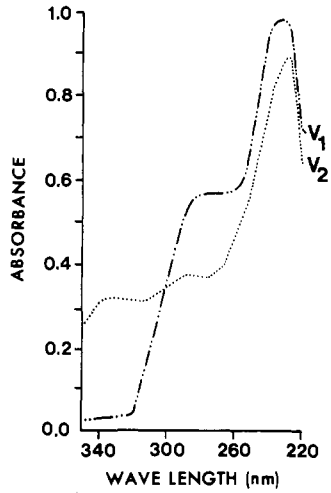

Figure 3. Ultraviolet absorption spectra of salt-extractable Commander polypeptide subfractions V_1 and V_2.

dure, appeared below the fractionation limit of the gel (Figure 2). Subfraction V_2 showed absorption at 328 nm as well as 280 nm and the ultraviolet scan showed absorption peaks at both wavelengths (Figure 3). Since 7 *M* urea did not dissociate the bonds between the chlorogenic acid and the polypeptides in subfraction V_2, covalent bonds appeared to be present. Reifer and Augustyniak (1968) have also reported the presence of covalent bonds between low molecular weight nitrogen compounds and chlorogenic acid in sunflower.

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Absorption, Translocation, and Metabolism of Metribuzin (BAY-94337) in Sugarcane

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Radioactivity from root-absorbed metribuzin, labeled with ^{14}C at position 5 in the ring, was deposited mainly in the leaves as unknown metabolites. Less than 10% of the ^{14}C could be accounted for and measured by gas chromatography as the parent herbicide and three known metabolites; acid hydrolysis increased recovery to about 10%. At 12 weeks, 84% of the radioactivity initially added to the nutrient solution was recovered—75% in the plant tissue, including abscised leaves, and 9% in the nutrient medium. Foliar treatment resulted in a distal movement within the leaves of 5-20% of the applied ^{14}C; another 10-20% remained at the treated site and 1-2% appeared proximally to the treatment area. Foliar residues averaged 25% of the amount applied, relatively independent of the length of time after surface treatment.

Metribuzin (BAY-94337), 4-amino-6-*tert*-butyl-3-(methylthio)-1,2,4-triazin-5(4*H*)-one, is a representative of a new class of *as*-triazinone herbicides (Eue *et al.*, 1969) with selective utility for sugarcane. We describe its absorption when applied to the foliage and, in nutrient culture solution, to the roots of sugarcane. The herbicide was diluted with radioactive metribuzin in order to follow translocation of the parent compound and its metabolites. Patterns of distribution and recovery of radioactive residues over a 12-week period are compared with those of the *s*-triazine herbicides atrazine and ametryne (Hilton *et al.*, 1970); with 2,4-D (Ashton, 1958); and with picloram (Hilton *et al.*, 1973). We determined by gas chromatography the known metabolites (DA, DK, and DADK) in various parts of the sugarcane plant and related their amounts to the radioactivity present in the same samples.

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MATERIALS AND METHODS

Root Application. Sugarcane cuttings of cultivar H50-7209 were established as small, uniform plants in individual containers in 3 l. of aerated Hoagland nutrient solution. To each container was added 29,630 μg of unlabeled metribuzin and 370 μg of metribuzin-^{14}C (labeled in position 5 of the ring, specific activity 1.4 mCi/mmol), resulting in herbicide concentrations of 10 μg/ml in contact with the plant roots at the start of the experiments. All plants were exposed to outdoor conditions; water and nutrient were added as needed to maintain volume.

Plants were removed after 1, 4, 8, and 12 weeks of continuous treatment and separated into green leaves, stalk, the apical meristem of the primary shoot, vegetative seedpiece, secondary basal shoots (suckers) if present, the ac-

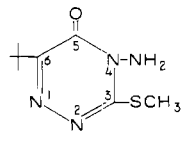
Table I. Distribution of ^{14}C-Labeled Residues from Metribuzin Introduced *via* the Roots of Sugarcane[a]

Plant section	Duration of ^{14}C-labeled herbicide treatment at no. of weeks							
	1		4		8		12	
	% of appl. act.	% of total intake	% of appl. act.	% of total intake	% of appl. act.	% of total intake	% of appl. act.	% of total intake
Leaves, green	15.4	57.8	36.6	52.7	13.0	16.6	8.5	11.4
Leaves, abscised	np	np	7.9	11.2	34.6	44.4	36.2	48.6
Meristem	0.4	1.6	0.2	0.2	ns	ns	ns	ns
Stalk	0.9	3.6	6.2	9.0	7.4	9.4	8.3	11.1
Root	8.5	31.9	15.6	22.5	20.2	25.9	19.5	26.2
Seedpiece (att.)	1.3	5.0	2.9	4.2	2.8	3.6	1.9	2.6
Secondary basal shoots	np	np	np	np	np	np	ns	ns
Nutrient culture solution	73.4		30.6		12.2		9.2	
Radioactivity unaccounted for	ns		ns		9.7		16.3	

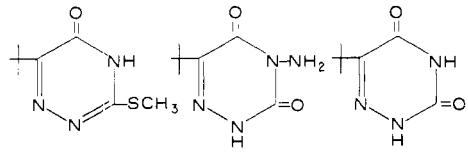
[a] np, none present; ns, no significant radioactivity.

cumulated dry abscised leaves, and roots. Each portion was coarsely chopped, dried in a desiccator over $CaCl_2$, and powdered in a Wiley mill. Subsamples were combusted in a Parr oxygen bomb. The $^{14}CO_2$ was collected and counted as $Ba^{14}CO_3$ by liquid scintillation spectrometry (Hilton *et al.*, 1972).

A portion of each sample was extracted, purified, and analyzed by gas chromatography by the general procedure of Stanley and Thornton (1972) (see also Thornton *et al.*, 1972). We adapted the procedure so that it could be applied to samples of 1 g (dry weight). Briefly, the samples were extracted under reflux with acetonitrile–water; the acetonitrile was evaporated, and the aqueous solution was extracted with chloroform. The three known metabolites, DA, DK, and DADK (more polar than metribuzin), were



METRIBUZIN



DA DK DADK

extracted from the chloroform with NaOH solution; the latter was acidified and the metabolites again extracted into chloroform. The original chloroform solution containing metribuzin and the second chloroform extract containing the metabolites were evaporated, dissolved in benzene–acetonitrile, passed through Florisil and silica gel, and gas chromatographed as separate solutions after removal of solvent and redissolving in benzene, on a 5% OV-225 column with an electron capture detector. Metribuzin and two of the three metabolites added to sugarcane fractions gave routine recoveries of 80–90%; 60–70% of the hydrolyzed diketo metabolite (DK) could be recovered. Gas chromatographic sensitivities for individual compounds varied between 0.01 and 0.001 ppm on dried plant material.

The extraction and partitioning of metribuzin and its metabolites resulted in three principal fractions that were investigated for recovery of radioactivity; the extracted fiber (residues insoluble in the acetonitrile–water extractant), the chromatographable portion soluble in chloroform containing metribuzin and the known metabolites, and the aqueous fraction from the original chloroform extraction (residues soluble in acetonitrile–water but not extracted from water by chloroform). Radioactivity in each fraction was determined by combustion and the combined total compared with the original radioactivity of the whole plant part.

Foliar applications were made by placing ethanol solutions of labeled metribuzin on 20–30-cm^2 portions near the center of blades of leaves 1, 2, and 3. The youngest expanded leaf (no. 1) at the apex received 75 μg in 50 μl; leaves 2 and 3 each received 150 μg in 100 μl. At 1, 2, 4, 8, and 12 weeks after treatment dried, ground leaf sections taken from the treated area, from a distal untreated area halfway to the leaf tip, and from a proximal untreated area halfway to the leaf sheath were combusted separately and counted as $Ba^{14}CO_3$.

RESULTS AND DISCUSSION

Root Absorption. *Radioactivity Distribution and Recovery.* Radioactivity from metribuzin entered the sugarcane plant through the roots and translocated readily through the stem into the leaves. The loss of ^{14}C from the nutrient solution took place with a half-rate of 18 days, identical with that of the *s*-triazine herbicides. In other work, we found that picloram absorbed more slowly, with 50% remaining in the nutrient after 25 days and 25% after 70 days.

Most of the metribuzin radioactivity was mobile and deposited in the green leaves (Table I). As these leaves became senescent and abscised, their residues were lost from the growing plant. The percentage of ^{14}C in the combined green and dry (abscised) leaves was remarkably constant during the 12-week period and accounted for about 60% of the total amount absorbed. During this same time the proportion of green to total leaf ^{14}C decreased from 100 to 19% as the dry leaf ^{14}C increased proportionally from 0 to 81%.

Root residues of about 25% based on the ^{14}C absorbed by the plant were constant with time. Since the roots were washed before drying and combustion, these residues appear to represent relatively immobile carbon compounds. Stalk radioactivity increased with time to 11% of the plant intake. There was no significant ^{14}C in the apical meristem of the primary stalk, nor in the secondary suckers when they appeared.

The concentration of residues, calculated from the radioactivity but including also the unlabeled portion, is shown in Figure 1 as parts per million of equivalent metribuzin on dry weight. Growth dilution and depletion of the nutrient solution supply of ^{14}C after the period of initial absorption account for the concentration maximum in the growing parts of the plant. A similar maximum occurred in the accumulated dry leaf trash at a later time. The re-

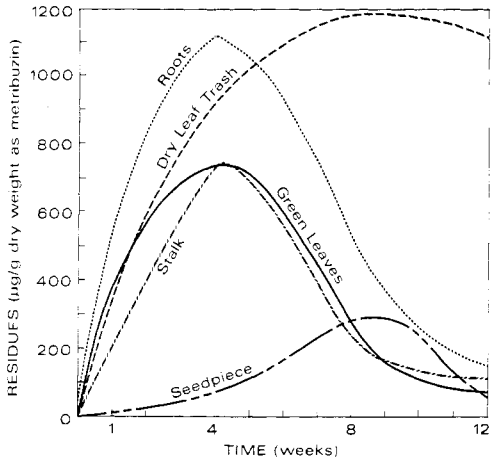


Figure 1. Concentration of residues from the absorption of metribuzin into sugarcane roots from nutrient culture solution. Residues are based on radioactivity, but are calculated to include the added unlabeled material.

markably high concentrations of residues were achieved by the large amounts of unlabeled metribuzin placed in the nutrient solution with the radioactive compound. Little visible phytotoxicity was noted from the initial root contact with the 10-ppm herbicide solutions, and recovery was rapid from an initial stunting effect.

We interpret the pattern of uptake, translocation, and deposition of mobile residues in the leaves as apoplastic transport in the water-nutrient stream. This interpretation is consistent with that from the residues from the ring-labeled *s*-triazine herbicides, although the recoveries of radioactivity differ greatly. Radioactive recovery from the metribuzin experiments was remarkable: 75% of the applied ^{14}C was recovered from the plants (including the abscised leaves) after 12 weeks, with an additional 9% remaining in the nutrient medium. Recoveries were quantitative at 1 and 4 weeks; analysis of the 8- and 12-week samples left 10 and 16% of the ^{14}C unaccounted for. While part of the loss could represent cumulative effects of plant growth dilution on the limit of ^{14}C detection, there are possible losses from aerating the nutrient solution, guttation losses from the leaves under conditions of high root pressure, leaching from leaves, and perhaps others. In addition, the losses may represent ring degradation and loss of volatile ^{14}C fragments. The substantial ^{14}C recovery must result from residues having considerable stability in the leaves and roots. Plant residues from atrazine, ametryne, and GS-14254, labeled in the *s*-triazine ring, contained 51, 62, and 16% of the initial radioactivity in about the same time period. The more stable picloram molecule, labeled at the carboxyl carbon, left 77 and 72% residue in 8 and 13 weeks' time.

Gas Chromatography of Known Metabolites. Preliminary gas chromatography of extracts from the nutrient solution and the plant tissue indicated that relatively small amounts of metribuzin were present as the parent molecule after the first week. At that time the radioactivity in the nutrient was soluble in acetonitrile, but could not be extracted from the nutrient medium into chloroform. Since metribuzin and the known metabolites were chloroform soluble, we investigated each plant fraction to determine those residues that could be identified by gas chromatography as well as soluble and insoluble ^{14}C not included in the chromatographable extracts from the analytical separation and purification. We deviated from the accepted residue method only in scaling down to 1-g samples, and in using plant material that had been dried in a desiccator, ground to a powder, and stored in a freezer. Water was added to restore the original green weight before extraction with water–acetonitrile.

The three known metabolites of metribuzin in addition to the parent compound that can be analyzed by the gas chromatograph are the products of deamination (DA), 6-*tert*-butyl-3-(methylthio)-1,2,4-triazin-5(4*H*)-one; hydrolysis (DK), 4-amino-6-*tert*-butyl-1,2,4-triazine-3,5(2,4*H*)-dione; and the combination of deamination and hydrolysis (DADK), 6-*tert*-butyl-1,2,4-triazine-3,5(2,4*H*)-dione. DA was found to be the primary product when metribuzin was irradiated with ultraviolet light (Pape and Zabik, 1972).

Sugarcane contained all three metabolites and the par-

Table II. Solvent Fractionation and Recovery of ^{14}C-Labeled Residues from Metribuzin Absorbed into Sugarcane *via* the Roots

	Recovery of ^{14}C as μg equiv of metribuzin (%)			
	1 week	4 weeks	8 weeks	12 weeks
Green leaves				
Soluble, not chromat.	1696 (27.4)	3,875 (18.5)	3,266 (12.7)	248 (1.1)
Chromatographable	91 (1.5)	123 (0.6)	62 (0.2)	501 (2.3)
Extracted fiber	2354 (38.0)	5,787 (27.7)	2,068 (8.0)	1,797 (8.3)
Dry trash leaves				
Soluble, not chromat.	np	1,634 (7.8)	5,154 (20.0)	6,898 (31.7)
Chromatographable	np	34 (0.2)	154 (0.6)	227 (1.1)
Extracted fiber	np	998 (4.8)	4,999 (19.4)	3,189 (14.6)
Stalk				
Soluble, not chromat.	63 (1.0)	322 (1.5)	1,738 (6.8)	453 (2.1)
Chromatographable	34 (0.6)	34 (0.2)	131 (0.5)	71 (0.3)
Extracted fiber	172 (2.8)	1,607 (7.7)	523 (2.0)	1,955 (9.0)
Roots				
Soluble, not chromat.	468 (7.6)	768 (3.7)	366 (1.4)	746 (3.4)
Chromatographable	210 (3.4)	115 (0.6)	44 (0.2)	83 (0.4)
Extracted fiber	945 (15.3)	4,140 (19.8)	4,708 (18.3)	5,179 (23.8)
Seedpiece				
Soluble, not chromat.	45 (0.7)	ns	523 (2.0)	11 (0.1)
Chromatographable	56 (0.9)	82 (0.4)	10 (0.04)	30 (0.1)
Extracted fiber	ns	437 (2.1)	1,803 (7.0)	541 (2.5)
Subtotals				
Soluble, not chromat.	2271 (36.7)	6,598 (31.5)	11,047 (42.9)	8,356 (38.4)
Chromatographable	391 (6.3)	387 (1.8)	402 (1.6)	912 (4.2)
Extracted fiber	3471 (56.0)	12,969 (62.0)	14,102 (54.8)	12,661 (58.1)
Total recovered[a]	6133 (99.0)	19,954 (95.4)	25,550 (99.2)	21,929 (100.6)

[a] Recovery based on original ^{14}C assay of the individual plant parts; np, none present; ns, not significant.

Table III. Residues of Metribuzin and Its Known Metabolites in the Chromatographable Portion of Sugarcane Extracts (in ppm of Dry Weight and Per Cent of Metribuzin Absorbed by the Plant)[a]

	Green leaves		Dry trash leaves		Stalk		Roots		Seedpiece	
	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%
Metribuzin										
1 week	0.56	0.43	np		1.68	0.14	16.20	1.81	2.78	0.85
4 weeks	0.44	0.14	2.64	0.04	9.41	0.12	5.98	0.35	8.05	0.28
8 weeks	<0.01	ns	0.52	0.02	0.71	0.28	0.98	0.04	0.02	0.01
12 weeks	0.03	0.01	<0.01	ns	0.02	0.01	0.04	0.02	0.05	0.02
DA										
1 week	0.22	0.17	np		4.16	0.34	3.66	0.41	0.14	0.04
4 weeks	1.03	0.32	4.79	0.07	3.16	0.04	2.73	0.16	3.16	0.11
8 weeks	0.11	0.06	4.55	0.15	0.81	0.22	2.57	0.06	0.37	0.02
12 weeks	0.19	0.08	0.31	0.13	0.10	0.04	0.35	0.14	0.15	0.06
DK										
1 week	0.16	0.12	np		0.89	0.07	10.31	1.15	<0.01	<0.01
4 weeks	0.02	0.01	4.51	0.06	0.35	<0.01	0.05	<0.01	0.01	<0.01
8 weeks	0.02	0.01	2.04	0.07	0.02	0.01	0.35	0.02	0.01	<0.01
12 weeks	0.13	0.05	0.18	0.07	0.08	0.03	0.29	0.12	0.05	0.02
DADK										
1 week	0.97	0.75	np		ns	ns	0.18	0.02	<0.01	<0.01
4 weeks	0.43	0.13	<0.01	ns	<0.01	ns	0.66	0.04	<0.01	ns
8 weeks	0.47	0.18	7.56	0.26	<0.01	ns	0.97	0.04	<0.01	ns
12 weeks	1.17	0.48	2.06	0.84	0.63	0.26	0.25	0.10	0.09	0.04

[a] np, none present; ns, not significant.

ent metribuzin, but the amounts were small (Tables II and III). Based on the ^{14}C in the total plant, this chromatographable fraction of four compounds amounted to 6.31% after 1 week of treatment. At 4 weeks 1.85% was recovered, at 8 weeks 1.56%, and at 12 weeks 4.2%. Unextracted radioactivity in the plant fiber averaged 58% after water–acetonitrile treatment. The remaining 31–43% of the ^{14}C was extractable in acetonitrile–water but not soluble in chloroform after removal of acetonitrile.

Assuming the chromatographable metabolites might be present as conjugates, we hydrolyzed the plant samples with HCl at concentrations ranging from 0.1 to 6 *N* and from 1 to 6 hr at 100°. The chromatographable fraction was increased to a maximum of 10% with some obvious degradation at the most vigorous conditions. Treatment with a crude beef liver enzyme preparation at pH values ranging from 4 to 8 increased the chromatographable fraction only slightly at pH 7–8.

Refluxing the leaf samples for 4 hr in aqueous acetic acid (20% acid by volume) increased extraction of ^{14}C to 80% in one instance and to 65% in another, indicating that a portion of the acetonitrile-insoluble residue could be extracted into acid solution. Hydrolysis with 6 *N* HCl following evaporation of the acetic acid failed to increase the chromatographable fraction, which constituted 9 and 7% of the two acid-extracted samples. Most of the dissolved ^{14}C remained in the aqueous phase during chloroform extraction.

Our conclusion from the chromatographic work carried out thus far is that most of the metabolites present in sugarcane as ^{14}C-labeled residues are unknown. The predominant water- or acid-soluble substances are highly polar; on the basis that acid hydrolysis did not increase the known metabolites to any great extent, it does not appear likely that they are present as conjugates. Authentic DADK is stable to the hydrolysis conditions. The possibility that ring cleavage could lead to labeled natural products has not been investigated. At the end of the 12-week period, 16.8% of the total radioactivity applied was left in the harvestable portion of the plant (green leaves and stalk). At least half that amount would be lost by abscission of the remaining leaves with residue. Sugarcane in Hawaii is harvested at 2 years of age.

Foliar Absorption. Metribuzin placed on sections of leaf blades of growing plants translocated distally toward the leaf tips with little apparent proximal movement toward the base (Table IV). Recoveries were variable, but about 25% of the total ^{14}C applied remained on or in the leaves, irrespective of the length of the treatment period. These data imply that weathering and other surface losses occurred in the first week, leaving an adsorbed layer—possibly an immobile metabolite or conjugate—of 10–20%, and a translocated fraction of 5–20%. The minor proximal movement of 1–2% of the ^{14}C could have been the result of diffusion or weathering contamination since the amount diminished with distance from the treatment zone, and no activity was found in the leaf sheaths at the base of the leaves.

Table IV. Distribution of ^{14}C-Labeled Residues from Metribuzin from Foliar Application[a]

	% of appl. act. at time after chemical application, weeks			
Leaf section	1	4	8	12
Top leaf				
Treated area	13.6	11.7	9.4	12.1
Untreated area	7.6	11.7	7.8	8.7
Distal	nd	6.7	6.4	7.3
Proximal	nd	5.0	1.4	1.4
Total recovery	21.2	23.4	17.2	20.8
Second leaf				
Treated area	16.5	13.4	11.7	17.3
Untreated area	11.6	9.5	20.6	12.6
Distal	nd	8.3	18.6	11.5
Proximal	nd	1.2	2.0	1.1
Total recovery	28.1	22.9	32.3	29.9
Third leaf				
Treated area	13.6	12.9	19.8	32.6
Untreated area	9.7	15.3	17.3	28.4
Distal	nd	14.3	15.8	27.0
Proximal	nd	1.0	1.5	1.4
Total recovery	23.3	28.2	37.1	61.0

[a] nd, not determined.

Leaf position affected residues. The youngest, most rapidly growing leaf lost radioactivity most readily. Lower leaves retained more residue both at the treated site and as translocated fractions. The solutions exhibited no phytotoxicity.

By 12 weeks, all treated leaves had become senescent and had abscised, eliminating all the residues from the

plant. Radioactivity did not move from treated leaves to stalk or to newer leaves emerging from the spindle.

The translocation behavior compares closely with that observed for the *s*-triazine herbicides and contrasts with picloram, which appears to move in the phloem from older to younger leaves. Movement of surface residues of metribuzin is probably that of diffusion in the extracellular spaces. Foliar residues of 2,4-D remained largely at the treated site, with small amounts apparently translocated to higher as well as lower leaves and other plant parts (Ashton, 1958).

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Metribuzin is being developed as Sencor herbicide by Chemagro, a division of Baychem Corp., Kansas City, Mo. We are indebted to that firm for the radioactive compound and samples of metabolite products. A. Maretski of this Experiment Station prepared the beef liver enzyme extract.

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Environmental Degradation of the Insect Growth Regulator Methoprene (Isopropyl (2*E*,4*E*)-11-Methoxy-3,7,11-trimethyl-2,4-dodecadienoate). I. Metabolism by Alfalfa and Rice

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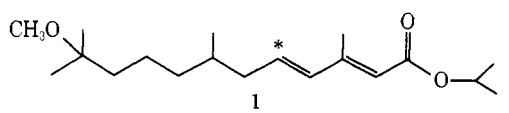
The metabolic fate of isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate, a new insect growth regulator (common name, methoprene; trademark, Altosid), was studied in alfalfa and rice as a function of time. The major metabolic pathways involved ester hydrolysis, O-demethylation, and oxidative scission of the 4-ene double bond. The principal nonpolar metabolite was 7-methoxycitronellal which was isolated from vapors evolved from the plants. Chromatographic evidence strongly suggests the incorporation of radiolabel from the extensively degraded (2*E*)-[5-^{14}C]methoprene molecule into carotenoids, chlorophylls, and other higher molecular weight plant constituents. Yields of primary metabolites were grossly inflated (10× too high) unless thin layer chromatography was accompanied by prior purification by gel permeation chromatography because much radioactivity was attributed to co-eluting natural products. Rapid metabolism of methoprene to biologically innocuous derivatives was characteristic of both rice and alfalfa.

The possibility of using insect growth regulators (IGR's) to control insect pests by disrupting metamorphosis has received considerable attention (Menn and Beroza, 1972). A new class of potent IGR's (alkyl 3,7,11-trimethyl-2,4-dodecadienoates) has shown efficacy in large-scale field tests (Henrick *et al.*, 1973). A slow-release formulation of methoprene IGR (**1**, isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate; trademark, Altosid) was effective in controlling mosquito larvae (Schaefer and Wilder, 1973), but produced no adverse biological effects on most nontarget aquatic organisms (Miura and Takahashi, 1973) and was rapidly degraded under natural field conditions (Schaefer and Dupras, 1973).



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Methoprene is the first IGR for which the Environmental Protection Agency has granted an experimental permit. In order to assess the biodegradability of methoprene when used as a mosquito larvicide, we report its metabolic fate in alfalfa and rice; this report represents part of a comprehensive study of the environmental fate of methoprene.

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MATERIALS AND METHODS

(2*E*,4*E*)-[5-^{14}C]Methoprene was radiosynthesized (by Dr. John C. Leak, ICN) by condensing [1-^{14}C]citronellal with diisopropyl 3-isopropoxycarbonyl-2-methyl-2-propenyl phosphonate (following the methods of Henrick *et al.*, 1973) to yield [5-^{14}C]isopropyl (4*E*)-3,7,11-trimethyl-2,4,10-dodecatrienoate. This substance was then subjected to methoxymercuration–borohydride reduction, generating [5-^{14}C]methoprene. The approximately 80:20 2*E*:2*Z* mixture was separated and purified by preparative high-resolution liquid chromatography (hrlc) to give (2*E*,4*E*)-[5-^{14}C]methoprene (58 mCi/mmol; 97.9% 2*E*,4*E*; 1.5% 2*Z*,4*E*). While tlc indicated 100% radiochemical purity as [5-^{14}C]methoprene, radio glc analysis revealed 0.6% of an unknown radioactive impurity which was chromatographically inseparable from methoprene by hrlc. The [5-^{14}C]methoprene was diluted with 98% (2*E*,4*E*)-methoprene to a specific activity of 5.0 mCi/mmol for these studies. Authentic metabolite standards were synthesized