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GREENSBORO, NC

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

M6-69-7PR, 7SR

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A B S T R A C T

Soybeans 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 at fourteen day intervals. Chemical names, codes and structures are in Figure 1. The first spraying was 48 weeks prior to planting the soybeans.

The level of radioactivity in the 0-3" soil layer decreased from approximately 0.34 ppm at the time of planting to 0.22 ppm twenty weeks later. During the twenty week growing season for soybeans, the extraction and partition data show a decrease in organic soluble radioactivity from 49.8% to 11.3% and an increase in nonextractable radioactivity from 45.8% to 76.9%. Since very little accumulation of radioactivity occurred in the polar fraction (<13%), nonpolar compounds, possibly some parent ϕ-^{14}C-CGA-48988, are being adsorbed to soil particles. The levels of radioactivity in lower soil layers were 0.19 ppm (3-6") and 0.16 ppm (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 slowly to $^{14}CO_2$.

The uptake of soil radioactivity by rotation soybeans was 0.40 ppm equivalent to ϕ-^{14}C-CGA-48988 after six weeks of growth, but increased to 0.59 ppm (leaves and stems) and 0.17 ppm (beans) at maturity. There was little variation in the radioactive extraction and partition characteristics throughout the growing season, except for mature beans. In these, metabolism proceeded further based on nonextractable radioactivity, 47.1% in beans compared to 21.4% in stalks.

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 soybeans, 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 soybeans 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 fourteen 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 soybeans by tilling to a depth of approximately 8". Seeds were planted at three inch intervals in two rows spaced eighteen 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. Soybeans were sampled at 6, 10, 13 and 20 weeks after planting. Soil was sampled at planting and at each soybean sampling. Soil cores were divided 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).

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.

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

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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 level in the 0-3" soil layer decreased from 0.34 ppm to 0.22 ppm during the twenty weeks. The 3-6" and 6-9" soil layers remained relatively constant at 0.21 ppm and 0.16 ppm, respectively, indicating that ϕ-^{14}C-CGA-48988 or its soil 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 49.8% to 11.3% accompanied by an increase in the nonextractable fraction from 45.8% to 76.9%. There was little accumulation in the polar fraction which never exceeded 12.8% of the total. These data and earlier data (8) 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.

Soybeans: The levels of radioactivity equivalent to ϕ-^{14}C-CGA-48988 in rotation soybeans are shown in Table IV. The level of radioactivity after six weeks of growth was 0.40 ppm equivalent to ϕ-^{14}C-CGA-48988. Uptake increased to 0.81 ppm at the ten week sampling and gradually decreased to 0.59 ppm in straw and 0.17 ppm in beans at maturity.

The balance data show that there is little variation in the partition characteristics of the radioactivity throughout the growing season, except for the mature beans. The organic fractions averaged approximately 16% of the total radioactivity, the polar fractions averaged approximately 73% and the nonextractable fractions averaged approximately 16%. The most obvious difference from this pattern is that for the mature beans. The radioactivity in the organic, polar and nonextractable fractions was 7.1%, 36.3% and 47.1%, respectively. These data indicate that metabolism of ϕ-^{14}C-CGA-48988 and its soil metabolites proceeded much further in beans than in the remainder of the plant.

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], "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."

8. [redacted], ABR-78077, "Uptake and Characterization of ϕ-^{14}C-CGA-48988 and Its Soil Metabolites in Rotation Winter Wheat."

TABLE I: PLANTING AND SAMPLING DATES

Date	Elapsed Time* (Weeks)	Action
6/5/78	0 (48)	Soybeans planted
7/19/78	6 (54)	25% sample
8/15/78	10 (58)	50% sample
9/6/78	13 (61)	75% sample
10/26/78	20 (68)	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; JUNE-OCTOBER, 1978

Month	Inches of Precipitation
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 (48)			6 (54)			10 (58)			13 (61)			20 (68)		
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	0.34	0.23	0.17	0.23	0.19	0.16	0.18	0.22	0.16	0.24	0.18	0.17	0.22	0.24	0.14
Balance (% of Total ^{14}C in 0-3" Layer)															
Organic	49.8			30.1			15.4			14.0			11.3	8.5	*<13.3
Polar	11.2			12.8			7.2			10.2			10.3	9.2	*< 8.0
Nonext.	45.8			63.9			70.6			70.5			76.9	85.0	90.7
Total	106.8			106.8			93.2			94.7			98.5	102.7	90.7

*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 (6).

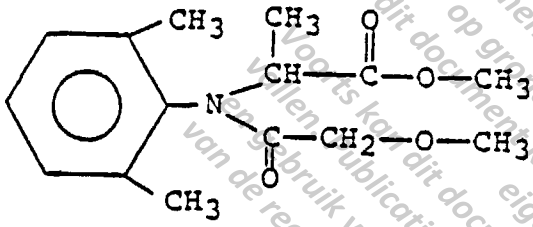
TABLE IV: UPTAKE AND BALANCE OF RADIOACTIVITY EQUIVALENT TO ϕ-^{14}C-CGA-48988 IN ROTATION SOYBEANS

Interval (weeks)	6	10	13	20	
Plant part	Whole plants	Whole plants	Whole plants	Leaves & stems	Beans
Total ppm	0.40	0.81	0.74	0.59	0.17
Balance (% of Total ^{14}C)					
Organic	Not enough Material	14.1	12.7	21.4	7.1
Polar		71.6	82.9	64.2	36.3
Nonext.		11.6	14.0	21.4	47.1
Total		97.3	109.6	107.0	90.5

TABLE V: CHARACTERISTICS OF FIELD PLOT SOIL

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

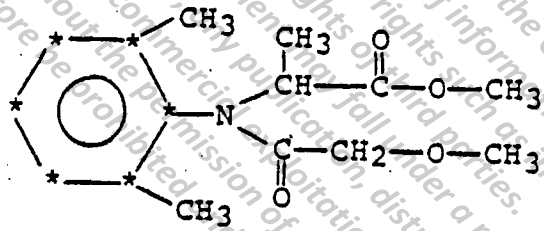
Texture	Silt Loam
pH	5.5
% Organic Matter	1.8
% Sand	44.4
% Silt	44.0
% Clay	11.6



CGA-48988

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

Radioactive Compound



ϕ-^{14}C-CGA-48988

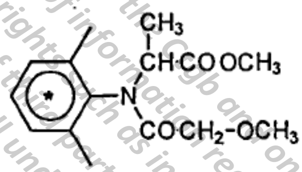
[U-ring-^{14}C] N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester

* = ^{14}C

Figure 1: CHEMICAL NAMES AND STRUCTURES

Annex II - 6.2. /06 : Uptake and characterization of Φ-^{14}C-CGA 48988 and its soil metabolites in field rotation soybeans

General Information	
Title of the study:	Uptake and characterization of Φ-^{14}C-CGA 48988 and its soil metabolites in field rotation soybeans
Report and/or project number: Author: Ciba File Number (Desire):	ABR-79003 [redacted] 5.1.2e Woo 48988/3567
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:	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):	soybeans planting: 48 weeks after the first treatment of target potatoes harvest: 20 weeks after planting the soybeans or 68 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

Soybeans 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 48 weeks prior to planting the soybeans.

The level of radioactivity in the 0 - 3" decreased from approximately 0.34 ppm at the time of planting to 0.22 ppm twenty weeks later. During the twenty week growing season for soybeans, the extraction and partition data show a decrease in organic soluble radioactivity fom 49.8% to 11.3% and an increase in nonextractable radioactivity from 45.8% to 76.9%. Since very little accumulation of radioactivity occurred in the polar fraction (<13%), nonpolar compounds, possibly some parent Φ-^{14}C-CGA 48988, are being adsorbed to soil particles. The level of radioactivity in lower soil layers were 0.19 ppm (3 - 6") and 0.16 ppm (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 slowly to $^{14}CO_2$.

The uptake of soil radioactivity by rotation soybeans was 0.40 ppm equivalent to Φ-^{14}C-CGA 48988 after six weeks of growth, but increased to 0.59 ppm (leaves and stems) and 0.17 ppm (beans) at maturity. There was little variation in the radioactive extraction and partition characteristics throughout the growing season, except for mature beans. In these, metabolism proceeded further based on nonextractable radioactivity, 47.1% in beans compared to 21.4% in stalks.

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

Plant part Soil Layer	Total Residues [ppm]	Organic Phase	Water Phase	Non extractable	Total
Leaves + Stems	0.59	21.4	64.2	21.4	107.0
Beans	0.17	7.1	36.3	47.1	90.5
0 - 3"	0.22	11.3	10.3	76.4	98.5
3 - 6"	0.24	8.5	9.2	85.0	102.7
6 - 9"	0.14	<*13.3	<*8.0	90.7	90.7

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

PP 2.52/ JK, 10.3.94