Metabolism of Imazethapyr (AC 263499) Herbicide in Corn

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Imazethapyr [5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid] is the active ingredient of imidazolinone PURSUIT herbicide. A confined plot field experiment was conducted to determine the metabolism of ^{14}C-6-pyridine-labeled imazethapyr in imidazolinone-tolerant corn after a single postemergence treatment at a rate of 0.25 lb ae/A, which is equivalent to 4 times the recommended use rate. The total radioactive residue in the corn plant was 15.35 ppm at 0 h after treatment and declined very rapidly to 0.28 ppm by 15 days. The residue in mature stalk/cob and seed at harvest was 0.08 and 0.02 ppm, respectively. The transport of radioactive residues to seed was minimal. Imazethapyr is metabolized very rapidly in corn. The major metabolic pathway for imazethapyr in corn was oxidative hydroxylation at the α-carbon atom of the ethyl substituent on the pyridine ring to yield α-(hydroxyethyl)-imazethapyr. Further glucosidation of the hydroxyl group was a very minor pathway to the terminal residue in corn. Only a trace amount of radioactive residue was identified as unchanged parent compound in corn. Since this study was conducted at 4 times the use rate, any one of the residue components in corn from application at normal use rate would be insignificant or negligible.

INTRODUCTION

Imazethapyr [5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid] (Figure 1) is the active ingredient of PURSUIT herbicide that is being developed for use in corn, soybeans, peanuts, beans, peas, alfalfa, and other leguminous crops by the American Cyanamid Co. (Los et al., 1984). Imazethapyr is a broad-spectrum imidazolinone that has been shown to have excellent activity against annual and perennial grass and broad-leaved weeds when applied either preplant incorporated or pre- or postemergence (Malefyt et al., 1984; Peoples et al., 1985) by inhibiting acetohydroxy acid synthase, the feedback enzyme in the biosynthesis of the branched-chain amino acids (Shaner et al., 1984, 1985; Anderson and Hibberd, 1985). Generally, the imidazolinone herbicidal selectivity between weed species and crops is attributable mainly to the differential metabolic rates or in some cases to the absorption rate at different growth stages rather than differential sensitivity of the target site (Shaner and Robson, 1985; Brown et al., 1987; Shaner and Mallipudi, 1991). In general, tolerant plant species are capable of metabolizing imidazolinone herbicides at a substantially faster rate than the susceptible weeds and crops. Metabolic reactions of imidazolinones in plants are similar to those of other herbicides, namely aliphatic hydroxylation and glucosidation. The focus of this paper will be on the level of imazethapyr degradation products detected in corn and their identification. Experiments were conducted under simulated field conditions with imidazolinone-tolerant corn.

MATERIALS AND METHODS

Isotopes and Chemicals. ^{14}C-6-Pyridine-labeled (Bullock, 1983a) and ^{13}C-6-pyridine-labeled (Bullock, 1983b) imazethapyr were obtained from the Agricultural Research Division, American Cyanamid Co., Princeton, NJ. The position of the ^{14}C label in the pyridine ring at the 6-carbon atom adjacent to the nitrogen atom is considered metabolically stable to allow determination of the corn plant metabolic profile (Figure 1). ^{14}C-Labeled imazethapyr had a specific activity of 21.2 μCi/mg and a radiochemical purity of 99.5% as determined by two-dimensional thin-layer chromatography and autoradiography. For identification of metabolites of imazethapyr, both ^{14}C- and ^{13}C-labeled imazethapyr were used. Experiments were performed by using a mixture of 1 part of ^{13}C-labeled imazethapyr and 1 part of nonradiolabeled imazethapyr containing sufficient ^{14}C-radiolabeled imazethapyr to allow ready detection and measurement of imazethapyr and its metabolites by conventional radiotracer techniques. By virtue of the ^{12}C/^{13}C ratio, the mixture provided doublet ion peaks in the mass spectra of isolated or derivatized imazethapyr and its metabolites. These doublets assist in distinguishing ions due to the metabolism products from those derived from nonradiolabeled contaminants (Mallipudi et al., 1991).

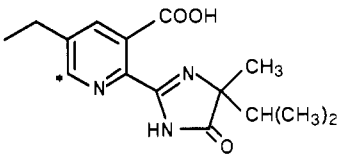

Figure 1. Chemical structure of imazethapyr (AC 263499) and positions of isotopic labels. The asterisk indicates the position of the ^{14}C- or ^{13}C-label in the pyridine ring at the 6-carbon atom adjacent to the nitrogen atom.

Samples of the following chemicals were obtained from the American Cyanamid Co. chemical library file: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(1-hydroxyethyl)-3-pyridinecarboxylic acid (metabolite 1) and nicotinic acid, 5-[1-(β-D-glucopyranosyloxy)ethyl]-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) (metabolite 2). ^{14}C-6-Pyridine-labeled metabolite 1 was also obtained from the American Cyanamid Co. (Ahmed and Bullock, 1988). The chemical purity of the ^{14}C-labeled metabolite 1 was about 95.7% by high-performance liquid chromatography (HPLC), and its radiopurity was greater than 98%. All other materials were obtained from commercial sources.

Experimental Design. Briefly, this experiment consisted of one control plot and one treated corn plot. The treatment plot was given [^{14}C]imazethapyr postemergence at a rate of 0.25 lb ae/A (pounds of acid equivalents per acre). The rate is equivalent to 4 times the recommended use rate. The control plot was treated with blank formulation only. The corn plants were harvested at various time intervals after treatment and were analyzed to determine total radioactive residues (TRR). Corn samples with radioactive residues were extracted and analyzed by HPLC to determine metabolic profile.

Test Crop and Test Plots. The corn seeds used in the field study were imidazolinone-tolerant field corn seeds which were obtained from Pioneer Hi-Bred International, Inc., Johnston, IA. The outdoor field experiment was conducted in a sandy

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loam soil at American Cyanamid Co., Princeton, NJ. The corn seeds were sown in a 5 ft × 10 ft plot at a depth of about 1.5 in. in five separate rows each spaced approximately 1 ft apart and 10 in. between plants within the row.

Dosing. The [^{14}C]imazethapyr was applied at the rate of 0.25 lb ae/A; thus, 130.16 mg of imazethapyr was required for a 50-ft^2 plot. The total amount of imazethapyr required for the study was perpared by mixing 65.88 mg of [^{14}C]imazethapyr and 65.13 mg of [^{13}C]imazethapyr to give a final specific radioactivity of 10.28 μCi/mg (22 822 dpm/μg). The radiolabeled imazethapyr preparation was formulated as the ammonium salt containing 0.25% nonionic surfactant X-77 before application. The mixture was diluted with water to a final volume of 750 mL, which was sufficient to give good coverage of the entire plot. The test substance was sprayed onto the corn crop at 4–5 leaf stage, which was at 34 days after initial seeding. Only one application was made to the crop. Application was made using a plastic pressure sprayer with an adjustable nozzle (Farnam Co. Inc., Phoenix, AZ). The environmental conditions during the treatment period were full sun, no precipitation, and a mean temperature of 65.8 °F (52.6–75.7 °F) during the day.

Plant Sampling Procedure. Corn plants were removed by cutting the plants at 1–2 in. above the soil surface. Samples were separated into stalk/cob and seeds. Husk surrounding the ears was combined with the stalk at the time of harvest. Samples taken at other growth stages were combined, *i.e.*, no separation of immature ears from plants. All plants samples were stored at approximately −20 °C until analysis.

Sample Preparation for Quantitation of Radioactivity. Green plant foliage or dry stalk/cob (harvest) was ground with dry ice using either a Hobart milling apparatus or a centrifugal grinding mill, Model ZM1 (Brinkman Instruments Co., Westbury, NY). Seeds were ground in the centrifugal grinding mill. After the carbon dioxide had dissipated, 250 (certain early stage plants)–500-mg aliquots of plant tissue were subjected to combustion analysis.

Extraction of Radioactivity from Plants. Finely ground samples (20–100 g) of green foliage, mature stalk/cob, or seed were extracted by processing with a Brinkman homogenizer (Model PT10/35) with a PTA 10 mm or PTA 20 mm generator in centrifuge bottles. The solvents were 1:1 aqueous acetone followed by reagent acetone. Between extractions the slurries were centrifuged at approximately 4000 rpm in an Accuspin FR centrifuge (Beckman Instrument Inc., Irvine, CA). The supernatant liquid was then decanted through a 600-mL Pyrex M filter-funnel. The aqueous acetone and acetone fractions were separated, reduced in volume, and, along with the postextraction solid (PES), subjected to liquid scintillation counting (LSC) and combustion analysis.

Aqueous fractions were washed with hexane or dichloromethane in a separatory funnel and then evaporated to low volume, methanol was added, and the precipitated solids were removed by centrifugation. The methanol was evaporated to produce the final solution used for HPLC analysis. Radioactivity present in precipitated solids was determined by combustion analysis, and the residues were added to the marc radioactive residues of that sample.

The radioactive residues, which were not extractable by above procedure, present in mature stalk/cob PES were extracted by the following procedure. The PES was extracted three times with 200 mL of a 2% methanol/hydrochloric acid solvent mixture. Solvent was removed by filtration, and the residue was heated at reflux for 4 h in the presence of 200 mL of 6 N aqueous sodium hydroxide solution. The reaction mixture was cooled to room temperature and centrifuged and the supernatant removed. The radioactive residues present in the methanol/HCl extract, 6 N aqueous sodium hydroxide extract, and final solid PES were analyzed either by direct counting in LSC or by combustion analysis.

Radioanalysis. Plant tissue (250–500 mg) samples were weighted into sample holders, and the samples were combusted in the presence of Combustaid in a Tri-Carb Model 306 oxidizer (Packard Instrument Co.). The counting solutions for the sample oxidizer were Oxisorb 2 absorber (9 mL) and Oxiprep 2 scintillator (12 mL) (New England Nuclear Co., Boston, MA). The level of radioactivity in the extract samples was determined by liquid scintillation cocktail (New England Nuclear) using a Beckman LSC Model LS 5801 (Beckman Instrument).

Table 1. Total Radioactive Residues (TRR) in Corn under Field Conditions after Postemergence Treatment with [^{14}C]Imazethapyr at 0.25 lb ae/A

days after treatment	TRR (ppm, imazethapyr equiv)[a] foliage or stalk/cob (95% CL)[b]	seed (95% CL)[b]
0	15.35 (±2.068)	
15	0.28 (±0.005)	
30	0.14 (±0.003)	
60	0.07 (±0.009)	
94 (harvest)	0.08 (±0.004)	0.02 (±0.002)

[a] ppm (parts per million) calcualted on a fresh weight basis. Values were rounded to the nearest 0.01 ppm. [b] 95% CL: 95% confidence limits.

High-Performance Liquid Chromatography (HPLC) Characterization of Radioactivity. Distribution of the radioactivity in the treatment solutions and aqueous extracts of foliage, stalk/cob, and seed was determined on an IBM LC/9533 liquid chromatograph fitted with a 4.5 × 250 mm IBM octadecyl column and variable-wavelength UV detector (254 nm) at a room temperature of about 25 °C. The column elution systems were linear gradients of either 1–70% methanol with 0.1–0.03% trifluoroacetic acid over a 100-min time span or a gradient of 1–70% methanol with 0.05–0.015 M H_3PO_4 (adjusted to pH 2.1) over a 100-min time span or a gradient of 25–70% methanol with 0.0375–0.015 M H_3PO_4 (adjusted to pH 2.1) over a 65-min time span. Flow rate was 1 mL/min. One-minute fractions of column eluate were collected in 7-mL scintillation vials with a Pharmacia Frac-100 fraction collector (Pharmacia Inc., Piscataway, NJ).

Gas Chromatography–Chemical Ionization Mass Spectrometry (GC–CIMS). The purified plant radioactive residue isolates were analyzed by gas chromatography–positive ion chemical ionization mass spectrometry (GC–PICIMS) on a Finnigan-MAT Model TSQ-70 GC–MS–MS–DS (Finnigan Corp., Sunnyvale, CA). The mass spectrometric parameters were as follows: source pressure, 9500 mTorr; reagent gas, methane; source temperature, 150 °C; electron multiplier voltage, 900 V; conversion dynode voltage, −5000 V; preamplifier range, 1 × 10^{-8} amp/V; ions monitored, m/z 318$^+$/319$^+$, m/z 348$^+$/349$^+$.

GC–CIMS analyses were carried out on a 5 m × 250 μm (i.d.) 0.25 μm DB-5 film, fused silica column (J&W Scientific, Folsom, CA). The following gas chromatographic operating conditions were used: injection temperature, 325 °C (split valve open at 0.5 min); column over temperature, 60 °C for 0.5 min then 30 °C/min to 225 °C, hold for 1 min; transfer line temperature, 200 °C; carrier gas, He at 4 psig head pressure; injection volume, 1 μL; 100 μL of trimethylanalinium hydroxide in methanol (TMAH; TMAH in 0.2 M methanol; Supelco, Inc., Bellefonte, PA) was added to each milliliter of isolate solution to form the methylated derivatives in the injection port, required for gas chromatography.

RESULTS AND DISCUSSION

^{14}C-Labeled Imazethapyr in the Spray Solution. The sample aliquots from the final [^{14}C]imazethapyr treatment solution were analyzed to determine radiochemical purity of imazethapyr and total radioactivity in the treatment solution. Imazethapyr in the spray solution was shown to be stable from the time of preparation to the time of application. HPLC analysis of spray solution showed one major radioactive peak which corresponded to the retention time of authentic nonradioactive imazethapyr. The radiopurity of [^{14}C]imazethapyr in the treatment solution preparation was 96.4%. The dosage of imazethapyr in the treatment was 0.25 lb ae/A, which is in agreement with the proposed dosage of 0.25 lb ae/A.

^{14}C Radioactive Residues in Corn Plants, Stalk/Cob, and Seeds. Corn plants treated with [^{14}C]-

Table 2. Results of Extraction and HPLC Analysis of the Major Extractable ^{14}C Radioactive Residue in Corn Samples[a]

			residue fractionation			HPLC analysis of aq acetone		
sample time (DAT) (A)	plant part (B)	TRR (ppm) (C)	name (D)	% (E)	ppm (F)	main ^{14}C components (G)	% of extractable (H)	concn in sample (ppm) (I)
0	plant	15.35	aq acetone	106.5	16.35	imazethapyr	87.9[b]	14.36
			$MeCl_2$			metabolite 1	2.6[b]	0.43
			PES	0.4	0.06	metabolite 2	ND[c]	ND
15	plant	0.28	aq acetone	90.6	0.25	imazethapyr	9.9[b]	0.03
			$MeCl_2$	6.3[d]	0.02	metabolite 1	55.9[b]	0.14
			PES	8.4	0.02	metabolite 2	2.5[b]	0.007
30	plant	0.14	aq acetone	93.3	0.13	imazethapyr	3.1	<0.005[e]
			$MeCl_2$	0.7	<0.005[e]	metabolite 1	63.9	0.08
			PES	7.3	0.01	metabolite 2	6.0	0.008
60	plant	0.07	aq acetone	92.0	0.06	imazethapyr	<0.01	<0.005
			$MeCl_2$	ND	ND	metabolite 1	67.0	0.04
			PES	10.9	0.008	metabolite 2	8.4	0.005
94 (harvest)	stalk/cob	0.08	aq acetone	77.7	0.06	imazethapyr	<1.0	<0.005
			$MeCl_2$	5.1	<0.005	metabolite 1	58.0	0.03
			PES	27.7[f]	0.02	metabolite 2	10.7	0.006
94 (harvest)	seed	0.02	aq acetone	93.3	0.019	imazethapyr	<1.0	<0.005
			$MeCl_2$	1.2	<0.005	metabolite 1	75.6	0.014
			PES	14.0	<0.005	metabolite 2	9.0	<0.005

[a] (A) DAT, days after treatment. (B) Stalk and cob were combined prior to analysis. (C) TRR (total radioactive residue) values from Table 1, which were rounded to the nearest 0.01 ppm. (D) $MeCl_2$, dichloromethane; PES (post extraction solid) includes precipitates obtained during fractionation. (E) Percent in the fraction was calculated by dividing actual residues in the fraction by the total residues started. (F) ppm = (E) × (C)/100 and rounded to two decimal places. (G) Residue component name. (H) Based on total recovered radioactivity in all fractions. (I) ppm = (H) × (F) for aq acetone/100 and rounded to either two or three decimal places. The remaining unaccounted for radiactivity included several minor radio-peaks and elevated baseline backgrounds. None of these minor radiopeaks exceeded 10% of the total residue. [b] Average of two replications. [c] ND, not detected. [d] Includes acetone extract of plant. [e] Sensitivity of the method is 0.005 ppm. [f] Further fractionation on only PES fraction without precipitates is shown in Table 3.

imazethapyr showed slight stunting and interveinal yellowing immediately after postemergence treatment. However, the treated plants recovered rapidly and were comparable in overall growth and vigor to untreated plants at maturity.

Radioactive residues in corn plants treated with [^{14}C]-imazethapyr are shown in Table 1. The TRR for samples shown in Table 1 represent net parts per million (ppm) values after correction for the control samples. Total radioactive residue values in the corn forage at 0, 15, 30, and 60 days after treatment (DAT) were 15.35, 0.28, 0.14, and 0.07 ppm, respectively. The TRR in the dry corn stalk/cob and seed at 94 DAT (harvest) were 0.08 and 0.02 ppm, respectively. The TRR in the corn plants declined steadily from the time of application to 60 days postemergence treatment and remained at a relatively constant level until the last plant sampling time. Generally, the significant [^{14}C]imazethapyr-related residues appeared in the vegetative portion of the early corn plants, whereas only small amounts of imazethapyr-related residues were in seeds.

Extraction of Radioactive Residues Present in Corn Plants and Seeds. Radioactive residue in the foliage, stalk/cob and seed was based on initial fresh weight and is shown in Table 2. Greater than 90% (range 90.6–106.5%) of the TRR in the green plant was extractable with aqueous acetone. In dry stalk/cob of mature corn, 77.7% of the total plant radioactivity was extractable with acetone/water. Only 1.0% or less of TRR residues was extracted with acetone following the acetone/water procedure. Unextractable radioactivity in green plant ranged from 0.4 to 10.9% in the preharvest sample to 27.7% (0.02 ppm) in dry mature stalk/cob.

The unextractable radioactivity in 94 DAT dry mature stalk/cob PES was further subjected to acid and base extraction procedure, and the results are shown in Table 3. The percents of TRR extracted into methanol/HCl extract and 6 N aqueous sodium hydroxide were 3.3% and 14.2%, respectively. Only 6.0% (0.005 ppm) of the TRR was left unextractable in the PES. The unextracted

Table 3. Distribution of ^{14}C-Labeled Imazethapyr-Derived Radioactivity from 94 DAT Stalk/Cob (Harvest) PES Sample[a]

residue fraction name	ppm imazethapyr equiv[b]	% of total sample[c]
methanol/HCl extract	<0.005	(3.3%)
6 N aq NaOH extract	0.01	(14.2%)
PES (unextractable)	0.005	(6.0%)

[a] ^{14}C residue concentration in stalk/cob PES (post extraction solid) = 0.02 ppm or 27.7% of the total residues (Table 2). [b] ppm = % of ^{14}C residues in a given fraction × the ppm of ^{14}C residues in plant samples [Table 2, (C)] and divided by 100. [c] % of total sample = actual residues in the fraction divided by the total residues started and multiplied by 100. This percentage number was multiplied with the total residues in the PES, 27.7% and divided by 100, which is the final percent of total sample.

radioactivity most likely consists of tightly bound components or incorporated natural plant constituents.

Radioactivity extractable with acetone/water in corn seeds was approximately 93.3%. Total ^{14}C residue in the unextracted fraction of the corn seed PES was 14.0% (<0.005 ppm).

Characterization of Extractable Radioactive Residues by HPLC and GC–CIMS. The extractable radioactive residue in corn (Table 2) was used to define the metabolic profile of imazethapyr. The nature of the radioactive components in each extract was initially identified by matching the HPLC profile with synthetic standard compounds imazethapyr, metabolite 1, and metabolite 2. The acetone and dichloromethane fractions contained less than 6% of the TRR in the sample; no attempts were made to further characterize these and other minor fractions from solvent-partitioning steps.

The results of sample fractionations and HPLC analyses are summarized in Table 2. HPLC radiochromatograms of the aqueous acetone extracts of corn samples are shown in Figure 2. The extracts of harvest stalk/cob and seed samples were also fortified with ^{14}C-labeled metabolite 1 for structure verification of the predominant radioactive

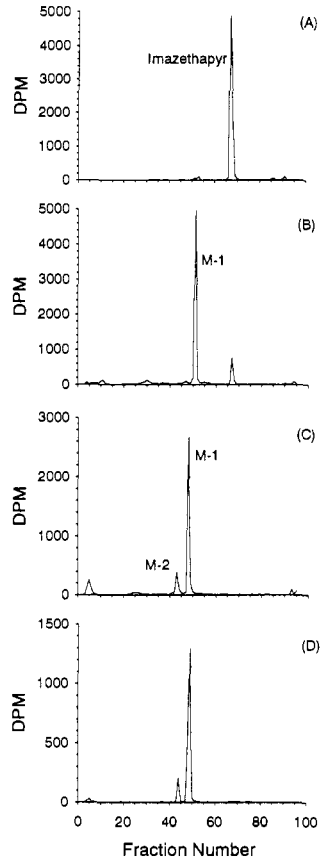


Figure 2. HPLC radiochromatogram of aqueous acetone extract of corn samples: (A) 0-h plant sample; (B) 15-day plant sample; (C) 94-day mature stalk/cob sample; (D) 94-day corn seed sample. Imazethapyr is the parent compound, whereas M-1 and M-2 represent metabolites 1 and 2, respectively. HPLC conditions: IBM C_{18} reversed-phase column, 4.5 × 250 mm; mobile phase; 1–70% methanol and 0.05–0.015 M phosphoric acid (pH 2.1), linear gradient over 100 min; flow rate; 1 mL/min. Individual 1-mL fractions were collected for liquid scintillation counting.

peak. The results of HPLC analyses showed that metabolite 1, the hydroxyethyl analog of imazethapyr, was the predominant metabolite at all time intervals. Metabolite 1 comprised about 55.9% (0.14 ppm) of the total plant foliage residue at 15 DAT and 58.0% (0.03 ppm) at harvest. Parent compound imazethapyr accounted for about 9.9% (0.03 ppm) of the total residue in early corn plant (15 DAT), declining to 3.1% (<0.005 ppm) at 30 DAT, and the compound was detected at <1.0% (<0.005 ppm) in the mature stalk/cob (94 DAT). The glucose conjugate of metabolite 1 (metabolite 2) was found in low amounts at all time intervals and constituted approximately 10.7% (0.006 ppm) of the TRR in the mature stalk/cob.

HPLC analysis of the ^{14}C radioactive residue released from PES of the 94 DAT dry stalk/cob by aqueous sodium hydroxide showed one discernible radioactive peak which has the same HPLC retention time as metabolite 2. This peak represents 24.5% of the total ^{14}C residue in the sodium hydroxide extract (or 0.002 ppm equivalent).

The metabolic profile in seed of mature corn showed that, as in plant, metabolite 1 was the predominant radiocomponent (Table 2). Extracts of corn seed showed that 75.6% (0.014 ppm) of the total residue was the hydroxylated metabolite (metabolite 1). About 9% (<0.005 ppm) of the total ^{14}C residue was identified by HPLC retention time as the glucose conjugate (metabolite 2). Approximately <1.0% (<0.005 ppm) of the radioactive residue was identified as unchanged parent compound (imazethapyr) in corn seed. Similarly, the sweet corn cell suspensions metabolized imazethapyr completely to metabolite 1 in 48 h (Robson et al., 1984).

^{14}C-Labeled residues of imazethapyr and metabolite 1 were extracted from the plant samples and were subjected to suitable cleanup involving solvent partitioning and solid-phase extraction techniques and analyzed by GC–CIMS. The GC–PICI (CH_4) analysis was performed on isolates from 15 DAT plant sample, 94 DAT (harvest) stalk/cob, and seed samples. The identities of GC peaks of imazethapyr and metabolite 1 were confirmed as dimethylated derivative of imazethapyr and trimethylated derivative of metabolite 1. The methylated derivatives were generated by methylation of compounds in the GC

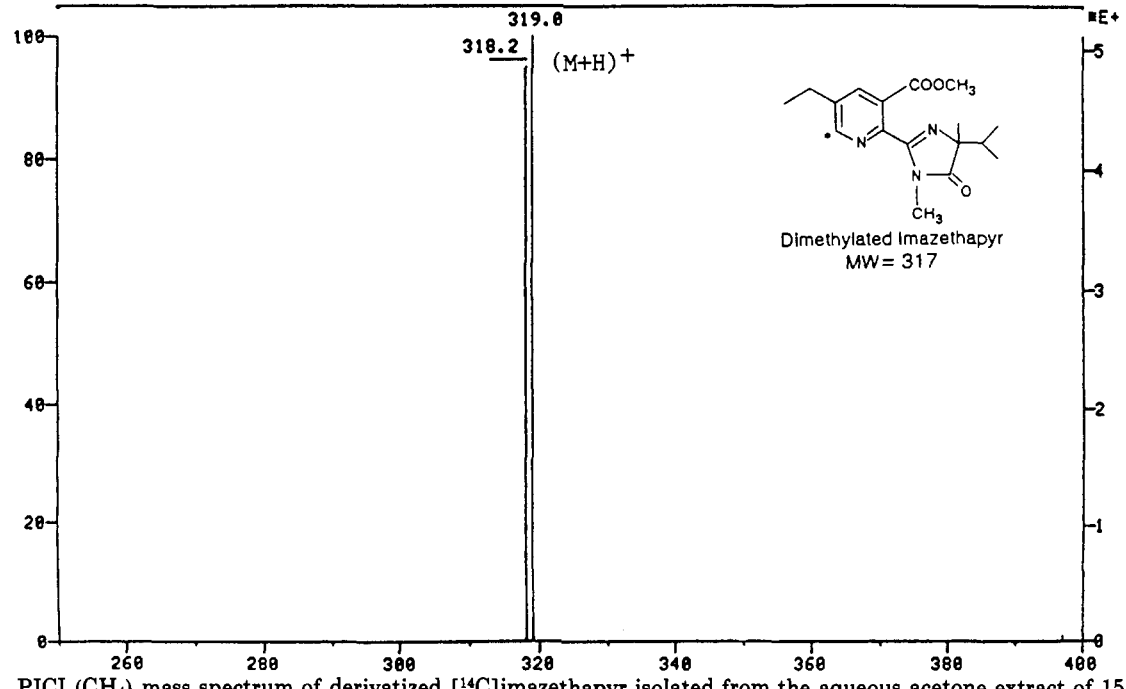


Figure 3. PICI (CH_4) mass spectrum of derivatized [^{14}C]imazethapyr isolated from the aqueous acetone extract of 15 days after treatment corn foliage.

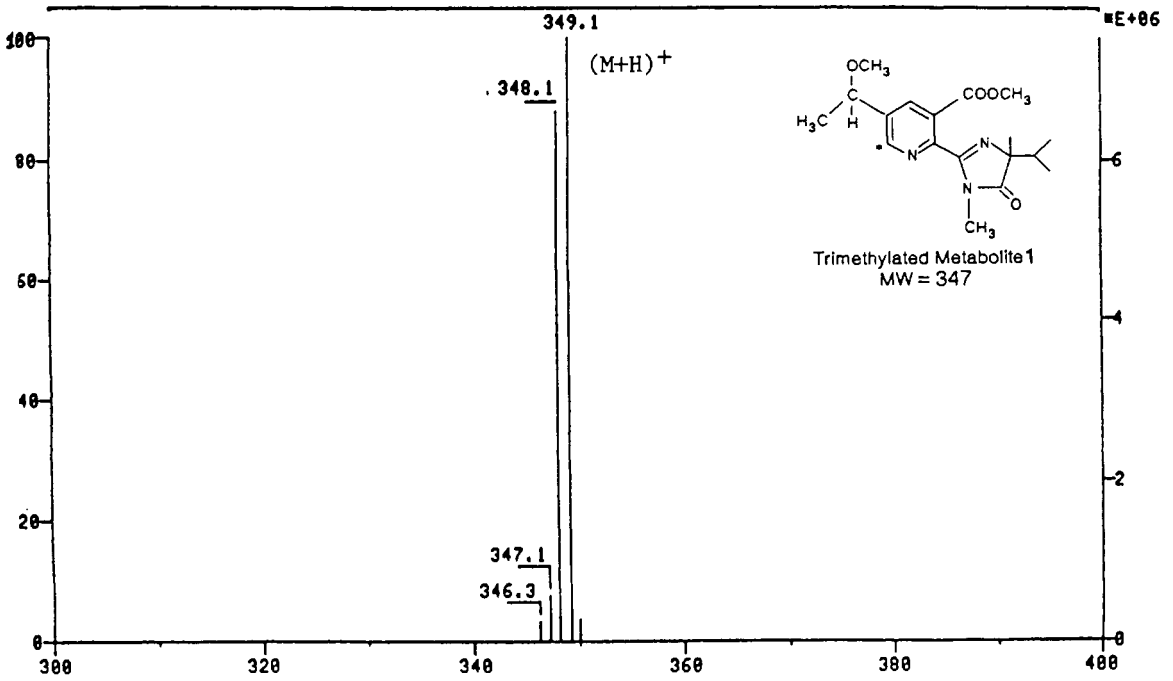
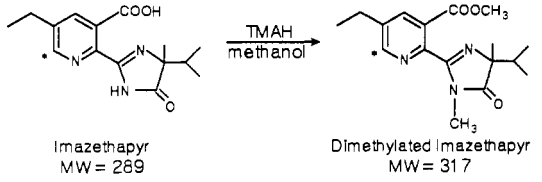


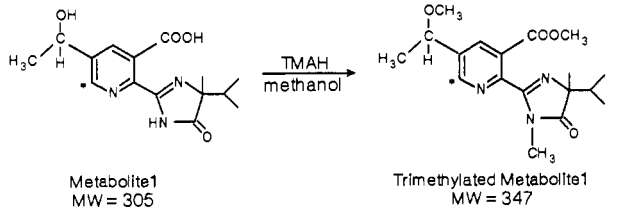
Figure 4. PICI (CH_4) mass spectrum of derivatized [^{14}C]metabolite 1 isolated from the aqueous acetone extract of 15 days after treatment corn seed.

injection port by the trimethylanilinium hydroxide methylating agent.

The GC–PICI (CH_4) showed an ion doublet for $(M + H)^+$ at m/z $318^+/319^+$ (Figure 3), which confirmed the identity of the GC peak as the dimethylated derivative of ^{13}C-labeled imazethapyr.



The GC–PICI (CH_4) showed an ion doublet for $(M + H)^+$ at m/z $348^+/349^+$ (Figure 4), which confirmed the identity of the GC peak as the trimethylated derivative of ^{13}C-labeled metabolite 1.



Conclusions. Results of this investigation showed very low TRR of imazethapyr-derived residue in the mature corn stalk/cob (0.08 ppm) and corn seed (0.02 ppm) at postemergence dose rate of 0.25 lb ae/A, which is equivalent to 4 times the recommended use rate. There was a rapid decline of TRR in the corn plant within a few days after postemergence application, and this was due to the plant growth dilution factor. Transport of plant TRR to seeds was minimal as is evident by very low residues in the seed of corn. On the basis of HPLC profiles of the aqueous acetone extracts it is apparent that the major metabolic pathway for imazethapyr is oxidative hydroxylation at the α-carbon atom of the ethyl side chain substituent on the pyridine ring to yield the α-hydroxyethyl analog of

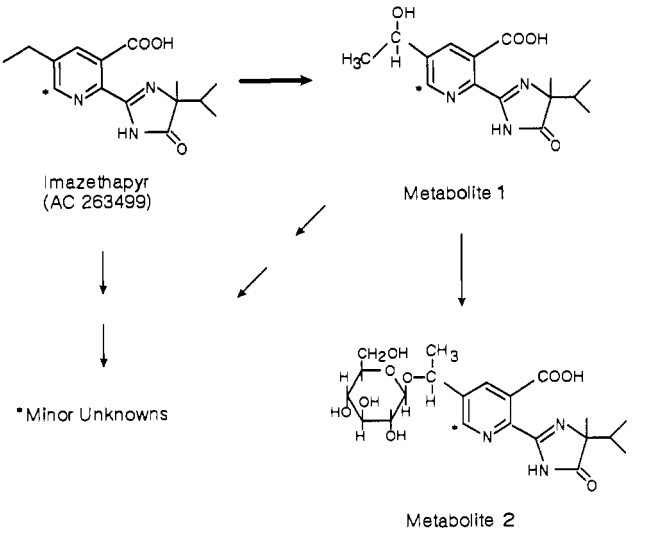


Figure 5. Suggested metabolic pathway of imazethapyr in corn. The scheme was derived from a field metabolism study using ^{14}C-6-pyridine-labeled imazethapyr.

imazethapyr (metabolite 1). Further glucosidation of the hydroxyl group of metabolite 1 was only a minor pathway to the terminal residue in corn. Since this study was conducted at 4 times the use rate, any one of the residue components in corn from application at the normal use rate would be insignificant or negligible. The metabolic profile showed very rapid metabolism of imazethapyr by the corn plant. The proposed metabolic pathway of imazethapyr in corn is shown in Figure 5.

Metabolite 1 is herbicidally active, although it is less active than the imazethapyr (Shaner and Mallipudi, 1991). Species with intermediate tolerance to imazethapyr, such as corn, appear to hydroxylate the herbicide imazethapyr but do not conjugate it. For hydroxylation alone to act as the primary detoxification mechanism, it has to be very rapid. If it occurs rapidly enough, hydoxylation of imazethapyr immobilizes the herbicide at the site of application. This reduces the phytotoxicity of imazethapyr

by limiting the amount of compound that reaches the meristematic regions of the plant.

Metabolite 1 and its glucose conjugate (metabolite 2) are not considered to be of any toxicological significance because metabolite 1 is a rat metabolite (Miller et al., 1991) and is present at 0.2% in the technical samples used in the chronic and oncogenicity rodent studies. The corn metabolites of imazethapyr are essentially metabolite 1 and traces of metabolite 2. The results of a lactating goat (Fung, 1989) and laying hen (Mallipudi, 1990) metabolism studies with ^{14}C-labeled metabolite 1 showed a rapid excretion of metabolite 1-derived residue and no detectable residues in egg, milk, and tissues. It is concluded from the residue values quantitated in these studies that there is no potential for secondary residues of imazethapyr-derived residues in meat or milk.

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