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GREENSBORO, N.C.

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

M6-69-4PR, 4SR

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A B S T R A C T

Spring oats 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 (chemical names, codes and structures are in Figure I) 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 oats.

The level of radioactivity equivalent to ϕ-^{14}C-CGA-48988 in the 0-3" and 3-6" soil layers decreased from 0.35 to 0.23 ppm in fourteen weeks. Radioactivity in the 6-9" layer increased slightly from 0.16 to 0.24 ppm. However, this increase is not enough to account for the decrease in the 0-3" and 3-6" layers by leaching. Therefore, some of the ϕ-^{14}C-CGA-48988 in the soil was probably being degraded to $^{14}CO_2$.

The balance data show that the radioactivity in the organic fraction decreased from 36.9% to 12.1%, and the nonextractable radioactivity increased from 52.0% to 79.4% during the course of the study. There was no increase of radioactivity in the polar fraction which

accounted for 10.1% or less of the total. These data indicate that nonpolar materials, possibly some parent ϕ-^{14}C-CGA-48988, are being adsorbed to soil particles.

The uptake of soil radioactivity was low, 0.09 ppm in mature grain and 0.19 ppm in mature straw. The balance data showed a decrease of radioactivity in the organic fraction from 23.4% to 6.0% and in the polar fraction from 48.5% to 27.6% in ten weeks. This is accompanied by an increase of radioactivity in the nonextractable fraction from 23.4% to 57.3%. These data show that rotation spring oats could further metabolize the soil metabolites of ϕ-^{14}C-CGA-48988.

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 spring oats, 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 spring oats grown as a rotation crop to potatoes (1).

EXPERIMENTAL

Preparation and Planting of the Field Plot: A 3'x19' 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'x3' subplot was prepared for rotation oats by tilling to a depth of approximately 8". Seeds were planted at one inch intervals in three rows spaced nine inches apart.

Radioactive Dose: A total of 1755 mg of ϕ-^{14}C-CGA-48988 was applied to the plot (sp. act. = 30μCi/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. Oats were sampled at 4, 7, 11 and 14 weeks after planting. Soil was sampled at planting and at each oat 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. Efficiencies 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 levels in the 0-3" and 3-6" soil layers decreased from 0.35 ppm to 0.23 ppm during the course of the study. Radioactivity in the 6-9" layer increased slightly from 0.16 ppm to 0.24 ppm. This indicates little leaching and this small increase does not account for the change in the level of radioactivity in the 0-3" and 3-6" layers. These data and prior data (7) indicate that the decrease of soil radioactivity is probably due to some degradation of the ring to $^{14}CO_2$.

The balance data show a decrease of radioactivity in the organic fraction, 36.9% to 12.1%, during the study and a concomitant increase of radioactivity in the nonextractable fraction, 52.0% to 79.4% There is no accumulation in the polar fraction, which is 10% or less total. These data indicate that nonpolar materials, possibly some parent ϕ-^{14}C-CGA-48988, were adsorbed to soil particles.

Spring Oats: The levels of radioactivity in rotation spring oats equivalent to ϕ-^{14}C-CGA-48988 are shown in Table IV. The uptake of soil radioactivity was moderate, 0.33 ppm, in the four week sample. However, growth rapidly outpaced uptake. At maturity, the level in the straw was 0.19 ppm and in the grain was 0.09 ppm. These data show that metabolites of CGA-48988 in the final agricultural product will be low.

The radioactive balance data show a trend from nonpolar to polar and nonextractable products. At maturity, radioactivity in the organic, polar and nonextractable fractions was 6.0%, 27.6% and 57.3%, respectively, compared to 23.4%, 48.5% and 23.4% at four weeks. Therefore, rotation spring oats are capable of further metabolizing ϕ-^{14}C-CGA-48988 and its soil metabolites.

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 Radio-active 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)	Oats Planted
6/12/78	4 (49)	25% Sample
6/29/78	7 (52)	50% Sample
7/28/78	11 (56)	75% Sample
8/17/78	14 (59)	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.

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

Month	Inches of Precipitation
May	4.9
June	4.1
July	3.4
August	5.5

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

Interval (weeks)*	0(45)			4(49)			7(52)			11(56)			14(59)		
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	0.36	0.16	0.29	0.28	0.18	0.32	0.31	0.20	0.21	0.32	0.28	0.20	0.25	0.24
Balance	(% of Total ^{14}C in 0-3" layer)														
Organic	36.9			35.3			28.8			19.5			12.1	9.5	<*3.0
Polar	6.8			2.3			10.1			5.8			<*3.2	3.5	2.7
Nonext.	52.0			53.0			59.6			72.2			79.4	85.0	77.6
Total	95.7			90.6			98.5			97.5			91.5	98.0	80.3

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

<*Indicates that the levels of radioactivity are detectable but are below the level of quantitation (6).

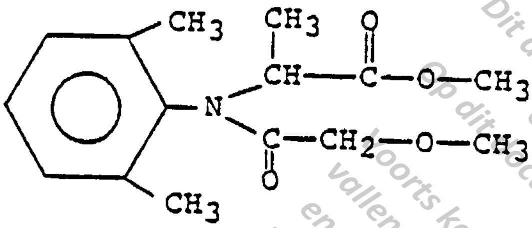
TABLE IV: UPTAKE AND BALANCE OF RADIOACTIVITY EQUIVALENT TO ϕ-^{14}C-CGA-48988 IN ROTATION SPRING OATS

Interval (weeks)	4	7	11	14	
Plant Part	Whole Plants	Whole Plants	Whole Plants	Grain	Straw
Total ppm	0.33	0.17	0.21	0.09	0.19
Balance		(% of Total ^{14}C)			
Organic	23.4	26.5	14.8		6.0
Polar	48.5	41.0	31.5		27.6
Nonext.	23.4	41.7	48.6		57.3
Total	95.3	109.2	94.9		90.9

TABLE V: CHARACTERISTICS OF FIELD PLOT SOIL

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

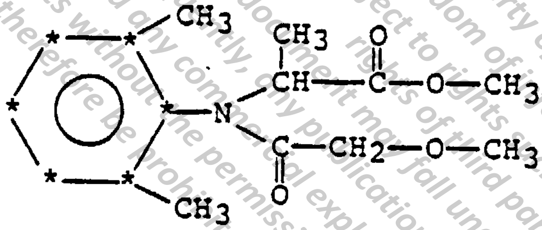
Texture	Silt Loam
pH	5.5
% Organic Matter	1.8
% Sand	44.4
% Silt	44.0
% Clay	11.6



CGA-48988

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

Radioactive Compound



ϕ-^{14}C-CGA-48988

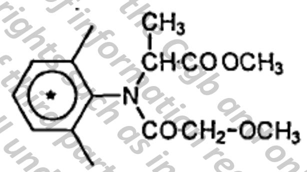
[U-ring-^{14}C] N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester

* = ^{14}C

FIGURE 1: CHEMICAL NAMES AND STRUCTURES

Annex II - 6.2. /05 : Uptake and characterization of Φ-^{14}C-CGA 48988 and its soil metabolites in field rotation spring oats

General Information	
Title of the study:	Uptake and characterization of Φ-^{14}C-CGA 48988 and its soil metabolites in field rotation spring oats
Report and/or project number: Author: Ciba File Number (Desire):	ABR-79002 5.1.2.e Woo 48988/3568
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 - 8/78
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 (= 8.77mCi), i.e. 1755 mg ^{14}C-CGA 48988/ 6 treatments (= 52.65 mCi for all 6 applications)
Rotational crop (planting / harvest):	spring oats planting: 45 weeks after the first treatment of target potatoes harvest: 14 weeks after planting the sping oats or 59 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

Spring oats 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 oats.

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The uptake of soil radioactivity was low, 0.09 ppm in mature grain and 0.19 ppm in mature straw. The balance data showed a decrease from 23.4% to 6.0% and in the polar fraction from 48.5% to 27.6% in ten weeks. This is accomplished by an increase of radioactivity in the nonextractable fraction from 23.4% to 57.3%. These data show that rotation spring oats could further metabolize the soil metabolites of Φ-^{14}C-CGA 48988.

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

Plant part Soil Layer	Total Residues [ppm]	Organic Phase	Water Phase	Non extractable	Total
Grain	0.09				
Straw	0.19	6.0	27.6	57.3	90.9
0 - 3"	0.20	12.1	<*3.2	79.4	91.5
3 - 6"	0.25	9.5	3.5	85.0	98.0
6 - 9"	0.24	<*3.0	2.7	77.6	80.3

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

PP 2.52/ JK, 10.3.94