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AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION
GREENSBORO, N. C.

UPTAKE AND CHARACTERIZATION OF ϕ-^{14}C-CGA-48988 AND ITS SOIL METABOLITES IN ROTATION LETTUCE

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ABSTRACT

Lettuce was grown the following spring as a rotation crop to white potatoes in a field plot on the CIBA-GEIGY Research Farm at Livingston, New York. The potato plot was sprayed over-the-top six times at a rate of 0.40 lb. a.i./A. The first spraying was 45 weeks prior to planting the lettuce. Subsequent sprayings were at 14 day intervals. The uptake of soil radioactivity by rotation lettuce was 0.11 ppm equivalent to ϕ-^{14}C-CGA-48988 after five weeks of growth and then decreased to 0.06 ppm after nine weeks and 0.05 ppm after 11 weeks (maturity). The extraction and partition data for the five week old lettuce indicate that as much as half of the recovered radioactivity was organic soluble and could be parent ϕ-^{14}C-CGA-48988 (40% of recovered ^{14}C). These data also show that metabolism is to polar (25.5%) and nonextractable (15.8%) products.

The level of radioactivity in the 0-3" soil layer remained at 0.30 ppm throughout the study. During the 11 week growing season for lettuce, the extraction and partition data show a decrease in organic soluble radioactivity from 40.5% to 13.4% and an increase in nonextractable radioactivity from 51.1% to 80.1%. Since no accumulation of radioactivity occurred in the polar fraction (<10%), nonpolar compounds, possibly parent ϕ-^{14}C-CGA-48988, are being adsorbed to soil particles. After settling (6 weeks), the level of radioactivity in lower soil layers is 0.21 ppm (3-6") and 0.16 (6-9") showing that ϕ-^{14}C-CGA-48988 and its soil metabolites do not leach.

INTRODUCTION

N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester* is a fungicide proposed for the control of late blight in potatoes. The objectives of this study were to: 1) determine the uptake of the soil degradation products of [U-ring-^{14}C] N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester** in rotation lettuce, 2) determine the movement and degradation of ϕ-^{14}C-CGA-48988 in field soil and 3) characterize the metabolites of ϕ-^{14}C-CGA-48988 in soil and lettuce grown as a rotation crop to potatoes (1).

EXPERIMENTAL

Preparation and Planting of the Field Plot: A 3' x 19' plot was prepared and planted with white potatoes at the CIBA-GEIGY Research Farm at Livingston, New York. The potatoes were sprayed over-the-top six times at 14 day intervals with ϕ-^{14}C-CGA-48988 at a rate of 0.40 lb. a.i./A. The first spray treatment was six weeks after plant emergence (7/1/77). The ϕ-^{14}C-CGA-48988 was dissolved in an ethanol/water (1:1) solution and applied with a miniature boom sprayer. Forty-five weeks after the first treatment, a 3' x 3' subplot was prepared for rotation lettuce by tilling to a depth of approximately 8". Seeds were densely planted in two rows spaced 12" apart.

Radioactive Dose: A total of 1755 mg of ϕ-^{14}C-CGA-48988 (specific activity = 30.0 µC/mg or 9.01 mC/mM) was sprayed on the primary crop of potatoes. No additional CGA-48988 or radioactive chemicals were applied for the duration of all rotational studies.

Sampling: Planting and sampling dates are given in Table I. Monthly rainfall data are in Table II. Lettuce was sampled 5, 8 and 10 weeks after planting. Soil was sampled at planting and at each lettuce sampling. Soil cores were separated into 0-3", 3-6" and 6-9" segments for analysis.

Sample Preparation and Analysis: Plants were homogenized with dry ice in a Wiley Mill (2) and 150-200 mg samples were combusted in a Harvey Oxidizer (3). Biphasic extractions were in accordance with AG-214 to produce organic, polar and nonextractable fractions (4).

* Chemical names and structures are given in Figure 1.
** Hereafter referred to as ϕ-^{14}C-CGA-48988.

Soil samples of approximately two grams each were combusted in a Harvey Oxidizer (3). Extractions were in accordance with AG-254 (5).

Samples were analyzed on arrival.

Radioactivity Measurements: Radioassays were done in a Beckman LS-255 or Searle Mark III liquid scintillation counter. Efficiencies were obtained by external standardization. Limits of detection and quantitation were determined in accordance with AG-276 (6).

RESULTS AND DISCUSSION

Lettuce: The levels of radioactivity equivalent to ϕ-^{14}C-CGA-48988 in rotation lettuce are shown in Table III. The radioactivity in the leaves decreased from 0.11 ppm at six weeks to 0.05 ppm at 11 weeks. These data show that the rate of growth of rotation lettuce exceeds the rate of uptake of ϕ-^{14}C-CGA-48988 or its soil metabolites and that the levels of CGA-48988 and its metabolites are low in mature spring lettuce.

There was only sufficient radioactivity in the six week sample for a balance determination and even this level was borderline. Despite the total not being 100 + 10%, the recovery does show that half of the accountable radioactivity is in the organic fraction. This could be parent ϕ-^{14}C-CGA-48988, about 0.05 ppm. The recovery of radioactivity in the polar (25.5%) and nonextractable (15.8%) fractions shows that metabolism proceeds through nonpolar to polar and finally to nonextractable products.

Soil: The level of radioactivity in the field soil (Table V) is shown in Table IV during the growing season for lettuce. The radioactivity in the 0-3" layer remained constant at 0.30 ppm equivalent to ϕ-^{14}C-CGA-48988. The balance data show a decrease of radioactivity in the organic fraction from 40.5% to 13.4% accompanied by an increase in the nonextractable fraction from 51.1% to 80.1%. There is no accumulation in the polar fraction which remained near the level of quantitation and never exceeded 10% of the total. These data indicate that nonpolar radioactive materials, possibly parent ϕ-^{14}C-CGA-48988, are being adsorbed to soil particles and are not extractable with methanol/water.

The levels of radioactivity in the 3-6" and 6-9" soil layers at planting were 0.40 and 0.23 ppm, respectively. After the soil has had time to settle, these levels remain relatively constant at 0.21 (3-6") and 0.16 (6-9") ppm. Therefore, neither ϕ-^{14}C-CGA-48988 nor its soil metabolites leached into lower soil layers during this study.

REFERENCES

1. [redacted] ABR-78013, "Uptake of ϕ-^{14}C-CGA-48988 in Potatoes Grown in a Field Plot - Preparation of Rotational Plots."

2. [redacted], AG-223, "Blending of Soils and Homogenization of Biological Materials for Radioassay and Extraction."

3. [redacted], AG-252, "Radioassay of ^{14}C in Biological Materials Using the Harvey Biological Material Oxidizer (BMO)."

4. [redacted], AG-214, "Biphasic Extraction of Radioactive Metabolites from Treated Biological Material."

5. [redacted], P. A., AG-254, "Extraction of CGA-10832 Residues in Soil."

6. [redacted] AG-276, "Statistical Methods in the Measurement of Radioactivity."

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TABLE I: PLANTING AND SAMPLING DATES

Date	Elapsed Time* (weeks)	Action
5/12/78	0 (45)	Lettuce Planted
6/27/78	6 (51)	5" Leaves
7/18/78	9 (54)	75% Harvest
7/31/78	11 (56)	Mature Harvest

* Numbers in parentheses indicate elapsed time (weeks) since the first treatment with ϕ-^{14}C-CGA-48988 (7/1/77). Subsequent treatments were at 14 day intervals: 7/15/77, 7/28/77, 8/11/77, 8/25/77 and 9/8/77.

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TABLE II: CIBA-GEIGY NEW YORK RESEARCH FARM
MONTHLY RAINFALL DATA; MAY-JULY 1978

Month	Inches of Precipitation
May	4.9
June	4.1
July	3.4

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TABLE III: UPTAKE AND BALANCE OF RADIOACTIVITY EQUIVALENT TO ϕ-^{14}C-CGA-48988 IN ROTATION LETTUCE

Interval (weeks)	6	9	11
Plant Part	leaves	leaves	leaves
Total ppm	0.11	0.06	0.05
Balance (% of total ^{14}C)			
Organic	40.4		
Polar	25.5		
Nonext.	15.8		
Total	81.7		

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TABLE IV: DISTRIBUTION AND BALANCE OF RADIOACTIVITY EQUIVALENT TO ϕ-^{14}C-CGA-48988 IN FIELD SOIL

Interval (weeks)*	0 (45)			6 (51)			9 (54)			11 (56)		
Depth (inches)	0-3	3-6	6-9	0-3	3-6	6-9	0-3	3-6	6-9	0-3	3-6	6-9
Total ppm	0.30	0.40	0.23	0.30	0.22	0.19	0.29	0.17	0.12	0.30	0.25	0.17
Balance in 0-3" layer												
Organic	40.5			25.8			20.4			13.4		
Polar	<*6.0			7.2			8.7			<*5.6		
Nonext.	51.1			64.4			61.6			80.1		
Total	91.6			97.4			90.7			93.5		

A <* indicates that the level of radioactivity is detectable but below the level of quantitation (6).

*Numbers in parentheses indicate the elapsed time (weeks) since the first treatment with ϕ-^{14}C-CGA-48988 (7/1/77).

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TABLE V: CHARACTERISTICS OF FIELD PLOT SOIL

Location: CIBA-GEIGY New York Research Farm
Livingston, New York

Texture	silt loam
pH	5.5
% Organic Matter	1.8
% Sand	44.4
% Silt	44.0
% Clay	11.6

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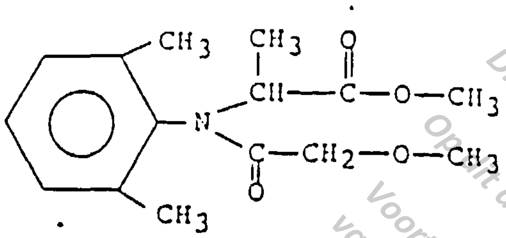
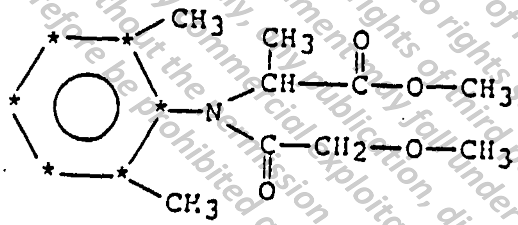


CGA-48988

N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester

Radioactive Compound



φ-^{14}C-CGA-48988

[U-ring-^{14}C] N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester

* = ^{14}C

FIGURE 1: CHEMICAL NAMES AND STRUCTURES

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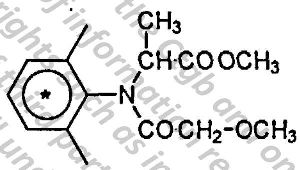
Annex II - 6.2. /04 : Uptake and characterization of Φ-^{14}C-CGA 48988 and its soil metabolites in rotation lettuce

General Information	
Title of the study:	**Uptake and characterization of Φ-^{14}C-CGA 48988 and its soil metabolites in rotation lettuce**
Report and/or project number: Author: Ciba File Number (Desire):	ABR-78078 5.1.2.e Woo 48988/3569
Name and address of testing facility:	Ciba-Geigy Corp., Livingston, NY, USA (Biological phase) Ciba-Geigy Corp., Greensboro, N.C., USA (Analytical phase)
Study period:	5/78 - 7/78
Date of report:	October 10, 1978
Compliance with GLP:	Yes [ ] No, but complies with sound scientific principles [X]
Test guideline(s) used:	-
Deviations from the test guideline:	-

Test substance	
Test substance (code number):	CGA 48988
Batch:	-
^{14}C-labeled test substance : Specific activity of [U-^{14}C-phenyl] label:	Yes [X] No [ ] 1.11 MBq/mg (= 30 µCi/mg)
Radiochemical purity of test substance:	not available
Structural formula: (Position of label)	[U-^{14} C-phenyl]-CGA 48988 * = ^{14}C 
Formulation used for study:	no

Test system	
Target crop:	field grown potatoes
Formulation (spray application): Formulation N° (spray application): Solvent for application (if used):	- - ethanol/water (1:1) solution
Application: Field experiment:	Spray applications with a miniature boom sprayer: 6 over-the-top sprays (starting 6 weeks after plant emergence) at 14 days intervals at a rate of 0.40 lb./A (= 292.3 mg ^{14}C-CGA 48988 / 3' X 19' plot/ treatment (= 8.77mCi), i.e. 1755 mg ^{14}C-CGA 48988/ 6 treatments (= 52.65 mCi for all 6 applications)
Rotational crop (planting / harvest):	lettuce planting: 45 weeks after the first treatment of target potatoes harvest: 11 weeks after planting the lettuce or 56 weeks after the first treatment of target potatoes

Soil:	Soil from Livingston, NY, USA	
	Texture:	Silt Loam
	pH:	5.5
	% Organic Matter:	1.8
	% Sand:	44.4
	% Silt:	44.0
	% Clay:	11.6

<u>Summary of findings</u>

Lettuce was grown the following spring as a rotation crop to white potatoes in a field plot on the CIBA-GEIGY Research Farm at Livingston, New York. The potato plot was sprayed over-the-top six times at a rate of 0.40 lb./A. The first spraying was 45 weeks prior to planting the lettuce. Subsequent sprayings were at 14 day intervals. The uptake of soil radioactivity by rotation lettuce was 0.11 ppm equivalent Φ-^{14}C-CGA 48988 after five weeks of growth and then decreased to 0.06 ppm after nine weeks and 0.05 ppm after 11 weeks (maturity). The extraction and partition data for the five week old lettuce indicate that as much as half of the recovered radioactivity was organic soluble and could be parent Φ-^{14}C-CGA 48988 (40% of recovered ^{14}C). These data also show that metabolitsm is to polar (25.5%) and nonextractable (15.8%) products.

The level of radioactivity in the 0 - 3" soil layer remained at 0.30 ppm throughout the study. During the 11 week growing season for lettuce, the extraction and partition data show a decrease in organic soluble radioactivity from 40.5% to 13.4% and an increase in nonextractable radioactivity from 51.1% to 80.1%. Since no accumulation of radioactivity occurred in the polar fraction (<10%), nonpolar compounds, possibly parent Φ-^{14}C-CGA 48988 , are being adsorbed to soil particles. After settling (6 weeks), the level of radioactivity in lower soil layers is 0.21 ppm (3 - 6") and 0.16 ppm (6 - 9") showing that Φ-^{14}C-CGA 48988 and its soil metabolites do not leach.

Tab 1 Uptake, distribution and balance of radioactivity equivalent to Φ-^{14}C-CGA 48988 in rotation lettuce and soil (at harvest)

Plant part Soil Layer	Total Residues [ppm]	Organic Phase	Water Phase	Non extractable	Total
Leaves	0.05				
0 - 3"	0.30	13.4	<*5.6	80.1	93.5
3 - 6"	0.25				
6 - 9"	0.25				

a <* indicates that the level of radioactivity is detectable but below the level of quantitation

PP 2.52/ JK, 10.3.94

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