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AGRICULTURAL DIVISION
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GREENSBORO, NC

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

M6-69-5PR, 5SR

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A B S T R A C T

Sugar beets were grown as a rotation crop to white potatoes in a field plot on the CIBA-GEIGY Research Farm at Livingston, New York. The plot was treated by spraying ϕ-^{14}C-CGA-48988 over-the-top six times at a rate of 0.40 lb. a.i./A and at fourteen day intervals. Chemical names, codes and structures are in Figure 1. The first spraying was 45 weeks prior to planting the sugar beets.

The level of radioactivity in the 0-3" soil layer decreased from approximately 0.33 ppm at the time of planting to 0.20 ppm twenty weeks later. During the twenty week growing season for sugar beets, the extraction and partition data show a decrease in organic soluble radioactivity from 36.5% to 11.1% and an increase in nonextractable radioactivity from 48.7% to 80.4%. Since no accumulation of radioactivity occurred in the polar fraction (<10%), nonpolar compounds, possibly parent ϕ-^{14}C-CGA-48988, were being adsorbed to soil particles. The level of radioactivity remained constant at approximately 0.17 (6-9"), showing that ϕ-^{14}C-CGA-48988 and its soil metabolites do not leach. Therefore, ϕ-^{14}C-CGA-48988 and its soil metabolites are probably being degraded to $^{14}CO_2$ slowly.

166

The uptake of soil radioactivity by whole rotation sugar beets was 0.16 ppm equivalent to ϕ-^{14}C-CGA-48988 after six weeks of growth. The radioactivity decreased to 0.06 and 0.03 ppm for tops and roots after fifteen weeks and to 0.02 ppm for both tops and roots after twenty weeks (maturity). The extraction and partition data for the six week old sugar beets indicate that as much as half of the recovered radioactivity was in the polar fraction (53.1% of recovered ^{14}C). These data also show that metabolism is from nonpolar to nonextractable products.

INTRODUCTION

N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester*, CGA-48988, 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 sugar beets, 2) determine the movement and degradation of ϕ-^{14}C-CGA-48988 in field soil and 3) characterize the radioactive metabolites of ϕ-^{14}C-CGA-48988 in soil and sugar beets 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 sugar beets by tilling to a depth of approximately 8". Seeds were planted at three inch intervals in three rows spaced twelve inches apart.

Radioactive Dose: A total of 1,755 mg of ϕ-^{14}C-CGA-48988 was applied to the plot (sp. act. = 30 µCi/mg or 9.01 mCi/mM). No additional CGA-48988 or radioactive chemicals were applied for the duration of the studies.

Sampling: Planting and sampling dates are given in Table I. Monthly rainfall data are in Table II. Sugar beets were sampled at 6, 9, 15, and 20 weeks after planting. Soil was sampled at planting and at each sugar beet sampling. Soil cores were divided into 0-3", 3-6" and 6-9" segments for analysis.

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

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).

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 upon arrival.

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

RESULTS AND DISCUSSION

Soil: The levels of radioactivity equivalent to ϕ-^{14}C-CGA-48988 in field soil (Table V) are shown in Table III. The level in the 0-3" and 3-6" layers showed a drop from approximately 0.33 ppm at the time of planting to 0.20 ppm twenty weeks later. The radioactivity in the 6-9" remained constant at approximately 0.17 ppm throughout the study. These data indicate that the radioactivity associated with the ϕ-^{14}C-CGA-48988 and its metabolites did not leach. The radioactive decrease may be due to slow degradation to $^{14}CO_2$ based on prior data (7).

The balance data show a trend of decreasing radioactivity in the organic fraction from 36.5% to 11.1% accompanied by an increase in the nonextractable fraction from 48.7% to 80.4%. There was 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 some parent ϕ-^{14}C-CGA-48988, were adsorbed to soil particles and are not extractable with methanol/water.

Sugar beets: The levels of radioactivity in rotation sugar beets equivalent to ϕ-^{14}C-CGA-48988 are shown in Table IV. The radioactivity at all samplings and in all plant parts is 0.16 ppm or less. Mature roots and tops contain 0.02 ppm. There was only sufficient radioactivity in the 6 week sample for a balance determination. The recovery does show that half of the radioactivity is in the polar fraction. The recovery of radioactivity in the organic (33.8%) and nonextractable (11.4%) fractions shows that metabolism proceeds through polar to nonpolar and finally to nonextractable products.

These data show that rotation sugar beets take up very little CGA-48988 or its metabolites from soil.

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], AG-254, "Extraction of CGA-10832 Residues in Soil."

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

7. [redacted], Project Report 08/78, "Degradation of CGA-48988 (Ridomil®) in Soil under Aerobic, Aerobic/Anaerobic and Sterile/Aerobic Conditions."

TABLE I: PLANTING AND SAMPLING DATES

Date	Elapsed Time* (weeks)	Action
5/12/78	0 (45)	Sugar beets planted
6/27/78	6 (51)	25% sample
8/7/78	9 (54)	50% sample
9/15/78	15 (60)	75% sample
10/26/78	20 (65)	Mature sample

*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.

172

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

Month	Inches of Precipitation
May	4.9
June	4.1
July	3.4
August	5.5
September	2.8
October	2.3

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

Interval (weeks)*	0 (45)			6 (51)			9 (54)			15 (60)			20 (65)		
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	0-3	3-6	6-9
Total ppm	0.33	0.30	0.19	0.26	0.25	0.19	0.19	0.15	0.13	0.16	0.16	0.16	0.20	0.20	0.18
Balance (% of Total ^{14}C in 0-3" Layer)															
Organic	36.5			30.6			14.1			12.9			11.1	9.8	<*6.7
Polar	9.1			5.0			5.4			<*6.3			5.7	7.4	<*5.3
Nonext.	48.7			61.2			76.7			85.8			80.4	87.5	95.8
Total	94.3			96.8			96.2			98.7			97.2	104.7	95.8

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

<*indicates that the level of radioactivity was below the level of quantitation.

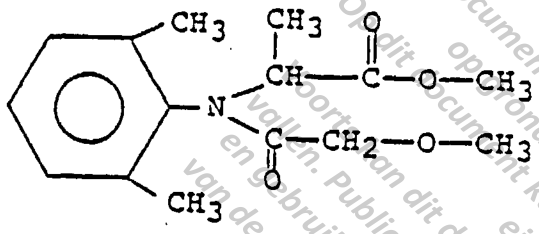
TABLE IV: UPTAKE AND BALANCE OF RADIOACTIVITY EQUIVALENT TO ϕ-^{14}C-CGA-48988 IN ROTATION SUGAR BEETS

Interval (weeks)	6	9	15		20	
Plant part	Whole plants	Whole plants	Tops	Roots	Tops	Roots
Total ppm	0.16	0.07	.06	.03	.02	.02
Balance	(% of Total ^{14}C)					
Organic	33.8					
Polar	53.1					
Nonext.	11.4					
Total	98.3					

TABLE V: CHARACTERISTICS OF FIELD PLOT SOIL

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

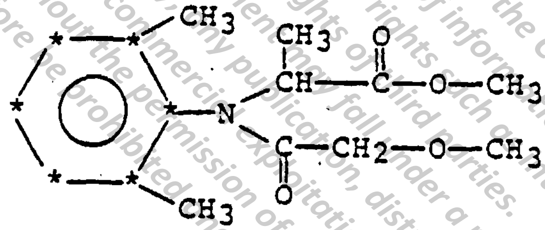
Texture	Silt Loam
pH	5.5
% Organic Matter	1.8
% Sand	44.4
% Silt	44.0
% Clay	11.6



CGA-48988

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

Radioactive Compound



ϕ-^{14}C-CGA-48988

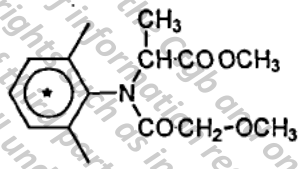
[U-ring-^{14}C] N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester

* = ^{14}C

Figure 1: CHEMICAL NAMES AND STRUCTURES

Annex II - 6.2. /08 : Uptake and characterization of Φ-^{14}C-CGA 48988 and its soil metabolites in field rotation sugar beets

General Information	
Title of the study:	Uptake and characterization of Φ-^{14}C-CGA 48988 and its soil metabolites in field rotation sugar beets
Report and/or project number: Author: Ciba File Number (Desire):	ABR-79005 5.1.2e Woo 48988/3565
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 - 10/78
Date of report:	February 12, 1979
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:	Yes [ ] No [X]

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 = 8.77 mCi), i.e. 1755 mg ^{14}C-CGA 48988/ 6 treatments (= 52.65 mCi for all 6 applications)
Rotational crop (planting / harvest):	sugar beets: planting: 45 weeks after the first treatment of target potatoes harvest: 20 weeks after planting the soybeans or 65 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

Summary of findings

Sugar beets were grown as a rotation crop to white potatoes in a field plot on the CIBA-GEIGY Research Farm at Livingston, New York. The plot was treated by spraying Φ-^{14}C-CGA 48988 over-the-top six times at a rate of 0.40 lb. a.i./A and at fourteen-day intervals. The first spraying was 45 weeks prior to planting the sugar beets.

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

Plant part Soil Layer	Total Residues [ppm]	Organic Phase	Water Phase	Non extractable	Total
Roots	0.02				
Tops	0.02				
0 - 3"	0.20	11.1	5.7	80.4	97.2
3 - 6"	0.20	9.8	7.4	87.5	104.7
6 - 9"	0.18	<*6.7	<*5.3	95.8	95.8

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

PP 2.52/ JK, 11.3.94