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AGRICULTURAL DIVISION
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GREENSBORO, N.C.

UPTAKE AND CHARACTERIZATON OF ϕ-^{14}C-CGA-48988 AND ITS SOIL METABOLITES IN ROTATION WINTER WHEAT

M6-69-5PR, 5SR

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A B S T R A C T

Winter wheat was planted in the fall 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 12 weeks prior to planting the wheat with subsequent sprayings at 14 day intervals.

The uptake of soil radioactivity in the fall grazing harvest was 3.97 ppm equivalent to ϕ-^{14}C-CGA-48988 but decreased to 0.56 ppm (straw) and 0.11 ppm (grain) at maturity. There was little variation in the radioactive extraction and partition characteristics throughout the growing season, except for mature grain. The organic fractions averaged approximately 37% of the total radioactivity, the polar fraction 40% and nonextractable radioactivity 27%. These data indicate that the metabolism of ϕ-^{14}C-CGA-48988 in rotation winter wheat is the same at all stages of growth except mature grain in which metabolism proceeds further to nonextractable materials, 66% nonextractable radioactivity in grain compared to 33% in stalks.

Some of the ϕ-^{14}C-CGA-48988 in the 0-3" and 3-6" soil layers is probably degraded to $^{14}CO_2$. This is supported by a decrease in 0-3" and 3-6" soil radioactivity from

0.74 ppm to 0.32 ppm during a 43 week period. There was no increase in radioactivity in the 6-9" soil layer (0.22 ppm), eliminating the possibility of leaching. Yet, in this time period, the balance data show a decrease of radioactivity in the organic fraction from 72.4% to 23.1% accompanied by an increase of radioactivity in the non-extractable fraction from 30.4% to 75.6%. Since no accumulation of radioactivity occurred in the polar fraction (<10%), nonpolar compounds, possibly parent ϕ-^{14}C-CGA-48988, is being adsorbed to soil particles and not all of the ring is being degraded to $^{14}CO_2$.

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 rotation 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 winter wheat, 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 winter wheat 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. Twelve weeks after the first treatment, a 3' x 3' subplot was prepared for rotation winter wheat 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 sprayed on the potato plot (sp. act. = 30.0 µCi/mg or 9.01 mCi/mM). 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. Winter wheat was sampled at 5, 35, 39 and 43 weeks after planting. Soil was sampled at planting and at each wheat sampling. Soil cores were separated 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 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

Winter Wheat: The levels of radioactivity equivalent to ϕ-^{14}C-CGA-48988 in rotation winter wheat are shown in Table III. The level of radioactivity after 5 weeks of growth, fall grazing stage, was 3.97 ppm equivalent to ϕ-^{14}C-CGA-48988. The level had decreased by one order of magnitude to 0.36 ppm equivalent to ϕ-^{14}C-CGA-48988 by the first spring sampling date, 35 weeks after planting. At maturity, 43 weeks after planting, the level in the grain was 0.11 ppm and in the straw was 0.56 ppm equivalent to ϕ-^{14}C-CGA-48988. These data show that the uptake of radioactivity from the soil by rotation winter wheat is rapid in the fall, but levels off in the spring and summer. The increased level in mature straw, 0.56 ppm, is probably a result of drying.

The balance data show that there is little variation in the partition characteristics of the radioactivity throughout the growing season, except for the mature grain. The organic fractions averaged approximately 37% of the total radioactivity, the polar fractions averaged approximately 40% and the nonextractable fractions averaged approximately 27%. The most obvious difference from this pattern is that of the mature grain, i.e., the organic fraction is <6.5% and nonextractable is 66.3%. These data indicate that metabolism of ϕ-^{14}C-CGA-48988 and its soil metabolites proceeds much further in grain than in the remainder of the plant.

Soil: The level of radioactivity in the field soil (Table V) is shown in Table IV. The fall samplings (0 and 5 week samplings) show that the level of radioactivity in the 0-3" and 3-6" layers was stabilized at approximately 0.7 ppm equivalent to ϕ-^{14}C-CGA-48988. After vernalization, the level of radioactivity in the 0-3" and 3-6" layers averages 0.32 ppm equivalent to ϕ-^{14}C-CGA-48988. These data attest to thorough soil tilling at the time of planting. They also indicate that ϕ-^{14}C-CGA-48988 is being degraded to $^{14}CO_2$ which is consistent with prior data (7). The level of radioactivity in the 6-9" layer remained consistent at approximately 0.22 ppm indicating that ϕ-^{14}C-CGA-48988 or its soil metabolites do not leach.

The balance data for the 43 week period show a steady decrease of radioactivity in the organic fractions from 72.4% to 23.1% in the 0-3" layer. There is a concomitant increase in the nonextractable radioactivity, 30.4% at the 0 week sampling to 75.6%. Little or no accumulation of radioactivity was found in the polar fractions. These remain near the level of quantitation and never exceed 10% of the total. These data suggest that nonpolar materials, possibly parent ϕ-^{14}C-CGA-48988, are being adsorbed to soil particles and these materials are not readily extracted with methanol/water.

The radioactive balance data for the 3-6" and 6-9" layers shows increased adsorption of ϕ-^{14}C-CGA-48988 or its soil metabolites to soil particles with aging.

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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 Radio-assay 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
9/23/77	0 (12)	Winter Wheat Planted
10/3/77	5 (17)	Fall Grazing Harvest
5/23/78	35 (47)	Spring Harvest
6/21/78	39 (51)	75% Harvest
7/18/78	43 (55)	Mature Harvest

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

TABLE II: CIBA-GEIGY NEW YORK RESEARCH FARM MONTHLY RAINFALL DATA; SEPTEMBER, 1977 - JULY, 1978

Month	Inches of Precipitation
September, 1977	8.0
October	5.4 + 2.0" snow
November	7.3
December	2.8 + 16" snow
January, 1978	4.3 + 34" snow
February	20" snow
March	3.1 + 9" snow
April	1.5
May	4.9
June	4.1
July	3.4

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

Interval (weeks)	5	35	39	43	
Plant Part	Whole Plants	Whole Plants	Whole Plants	Grain	Straw
Total ppm	3.97	0.36	0.34	0.11	0.56
Balance (% of total ^{14}C)					
Organic	37.8	44.6	36.1	< 6.5	27.5
Polar	42.7	40.7	30.9	23.9	44.3
Nonext.	23.2	23.1	28.6	66.3	33.0
Total	103.7	108.4	95.6	90.2	104.8

A < indicates that the level of radioactivity was below the level of detectability (6).

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

Interval (Weeks)*	0 (12)			5 (17)			35 (47)			39 (51)			43 (55)		
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	0.74	0.47	0.14	0.75	0.61	0.22	0.32	0.33	0.22	0.33	0.28	0.22	0.32	0.33	0.25
Balance															
Organic	72.4			52.0			43.3			31.8			23.1	16.6	<*7.9
Polar	<*5.7			9.9			5.4			<*5.0			<*4.9	<*4.9	< 3.9
Nonext.	30.4			34.8			58.6			62.4			75.6	86.3	99.8
Total	102.8			96.7			107.3			94.2			98.7	99.9	99.8

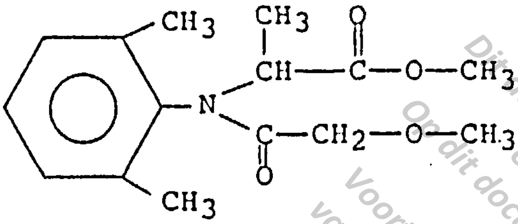
*Numbers in parentheses indicate the elapsed time (weeks) since the first treatment with ϕ-^{14}C-CGA-48988 (7/1/77).

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

TABLE V: CHARACTERISTICS OF FIELD PLOT SOIL

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

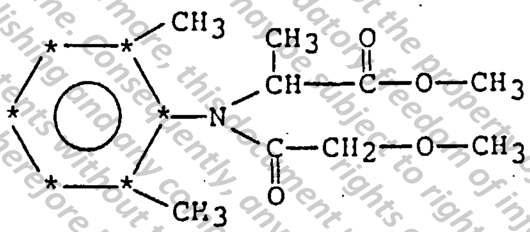
Texture	Silt Loam
pH	5.5
% Organic Matter	1.8
% Sand	44.4
% Silt	44.0
% Clay	11.6



CGA-48988

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

Radioactive Compound



φ-^{14}C-CGA-48988

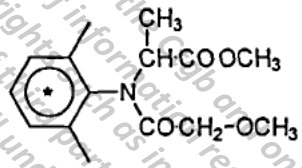
[U-ring-^{14}C] N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester

* = ^{14}C

FIGURE 1: CHEMICAL NAMES AND STRUCTURES

Annex II - 6.2. /03 : Uptake and characterization of Φ-^{14}C-CGA 48988 and its soil metabolites in rotation winter wheat

General Information	
Title of the study:	Uptake and characterization of Φ-^{14}C-CGA 48988 and its soil metabolites in rotation winter wheat
Report and/or project number: Author: Ciba File Number (Desire):	ABR-78077 5.1.2.e Woo 48988/3570
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:	9/77 - 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:	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):	winter wheat planting: 12 weeks after the first treatment of target potatoes harvest: 43 weeks after planting the winter wheat or 55 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

Winter wheat was planted in the fall as a rotation crop to white potatoes in a field plot on the CIBA-GEIGY Research Fram at Livingston, New York. The potatoe plot was sprayed over-the-top six times at a rate of 0.40 lb./A. The first spraying was 12 weeks prior to planting the wheat with subsequent sprayings at 14 days intervals.

The uptake of soil radioactivity in the fall grazing harvest was 3.97 ppm equivalent to Φ-^{14}C-CGA 48988 but decreased to 0.56 ppm (straw) and 0.11 ppm (grain) at maturity. There was little variation in the radioactive extraction and partition characteristics throughout the growing season, except for mature grain. The organic fractions averaged approximately 37% of the total radioactivity, the polar fraction 40% and nonextractable radioactivity 27%. These data indicate that the metabolism of Φ-^{14}C-CGA 48988 in rotation winter wheat is the same at all stages of growth except mature grain in which metabolism proceeds further to nonextractable materials, 66% nonextractable radioactivity in grain compared to 33% in stalks.

Some of the Φ-^{14}C-CGA 48988 in the 0 - 3" and 3 - 6" soil layer is probably degraded to $^{14}CO_2$. This is supported by a decrease in 0 - 3" and 3 - 6" soil radioactivity from 0.74 ppm to 0.32 ppm during a 43 week period. There was no increase on radioactivity in the 6 - 9" soil layer (0.22 ppm), eliminating the possibility of leaching. Yet, in this time period, the balance data show a decrease of radioactivity in the organic fraction from 72.4% to 23.1% accompanied by an increase of radioactivity in the non-extractable fraction from 30.4% to 75.6%. Since no accumulation of radioactivity occured in the polar fraction (<10%), nonpolar compounds, possibly parent Φ-^{14}C-CGA 48988, is being adsorbed to soil particles and not all of the ring is being degraded to $^{14}CO_2$.

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

Plant part Soil Layer	Total Residues [ppm]	Organic Phase	Water Phase	Non extractable	Total
Grain	0.11	< 6.5	23.9	66.3	90.2
Stalks	0.56	27.5	44.3	33.0	104.8
0 - 3"	0.32	23.1	<*4.9	75.6	98.7
3 - 6"	0.33	16.6	<*4.9	86.3	99.9
6 -8"	0.25	<*7.9	< 3.9	99.8	99.8

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

PP 2.52/ JK, 10.3.94