0.3	0.001
Metabolite 3EX	ND	--	ND	--	0.4	0.001	1.6	0.068	0.8	0.003
Metabolite 4EX [b]	3.8	0.002	0.9	0.001	0.7	0.002	1.2	0.047	0.4	0.001
Metabolite 5EX	ND	--	ND	--	ND	--	ND	--	0.5	0.002
Metabolite 7EX	ND	--	ND	--	ND	--	0.2	0.007	0.6	0.002
Metabolite 8EX [c]	1.3	0.001	2.1	0.002	ND	--	ND	--	ND	--
Metabolite 9EX	ND	--	0.2	<0.001	0.2	<0.001	ND	--	ND	--
Total Identified (TI)	10.3	0.006	5.8	0.007	2.3	0.007	7.7	0.322	8.2	0.031
Minor unknowns [d]	66.8	0.039	64.9	0.076	69.9	0.206	79.0	3.371	76.3	0.292
Unanalyzed solvent fractions	0.9	<0.001	1.4	0.002	NA	--	4.3	0.183	0.3	0.001
Precipitates [e]	NA	--	8.0	0.009	4.2	0.012	NA	--	2.7	0.010
Total Characterized (TC)	67.7	0.040	74.3	0.087	74.1	0.218	83.3	3.554	79.3	0.304
Total Bound (TB)	22.1	0.013	21.4	0.025	23.1	0.068	9.0	0.384	12.5	0.048
% Mass Balance	100.1		101.5		99.5		100.0		100.0	

[a] Metabolite 1EX was actually composed for two glucuronic acid metabolites, 1aEX and 1bEX, in a ratio of 2:1.
[b] Metabolite 4EX was considered to be an artifact derived from Metabolite 3EX during processing or MS analysis.
[c] Metabolite 8EX was considered to be an artifact derived from Metabolite 9EX during processing or MS analysis.
[d] Comprised of 19-30 unknown HPLC peaks or fractions that each accounted for ≤5.8% of the TRR in egg whites, ≤ 2.9% of the TRR in yolks, ≤3.4% of the TRR in muscle, ≤8.3% of the TRR in skin/fat, and ≤3.7% of the TRR in liver.
[e] Includes radioactivity that precipitated out with solids during work-up of an extract fraction.
ND = not detected
NA = not applicable
TC = Sum of all unidentified, extractable resides
% Mass Balance = TI (%TRR) + TC (%TRR) + TB (%TRR)

Figure 1. Proposed Metabolic Fate of *S*-Metolachlor in Poultry.



* hydroxylation in α or β position of the ethyl group
** identified as decarboxylated N-ethyl metabolite
[ ] proposed intermediate

Table 2.4. Metabolites of S-Metolachlor Identified in Poultry Tissues and Eggs.

Metabolite Identifier	Chemical Name	Structure	Comments
S-Metolachlor (CGA 77102)	(S)-2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-actemide	N, O, O, Cl	found in eggs and fat
Metabolite 1aEX	Glucuronic acid conjugate of 2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-hydroxy-1-methyl-ethyl)-acetamide	N, O-Gluc, O, Cl	found in eggs and liver (also found in goats as Metabolite 1aN)
Metabolite 1bEX	Glucuronic acid conjugate of 2-[chloroacetyl-(2-ethyl-6-methyl-phenyl)-amino]-propionic acid	O, N, O-Gluc, O, Cl	found in eggs and liver (also found in goats as Metabolite 1aN)
Metabolite 2EX	2-[(2-ethyl-6-methyl-phenyl)-oxalyl-amino]-propionic acid	COOH, N, COOH, O	found in eggs and liver
Metabolite 3EX	2-chloro-N-[(2-(1-hydroxy-ethyl)-6-methyl-phenyl]-N-(2-hydroxy-1-methyl-ethyl)-acetamide	HO, N, OH, O, Cl	found in eggs, muscle, liver, and fat. (includes Metabolite 4EX)

Table 2.4 Continued.

Metabolite Identifier	Chemical Name	Structure	Comments
Metabolite 4EX	2-chloro-N-(2-ethenyl-6-methyl-phenyl)-N-(2-hydroxy-1-methyl-ethyl)-acetamide	N OH O Cl	artifact derived from Metabolite 3EX during processing or MS analysis
Metabolite 5EX	Glucuronic acid conjugate of 2-chloro-N-(2-ethyl)-6-hydroxymethyl-phenyl)-N-(2-hydroxy-1-methyl-ethyl)-acetamide	N OH O Cl Gluc—O	found in fat
Metabolite 7EX	2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-hydroxy-1-methyl-ethyl)-acetamide	N OH O Cl	found in liver and fat
Metabolite 8EX	2-[(2-ethenyl-6-methyl-phenyl)-amino]-propan-1-ol	N OH H	artifact derived from Metabolite 9EX during processing or MS analysis

Table 2.4 Continued.

Metabolite Identifier	Chemical Name	Structure	Comments
Metabolite 9EX	2-[2-(1-hydroxy-ethyl)-6-methyl-phenyl-amino]-propan-1-ol	HO, N, H, OH	found in eggs and muscle. (includes Metabolite 8EX)

3. Discussion

3.1. Methods

A group of 5 laying hens were dosed orally once a day via gelatin capsules containing [^{14}C-phenyl]*S*-metolachlor at 7.2 mg ai/kg body weight/day for 4 consecutive days. Based on feed consumption, this dose level was equivalent to 100.3 ppm in the diet. Eggs and excreta were collected daily from each hen and tissue samples were collected within 6 hours of administering the final dose. Radioactivity in collected samples was determined in triplicate either directly by LSC or by LSC following combustion or treatment with a tissue solubilizer.

The initial solvent extractions released 35.3-77.0% of the TRR from egg and tissue samples, and subsequent microwave extractions released an additional 39.8-43.9% of the TRR from egg yolk and tissue samples. Radioactivity remaining in the residual solids accounted for 9.0-23.1% of the TRR, but only in the case of muscle did the residual solids contain ≥10% of the TRR and ≥0.05 ppm. Muscle residual solids accounted for 23.1% of the TRR and contained 0.068 ppm. Extracted ^{14}C-residues were generally fractionated into polar and non-polar fractions and were then analyzed by reverse-phase HPLC. Metabolite identities were confirmed by LC/MS analysis of the extract from excreta. Although only 2.3-10.4% of the TRR was identified in any egg or tissue sample, the majority of the TRR (64.9-79.0% TRR) was characterized as consisting of a large number (19-30) of minor unknown metabolites, which each accounted for <10% of the TRR. The mass balance for the analysis of eggs and tissues was 99.5-101.5%.

To demonstrate the stability of ^{14}C-residues in frozen eggs and tissues over the course of the study, the analytical laboratory extracted stored samples of egg whites and liver after 1, 5, and 11 months of frozen storage and analyzed the solvent extracts by 2D-TLC. Example chromatograms for each analysis were presented along with data quantifying the %TRR associated with each TLC zone. Extractability of the ^{14}C-residues remained unchanged in egg whites (76.6-84.4% TRR) and liver (34.7-45.6% TRR) during storage, and the metabolite profile was qualitatively similar at each storage interval. Some quantitative changes were noted over time in the % TRR associated with certain TLC zones. However, these changes were minor and did not adversely affect the integrity of the study. No additional storage stability data are require to support this poultry metabolism study.

The methods used to extract, fractionate, and identify ^{14}C-residues in eggs and tissues of laying hens were adequate.

3.2. Results

Following four days of oral dosing with [^{14}C]*S*-metolachlor at a level equivalent to 100.3 ppm in the diet, 86.8% of the dosed radioactivity was recovered from poultry. The majority of the dosed radioactivity was recovered in the excreta (81.5% dose), and less than 0.1% of the dose was contained in the eggs.

At sacrifice, concentrations of radioactivity were highest in the bile (110.5 ppm), blood (8.3 ppm), liver (4.3 ppm), and kidney (4.5 ppm). Concentrations in meat, fat, and skin with attached fat

were similar (0.294-0.389 ppm). Radioactivity in egg whites plateaued at 0.082 ppm by Day 3, but radioactivity in egg yolks continued to increase to 0.288 ppm by Day 4.

Solvent extraction and microwave extraction released 76.4-91.0% of the TRR from egg and tissue samples, and HPLC analyses identified a total of 10 metabolites, including parent. Each of these compounds accounted for ≤5.6% of the TRR in any sample. Two of the identified metabolites, 4EX and 8EX, were also considered to be artifacts that were produced during sample processing or MS analysis from Metabolites 3EX and 9EX, respectively. For purposes of this report, ^{14}C-residues of 4EX and 8EX will be summed with 3EX and 9EX, respectively. Including parent, only a total of 2.3-10.4% of the TRR was identified in eggs and tissues.

Parent compound was detected only in egg whites (1.4% TRR), egg yolks (1.8% TRR), and skin/fat (5.6% TRR), and the O-demethylated metabolite, 7EX, was detected only in liver (0.2% TRR) and skin/fat (0.6% TRR). Trace amounts of the dicarboxylic acid metabolite 2EX (0.3-2.3% TRR) and the dihydroxy metabolite 3EX (0.9-3.9% TRR) were detected in all tissues, and the other dihydroxy metabolite 9EX was detected at 0.2% of the TRR only in egg yolks and muscle. Glucuronic acid conjugated metabolites (1aEX, 1bEX, and 5EX) were detected at low levels in eggs (0.5-1.9% TRR), liver (2.4% TRR), and skin/fat (0.5% TRR).

In all tissues and excreta, the majority of radioactivity (66.8-79.0% TRR) was characterized as consisting for numerous (+20) minor metabolites, each of which accounted for ≤8.3% of the TRR. The majority of these metabolites were more polar than the parent compound.

Considering the high levels of radioactivity present in blood and bile at the time of sacrifice and the complexity of the metabolite profile both in excreta and tissues, S-metolachlor is readily absorbed and extensively metabolized by poultry. The study author proposes that metabolism of S-metolachlor in poultry proceeds via O-demethylation to produce the primary metabolite 7EX. This metabolite is then either conjugated with glucuronic acid or undergoes further oxidation to produce dihydroxy metabolites (e.g. 3EX) or carboxylic acid metabolites that may also be conjugated with glucuronic acid (1bEX and 5EX). Further oxidation and loss of the chloro-acetamide moiety yields Metabolites 2EX and 9EX.

The submitted poultry metabolism study is adequate.

4. Deficiencies

The only deficiency noted in this poultry metabolism study was that ^{14}C-residues in the residual solids of muscle (23.1% TRR, 0.068 ppm) were not extracted down to levels specified in the Agency's guidelines (860.1300). However, considering the level of radioactivity remaining in the muscle solids, and the complexity of the metabolite profile in all egg and tissue samples, this deficiency will not adversely affect the adequacy of the poultry metabolism study. No additional data are required for this metabolism study.

5. References

None

F. Document Tracking

PC Code: 108800
DP Barcode(s): D278742

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.

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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: August 15, 2003

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

Alan Nielsen, Branch Senior Scientist *Al Nielsen 8/15/03*
Reregistration Branch 2
Health Effects Division, 7509C

DP Barcode: D283235

Citation: 45672302 Sandmeier, P. (2001) Outdoor Confined Accumulation Study on Rotational Crops after Bareground Application of [Phenyl-U-^{14}C] CGA77102: Lab Project Number: 99PSA53; 1287-99. Unpublished study prepared by Syngenta Crop Protection, Inc. 252 p.

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.

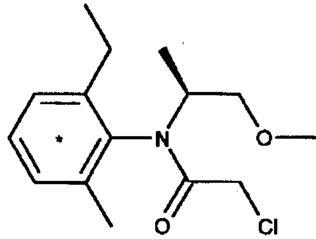
GLP Compliance

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)

80

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.

(continued; footnotes follow)

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 364-Day PBI (0.014 ppm) [a]			
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	<0.00 1	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	<0.00 1	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	N, O, O, Cl	lettuce soil
NOA 443156	3-{[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methylsulfanyl}-2-hydroxy-propionic acid	O, O, N, S, OH, O, OH	lettuce wheat
NOA 443819	3-{[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfinyl}-2-hydroxy-propionic acid	O, O, N, S, OH, O, O, OH	lettuce radish wheat
Metabolite WH-7		O, O, N, O, S, O, O, HO, OH	radish wheat

(continued)

88

Table 2.5. Continued.

Metabolite Identifier	Chemical Name	Structure	Crop/matrix
NOA 413173	2-[(2-ethyl-6-methyl-phenyl)-sulfoacetyl-amino]-propionic acid	O, OH, N, O, S, O, O, OH	wheat soil
CGA 368208	(2-ethyl-6-methyl-phenylcarbamoyl)-methanesulfonic acid	H N, O, S, O, O, OH	soil
CGA 380168	[(2-ethyl-6-methyl-phenyl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfonic acid	O, N, O, S, O, O, OH	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	O, N, S, NH_2, O, O, OH	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	O, OH, N, OH, O	soil
CGA 49750	4-(2-ethyl-6-methyl-phenyl)-5-methyl-morpholine-2,3-dione	O, N, O, O	radish
CGA 217498	N-(2-ethyl-6-methyl-phenyl)-2-methanesulfonyl-N-(2-methoxy-1-methyl-ethyl)-acetamide	O, N, S, O, O, O	wheat soil
CGA 133275 Metabolite I_{28}	N-(2-ethyl-6-methyl-phenyl)-N-(2-hydroxy-1-methyl-ethyl)-2-methanesulfonyl-acetamide	OH, N, S, O, O, O	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 ($\leq$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 $\leq$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.

CGA 46129 SO

Oxidative Pathways

CGA 77102 (CGA 24705)

Glutathione Pathway

CGA 380168 (CGA 354743)
RA, WH, SO

CGA 368208
SO

CGA 351916 (CGA 51202)
LE, WH, SO

Glutathione-conjugate

NOA 413173
WH, SO

CGA 49750 RA

CGA 46576
LE, RA

Malonyl-cysteinyl-conjugate (= I_{17})
RA

sugar conjugation (LE), RA, WH
(WH-9, WH-10)

R = H: CGA 133275 (= I_{20}) RA, WH
R = Me: CGA 217498 WH, SO

R = H, H NOA 443819 (CGA 118243)
LE, RA, WH
R = O: WH-7
WH

NOA 436611
LE, WH, SO

R = H: I_{12} WH

LE: Lettuce WH: Wheat
RA: Radish SO: Soil

[34] 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

98