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3599

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Ciba Plant Protection
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Greensboro, North Carolina

FINAL REPORT AMENDMENT ON THE UPTAKE AND METABOLISM OF METALAXYL IN GREENHOUSE ROTATIONAL CROPS FOLLOWING TARGET TOBACCO GROWN IN SOIL TREATED WITH [PHENYL-^{14}C]-METALAXYL

AMENDMENT 1

Registration Category: Pesticide Assessment Guidelines
Subdivision N
Environmental Fate
EPA Guideline No. 165-1
Section 158.290
Confined Accumulation Studies on Rotation Crops

Report No.: ABR-91084 **Project No.:** 409925

Study Director: 5.1.2.e Woo

Title: Research Specialist

Signature: 5.1.2.e Woo

Date: 7/21/94

Approved By: 5.1.2.e Woo

Title: Manager, Metabolism

Signature: 5.1.2.e Woo

Date: 7/21/94

Submitted by: 5.1.2.e Woo

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Study Initiation Date: September 11, 1989

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The supplemental portion of this study was conducted in accordance with the applicable EPA Good Laboratory Practice Standards (40 CFR Part 160) with the following exception:

1. Standards CGA-62826 and CGA-67869 were qualitative standards used to aid calibration of the HPLC instrument and were not used for quantitative or qualitative purposes.

[redacted] ____________ 7-21-94
Date

Study Director

[redacted] ____________ 7-21-94
Date

Manager, Metabolism
Representative of Submitter/Sponsor

Submitter/Sponsor: Biochemistry Department
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GENERAL INFORMATION

Protocol Number: 203-89[1] with Amendment Lists (Tobacco), 203-89-Part A with Amendment Lists (Rotational Crops), and 203-89-Part B with Amendment Lists (Non-extractable Residues)

Guidelines: This study is being conducted to support the requirements outlined in: Pesticide Assessment Guidelines, Subdivision N, Environmental Fate EPA Guideline No. 165-1, Section 158.290, Confined Accumulation Studies on Rotational Crops.

Sponsor:
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Test Substance:
[Phenyl-^{14}C]-Metalaxyl
Code: ^{14}C-CGA-48988
Common Name: Metalaxyl
Trade Name: Ridomil®
Specific Activity: 29.8 µCi/mg
Lot No.: CL-XX-34
Radiochemical Purity: 98.4%
Chemical Purity: 99.7%

Carrier: Acetone - HPLC Grade
Fisher Scientific Lot No.: 891019

Experiment Numbers:
M90-409-001P (Lettuce)
M90-409-002P (Spring Wheat)
M90-409-003P (Soybeans)
M90-409-004P (Sugar Beets)
M90-409-005S (Soils)

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Supplier of the Test Substance and Reference Materials: [redacted] Manager
Chemical Synthesis Group
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Testing Periods: Tobacco Biology Phase (Target Crop)

Protocol Approval - 09/11/89
Soil Treatment - 09/13/89
Tobacco Transplant - 09/13/89
Mature Tobacco Harvest - 04/27/90

Rotational Crop Biology Phase

Protocol Approval - 05/03/90
Crop Planting - 05/03/90
Final Harvest - 11/19/90

Supplemental Analytical Phase

Start Date - 01/21/94
Termination Date - 05/10/94

Archives:

The protocol, raw data, biology reports, final report, final report amendment and other pertinent records and reports will be filed and archived at:

Biochemistry Group Archives
Ciba Crop Protection
Ciba-Geigy Corporation
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

Specimens, if not depleted during the study will be retained at Ciba-Geigy Corporation, Greensboro, NC for as long as the quality of the preparation affords evaluation, at which time the specimens will be discarded after Quality Assurance verification.

I. ABSTRACT

A. Chemical

Metalaxyl, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester, the active ingredient in Ridomil®, Apron® and Subdue® fungicides, is used in numerous food and non-food crops for the control of diseases, such as late blight, downy mildew and seedling diseases caused by oomycetes.

The structure of metalaxyl is included in Figure 1. As reported in ABR-91084[2], the uptake and metabolism of [phenyl-^{14}C]-metalaxyl was investigated in four rotational crops grown in soil previously used for growing tobacco. The tobacco target crop was treated with one soil application of [phenyl-^{14}C]-metalaxyl at the rate of 3 lbs. a.i./A which is the maximum treatment rate used in tobacco[3]. The active ingredient was mixed with acetone prior to incorporation into the soil.

B. Maintenance of Treatment Area

Details concerning the biological phase of this study are documented in ABR-91084[2], BIOL-90016 (rotational crops)[4] and BIOL-90017 (target tobacco)[5].

C. Residues

The uptake and metabolism of [phenyl-^{14}C]-metalaxyl in leaf lettuce, spring wheat, soybeans and sugarbeets planted 232 days after soil treatment is given in ABR-91084[2].

Storage stability data for homogenates and extracts is presented in this final report amendment, showing that metalaxyl and its metabolites are stable under frozen storage conditions. These results support the metabolic pathways of metalaxyl as reported in ABR-91084[2].

D. Problems

No problems occurred that would adversely affect the results of the study.

E. Contact Person

5.1.2.e Woo

II. INTRODUCTION

This study was initiated to support the reregistration of metalaxyl. Metalaxyl, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester, the active ingredient in Ridomil®, Apron® and Subdue® fungicides, is used to control specific fungi in several crops. The objectives of this study were to investigate the uptake and metabolism of [phenyl-^{14}C]-metalaxyl in rotational crops to support the data requirements in the Environmental Fate Chemistry Section 158.290, Guideline No. 165-1 (Confined Accumulation Studies in Rotational Crops).

Earlier, a final report, ABR-91084[2], was submitted to support the reregistration of metalaxyl according to Metabolism Protocol Number 203-89 (203-89, 203-89 Part A and 203-89 Part B). The objectives were to investigate the uptake and metabolism of [phenyl-^{14}C]-metalaxyl in lettuce, spring wheat, soybean and sugarbeet rotational crops grown in the greenhouse following a target crop of tobacco. The tobacco was grown in soil treated with a 3.0 lbs. a.i./A preemergent application. Data on the uptake and characterization of metabolites in the tobacco target crop treated prior to the planting of the greenhouse rotational crops were also included in ABR-91084[2]. Details on the biological phase are documented in biological report numbers BIOL-90016 (rotational crops)[4] and BIOL-90017 (target tobacco)[5].

The objective of the present final report amendment is to give storage stability data for rotational crop plant and soil homogenates and extracts. Stability data is given here because it was omitted in the final report, ABR-91084[2], and was cited as a deficiency in the Environmental Fate and Ground Water Branch (EFGWB) review of the original study. The EFGWB reviewer ([redacted]) concluded that this study provides acceptable data that shows metalaxyl residues are present in rotational crops planted 232 days after treatment. A storage stability test needs to be performed in order to determine whether metalaxyl residues in the samples degraded during handling and storage, since no data was presented relating to freezer stability during storage.

III. MATERIALS AND METHODS

Methods were as previously described in ABR-91084[2]. In this final report amendment, the following additions were made:

A. Reference Materials

In addition to the reference standards listed in Table I from ABR-91084[2], Table I in this final report amendment presents information on standards used after the submission of ABR-91084[2]. Radiolabeled and non-radiolabeled structures of metalaxyl and possible metalaxyl metabolites are shown in Figure 1. Standard stock solution concentrations, storage conditions and usage are described in ABR-91084[2].

B. Data Evaluation

Data reported as dpm, dpm/g, dpm/ml, histograms and other quantitative data calculated from dpm values were processed by the VAX Metabolism Data Base System (VAX) for the original study and by the Talisman System for the supplemental work presented. The calculations used were the same for both systems. The only difference is the definition of quantifiable DPMs.

Quantifiable DPMs as defined by the VAX Metabolism Data Base System are the DPMs that are greater than $[2 \times DPM_{bkg}]$. Quantifiable DPMs as defined by the Talisman System are the DPMs that are greater than Minimum Quantifiable Amount (MQA). MQA is defined as:

$$MQA = \{(14.1)SQRT[(avg\ DPM_{bkg})(avg\ count\ time)]\}/(avg\ count\ time)$$

For this final report amendment, VAX histogram data from ABR-91084[2] has been converted to the Talisman histogram format. The new figures were generated by using the dpm values collected by the VAX System and regraphing the data through the Talisman System. This was done to facilitate comparison of histograms from both systems.

C. Storage Stability

All of the sample homogenates and extracts were maintained in a freezer at approximately -20°C.

Extract stability was addressed by comparing the initial HPLC profiles of selected mature extracts which were analyzed within 8 months of harvest to HPLC profiles performed 3 to 4 years later.

Homogenate stability was addressed by extracting two subsamples each of 50% mature lettuce foliage, mature wheat stalk and mature sugarbeet root homogenates and comparing HPLC profiles of the extracts. Time in frozen freezer conditions between extractions ranged from 4.5 to 11.5 months.

IV. RESULTS AND DISCUSSION

A. Extract Stability

All mature plant samples and the last soil sampled were extracted and analyzed within 5 months of harvest. Harvest, extraction and analysis dates for each of these samples are given in Table II. Initial 2D-TLC analyses of the extracts were completed within approximately 1 to 5 months of harvest. The gradient for HPLC analysis was not developed until a later date. Initial HPLC analyses of the mature plant extracts were completed within approximately 3.5 to 8 months of harvest (Table II). Comparison of the HPLC analyses presented in ABR-91084[2] and HPLC analyses performed 3 to 4 years later show that metalaxyl and its metabolites are stable under frozen storage conditions in plant and soil extracts (Tables III-X and Figures 2-9).

B. Plant Homogenate Stability

Several plant samples were reextracted for identification purposes. Comparisons of HPLC profiles from these extracts were used to show that metalaxyl and its metabolites are stable under frozen storage conditions in plant homogenates.

Subsamples of 50% mature lettuce foliage were extracted 298 and 432 days after harvest. The amount extractable was 92.6% and 90.8%, respectively. Each extract was partitioned with ethyl acetate. The organic and aqueous fractions from both extracts were analyzed by HPLC. Profiles of the organic and aqueous fractions are qualitatively and quantitatively similar, thus showing stability of metalaxyl and its metabolites in frozen lettuce homogenates (Figures 10 and 11 and Table XI).

Subsamples of mature wheat stalks were extracted 22 and 285 days after harvest. The amount extractable was 79.2% and 74.5%, respectively. The extracts were analyzed by HPLC. Comparison of the HPLC quantitation shows quantitative variances in composition, particularly for peaks WS1, WS4, WS5 and WS7. (Figure 12 and Table XII). However, it can be concluded that the qualitative nature of the metalaxyl residues were preserved during the freezer storage conditions employed in this study.

Subsamples of mature sugarbeet roots were extracted 45 and 386 days after harvest. The amount extractable was 62.5% and 61.5%, respectively. The extracts were analyzed by HPLC. Comparison of the HPLC quantitation shows peak shifting and differing peak resolution, probably due to the use of different HPLC instrument systems (Figure 13 and Table XIII). However, the quantitation is similar for corresponding regions; thus, it is concluded that metalaxyl and its metabolites are stable under frozen storage conditions in sugarbeet homogenates.

V. CONCLUSION

Comparison of HPLC profiles of extractable radioactivity from various rotational crop plant and soil samples with the profiles of extracts stored in the freezer for approximately 3 to 4 years showed that the metalaxyl metabolites in rotational crops are stable during frozen storage.

TABLE I. SYNTHESIS REFERENCE NUMBERS AND PURITY INFORMATION FOR METALAXYL STANDARDS

STANDARD	RECEIPT DATE	SYNTHESIS REFERENCE NUMBER	PURITY	INFORMATION REANALYSIS DATE
CGA-78532	02/02/94	JAK-IV-15-1	>99.9	01/96
CGA-68124	02/02/94	BPM-X-31	>99.9	01/96
CGA-108906	02/02/94	WFH-VII-96	>99.9	01/96
CGA-119857	02/02/94	GB-XLV-3	>99.9	06/94
CGA-62826	02/02/94	BPM-I-4B	>99.9	03/94
CGA-37734	02/02/94	BPM-I-8	96.9	02/96
CGA-67867	02/02/94	RAF-IX-58	>99.9	01/96
CGA-100255	02/02/94	WFH-VII-86	99.7	01/96
CGA-94689A/B	02/02/94	JAK-II-21	96.5	06/94
CGA-67868	02/02/94	BPM-X-26	>99.9	01/96
CGA-67869	02/02/94	DAH-XV-35	>99.9	04/94
CGA-48988	02/02/94	BPM-XIII-58	>99.9	01/96
CGA-67866	02/02/94	RAF-IX-91	99.4	01/96
CGA-107955	02/02/94	GB-XLVI-7	>99.9	02/95
CGA-79353	02/02/94	JAK-IV-14-1	99.6	01/96
CGA-108905	02/02/94	GB-XLIV-78	95.5	07/94

TABLE II. HARVEST, EXTRACTION AND ANALYSIS DATES FOR MATURE PLANT AND SOIL SAMPLES

	Harvest Date	First Extraction Date	Days After Harvest	TLC Analysis Date	Days After Harvest	First HPLC Analysis Date	Days After Harvest	Second HPLC Analysis Date	Days After Harvest
Lettuce									
Mature Foliage	07/02/90	09/14/90	74	09/18/90	78	03/07/91	248	02/08/94	1317
Spring Wheat									
Mature Stalk	08/02/90	08/24/90	22	08/24/90	22	03/07/91	217	--	--
Mature Grain	08/02/90	09/07/90	36	09/18/90	47	03/15/91	225	05/10/94	1377
Mature Hull	08/02/90	08/28/90	26	09/07/90	36	02/28/91	210	02/08/94	1286
Soybean									
Mature Stalk	11/17/90	01/11/91	55	01/11/91	55	03/07/91	110	02/08/94	1179
Mature Pod	11/17/90	01/15/91	59	01/16/91	60	03/15/91	118	02/08/94	1179
Mature Bean	11/17/90	01/17/91	61	--	--	--	--	--	--
Sugarbeet									
Mature Foliage	10/29/90	03/27/91	149	04/03/91	156	05/16/91	199	02/08/94	1198
Mature Root	10/29/90	12/13/90	45	12/19/90	51	03/15/91	137	--	--
Mature Soybean Soil									
0-3"	11/17/90	01/18/91	62	01/23/91	67	03/15/91	118	02/08/94	1179
6-8"	11/17/90	04/05/91	139	04/10/91	144	05/27/91	191	02/08/94	1179

TABLE III. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE LETTUCE FOLIAGE

Harvest Date	07/02/90
Extraction Date	09/14/90
% Extractable	90.8

Peak[1]	Injection #1 03/07/91 % TRR	Injection #2 02/08/94 % TRR
L1	20.4	21.1
L2	5.0	5.0
L3	37.1	37.8
L4	13.1	12.0
L5	6.3	5.2
L6	3.0	4.5
L7	5.0	4.8

[1]See Figure 2 for corresponding peaks.

TABLE IV. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SPRING WHEAT GRAIN

Harvest Date	08/02/90
Extraction Date	09/07/90
% Extractable	22.0

Peak[1]	Injection #1 03/15/91 % TRR	Injection #2 05/10/94 % TRR
WG1	2.0	2.4
WG2	6.0	6.3
WG3	1.6	1.6
WG4	1.6	2.6
WG5	2.6	1.2
WG6	2.7	2.4
WG7	5.6	5.6

[1]See Figure 3 for corresponding peaks.

TABLE V. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SPRING WHEAT HULL

Harvest Date	08/02/90
Extraction Date	08/28/90
% Extractable	60.8

Peak[1]	Injection #1 02/28/91 % TRR	Injection #2 02/08/94 % TRR
WH1	3.1	2.3
WH2	7.8	8.3
WH3	5.5	6.1
WH4	7.1	5.5
WH5	10.7	10.8
WH6	16.8	16.3
WH7	2.8	1.9
WH8	1.9	2.0

[1]See Figure 4 for corresponding peaks.

TABLE VI. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN STALK

Harvest Date	11/17/90
Extraction Date	01/11/91
% Extractable	88.2

Peak[1]	Injection #1 03/07/91 % TRR	Injection #2 02/08/94 % TRR
SS1	14.3	14.8
SS2	24.5	23.8
SS3	5.2	6.0
SS4	19.6	19.4
SS5	20.6	20.5
SS6	2.1	2.3

[1]See Figure 5 for corresponding peaks.

TABLE VII. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN POD

Harvest Date	11/17/90
Extraction Date	01/15/91
% Extractable	81.6

Peak[1]	Injection #1 03/15/91 % TRR	Injection #2 02/08/94 % TRR
SP1	5.9	5.1
SP2	4.7	9.8
SP3	14.7	13.2
SP4	1.7	1.8
SP5	19.3	18.9
SP6	2.8	5.1
SP7	12.1	13.3
SP8	5.5	—
SP9	13.8	14.4

[1]See Figure 6 for corresponding peaks.

TABLE VIII. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-[14]C-METALAXYL MATURE SUGARBEET FOLIAGE

Harvest Date	10/29/90
Extraction Date	03/27/91
% Extractable	88.1

Peak[1]	Injection #1 05/16/91 % TRR	Injection #2 02/08/94 % TRR
SBF1	44.6	54.4
SBF2	10.1	9.3
SBF3	6.9	7.1
SBF4	4.8	2.1
SBF5	7.8	3.9
SBF6	4.7	6.3

[1]See Figure 7 for corresponding peaks.

TABLE IX. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN HARVEST 0-3" SOIL

Harvest Date	11/17/90
Extraction Date	01/18/91
% Extractable	29.6

Peak[1]	Injection #1 03/15/91 % TRR	Injection #2 02/08/94 % TRR
0-3S1	1.7	1.4
0-3S2	1.3	1.1
0-3S3	0.9	0.9
0-3S4	0.9	1.7
0-3S5	2.1	1.4
0-3S6	8.3	8.0
0-3S7	11.8	11.9

[1]See Figure 8 for corresponding peaks.

TABLE X. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN HARVEST 6-8" SOIL

Harvest Date	11/17/90
Extraction Date	04/05/91
% Extractable	20.7

Peak[1]	Injection #1 05/27/91 % TRR	Injection #2 02/08/94 % TRR
6-8S1	1.0	1.3
6-8S2	0.8	0.6
6-8S3	1.8	1.8
6-8S4	1.1	1.6
6-8S5	0.9	0.7
6-8S6	9.9	9.8
6-8S7	4.2	4.3

[1]See Figure 9 for corresponding peaks.

TABLE XI. COMPARISON OF HPLC QUANTITATION FROM TWO SEPARATE EXTRACTIONS OF 50% MATURE LETTUCE FOLIAGE

	50% Mature Lettuce Foliage	
	Extraction #1	Extraction #2
Date Harvested	06/01/90	06/01/90
Date Extracted	03/26/91	08/07/91
Days Post Harvest	298	432
PPM	0.877	0.877
% Extractable	92.6	90.8
% Organic	24.9	23.1
% Aqueous	75.1	76.9

Peak[3]	% TRR[1] #1	% TRR[1] #2	Peak[4]	% TRR[2] #1	% TRR[2] #2
LO1	0.4	0.5	LA1	15.5	17.0
LO2	0.6	0.6	LA2	6.9	6.0
LO3	0.3	0.5	LA3	35.9	35.8
LO4	1.0	1.4	LA4	7.2	7.6
LO5	1.9	2.3	LA5	1.9	1.9
LO6	1.2	1.2	LA6	2.0	1.6
LO7	16.6	13.3			

[1]The organic fractions from both extracts were analyzed 502 days after harvest.
[2]The aqueous fractions from both extracts were analyzed 497 days after harvest.
[3]See Figure 10 for corresponding peaks.
[4]See Figure 11 for corresponding peaks.

TABLE XII. COMPARISON OF HPLC QUANTITATION FROM TWO SEPARATE EXTRACTIONS OF MATURE SPRING WHEAT STALK

	Mature Spring Wheat Stalk	
	Extraction #1	Extraction #2
Date Harvested	08/02/90	08/02/90
Date Extracted	08/24/90	05/14/91
Days Post Harvest	22	285
PPM	7.171	7.171
% Extractable	79.2	74.5

	% TRR[1]	
Peak[2]	#1	#2
WS1	5.1	2.6
WS2	9.2	11.0
WS3	8.7	6.8
WS4	14.7	2.6
WS5	11.2	17.5
WS6	15.9	13.4
WS7	3.7	7.8
WS8	1.6	2.5
WS9	4.0	7.3
WS10	1.7	1.3
WS11	3.4	1.5

[1]The extractable fractions from both extracts were analyzed 217 and 286 days after harvest, respectively.
[2]See Figure 12 for corresponding peaks.

TABLE XIII. COMPARISON OF HPLC QUANTITATION FROM TWO SEPARATE EXTRACTIONS OF MATURE SUGARBEET ROOT

	Mature Sugarbeet Root	
	Extraction #1	Extraction #2
Date Harvested	10/29/90	10/29/90
Date Extracted	12/13/90	11/19/91
Days Post Harvest	45	386
PPM	0.275	0.275
% Extractable	62.5	61.5

	% TRR[1]	
Peak[2]	#1	#2
SBR1	35.9	31.9
SBR2	9.4	8.8
SBR3	5.4	5.5
SBR4	5.2	4.2
SBR5	3.9	4.4
SBR6	2.8	2.8

[1]The extractable fractions from both extracts were analyzed 137 and 399 days after harvest, respectively.
[2]See Figure 13 for corresponding peaks.

FIGURE 1. CHEMICAL NAMES AND STRUCTURES

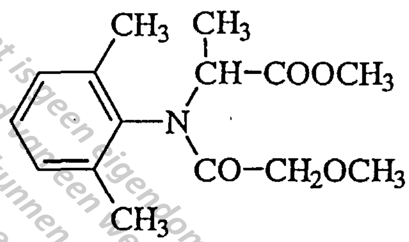
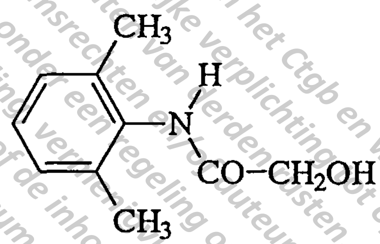
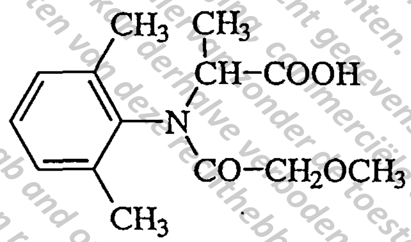
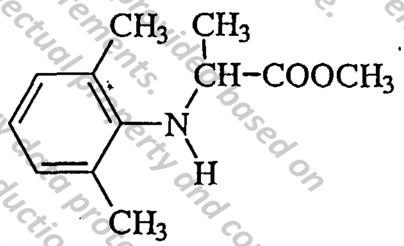
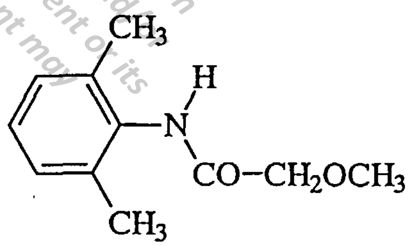
Company Code Chemical Name	Structure
CGA-48988 N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester	
CGA-37734 N-(2,6-dimethylphenyl)-2-hydroxyacetamide	
CGA-62826 N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine	
CGA-67866 N-(2,6-dimethyphenyl)-alanine methyl ester	
CGA-67868 N-(2,6-dimethylphenyl)-2-methoxyacetamide	

FIGURE 1. <u>CHEMICAL NAMES AND STRUCTURES</u> (Continued)

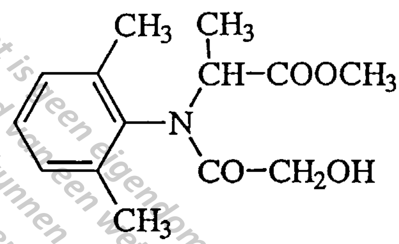
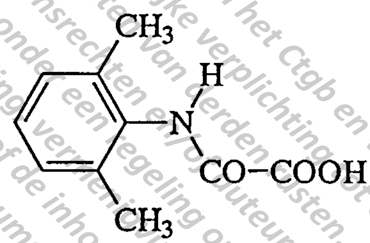
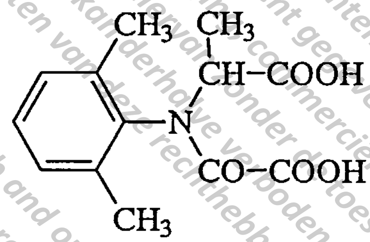
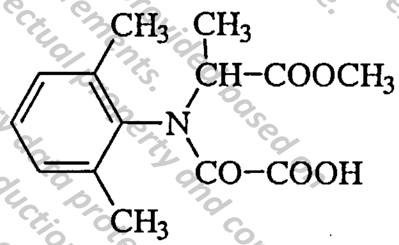
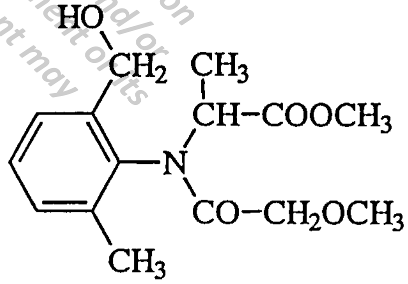
Company Code Chemical Name	Structure
CGA-67869 N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-alanine methyl ester	
CGA-68124 [(2,6-dimethylphenyl)-amino]oxoacetic acid	
CGA-78532 N-(carboxycarbonyl)-N-(2,6-dimethylphenyl) alanine	
CGA-79353 N-(carboxycarbonyl)-N-(2,6-dimethylphenyl) alanine methyl ester	
CGA-94689 N-[(2-hydroxymethyl)-6-methylphenyl]-N-(methoxy acetyl)-alanine methyl ester	

FIGURE 1. CHEMICAL NAMES AND STRUCTURES (Continued)

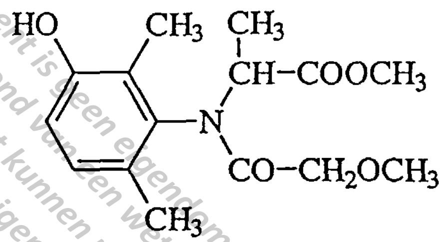
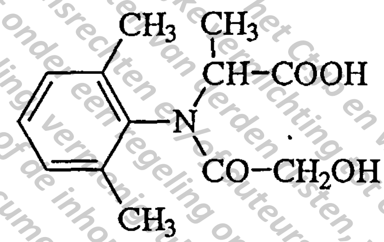
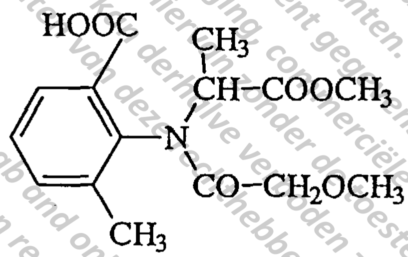
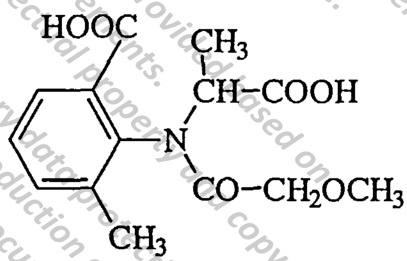
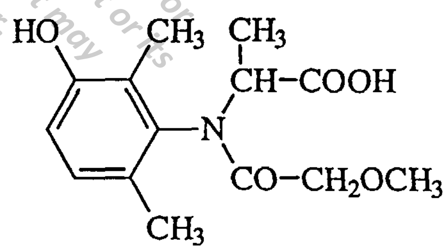
Company Code Chemical Name	Structure
CGA-100255 N-(3-hydroxy-2,6-dimethyl phenyl)-N-(methoxyacetyl) alanine methyl ester	
CGA-107955 N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-alanine	
CGA-108905 2-[(methoxyacetyl)(2-methoxy-1-methyl-2-oxoethyl)amino]-3-methylbenzoic acid	
CGA-108906 2-[(1-carboxyethyl)(methoxy acetyl)amino]-3-methyl benzoic acid	
CGA-119857 N-(3-hydroxy-2,6-dimethyl phenyl)-N-(methoxyacetyl)-alanine	

FIGURE 1. CHEMICAL NAMES AND STRUCTURES (Continued)

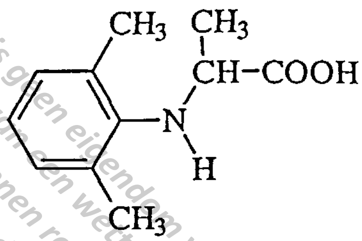
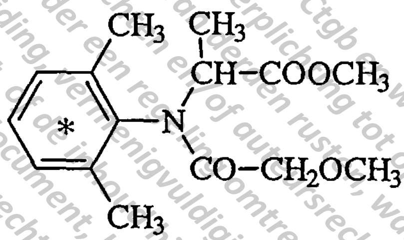
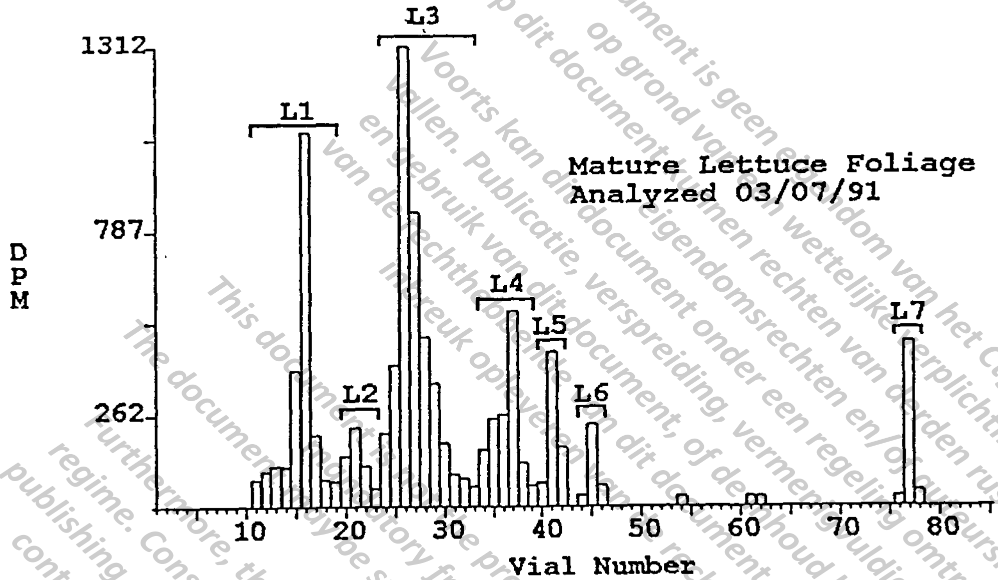
Company Code Chemical Name	Structure
CGA-67867 N-(2,6-dimethylphenyl)-alanine	
[phenyl-^{14}C]-metalaxyl	 * indicates position of radiolabeled atom

FIGURE 2. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE LETTUCE FOLIAGE.



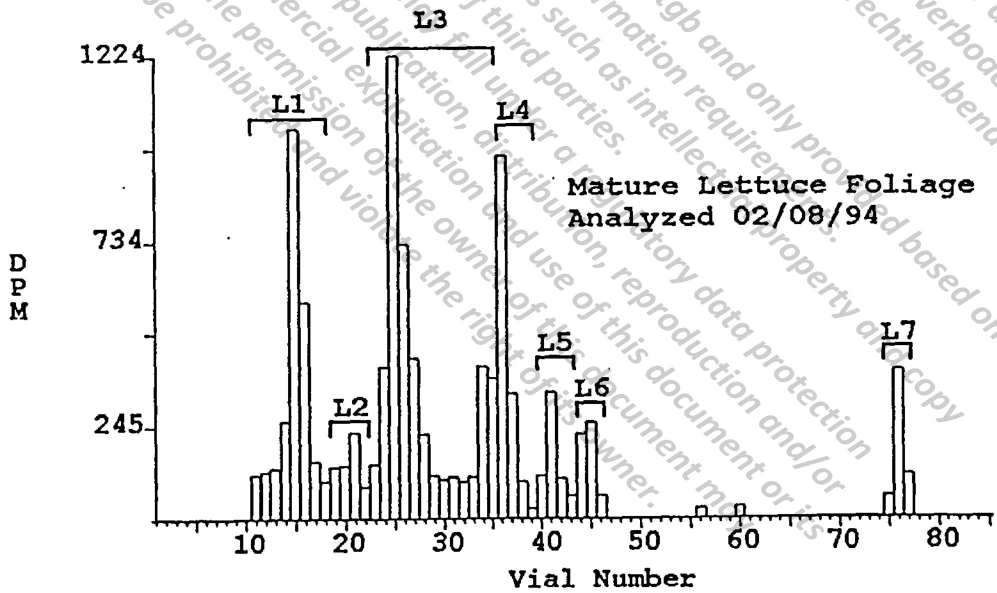
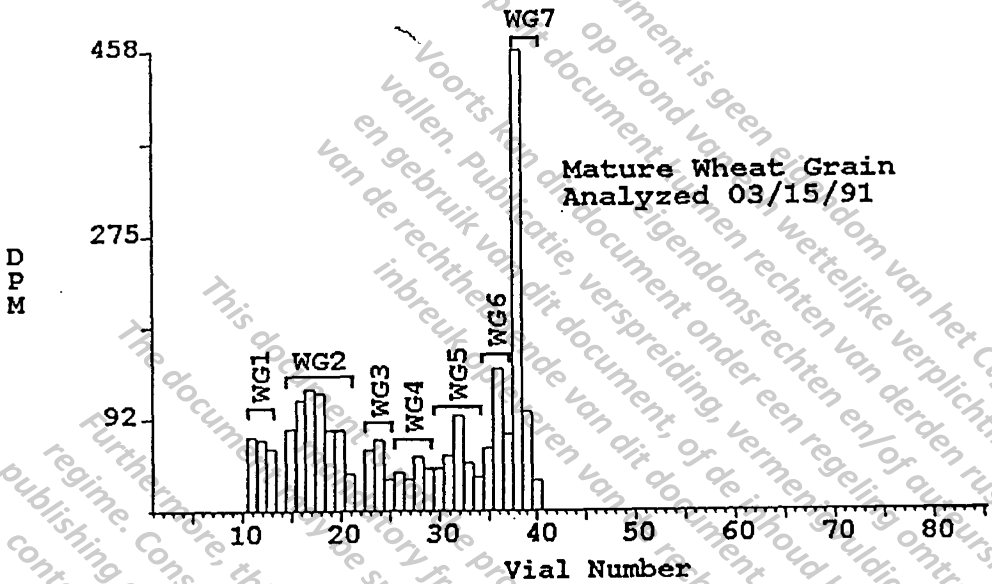


FIGURE 3. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SPRING WHEAT GRAIN



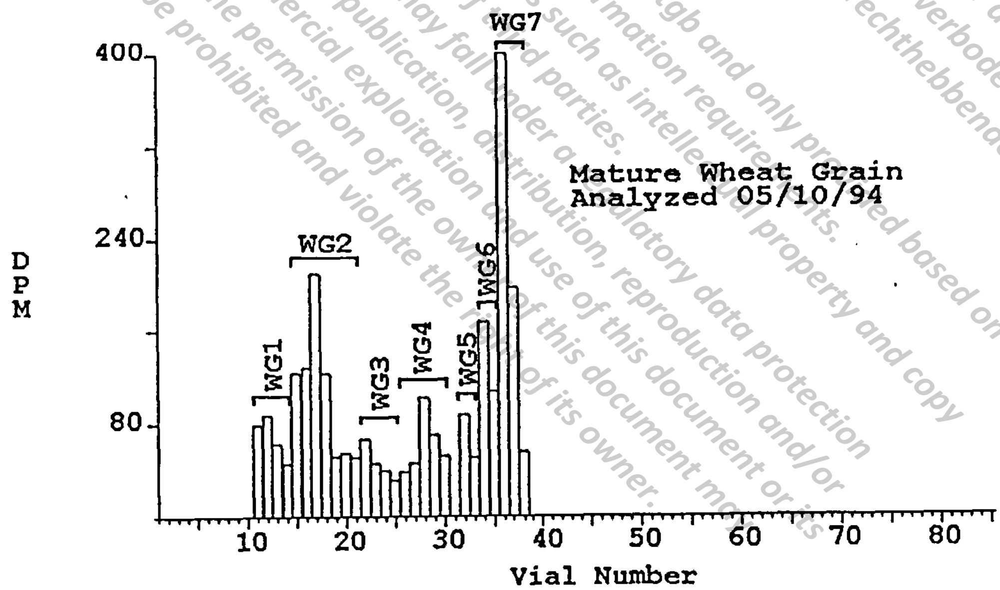
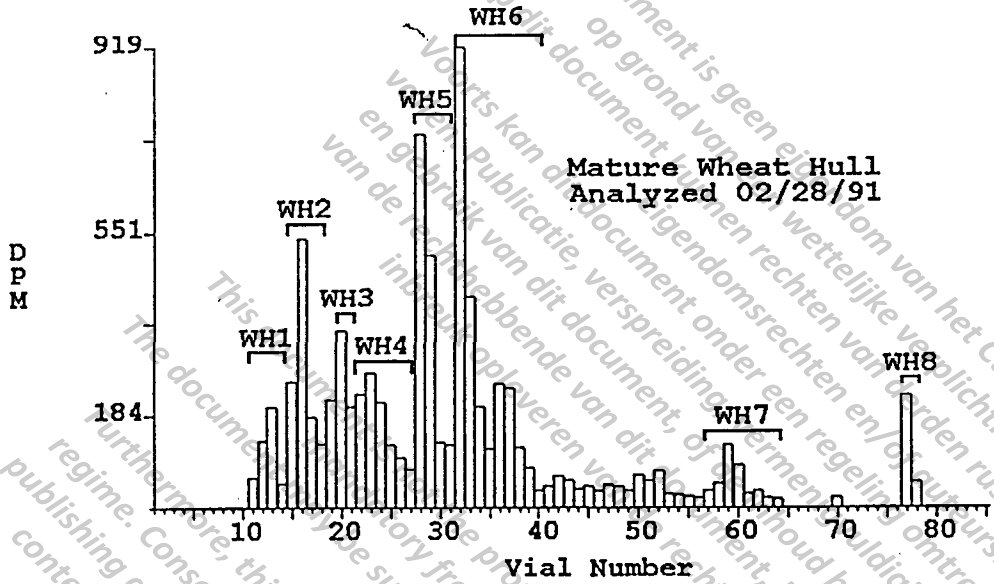


FIGURE 4. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SPRING WHEAT HULL



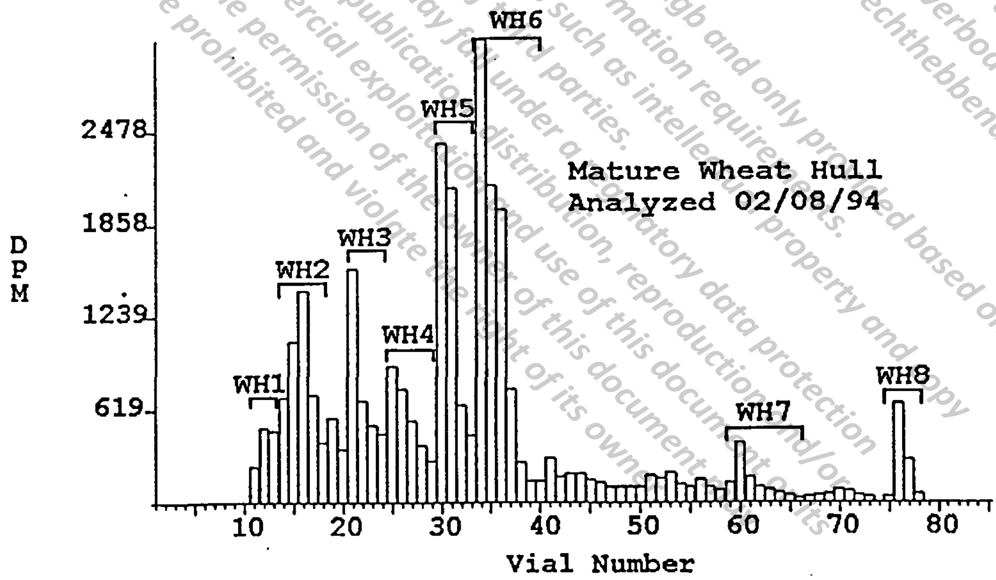
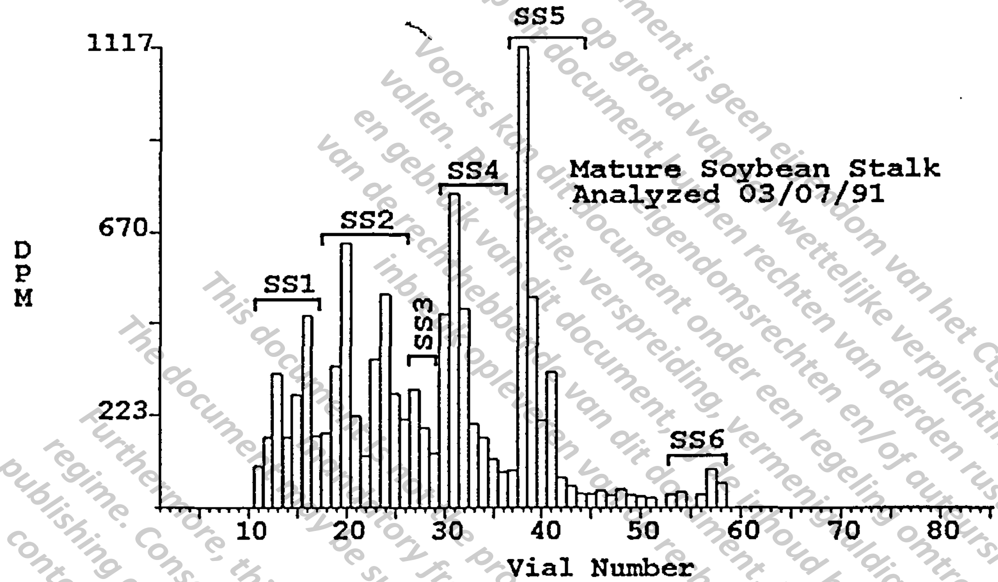


FIGURE 5. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN STALK



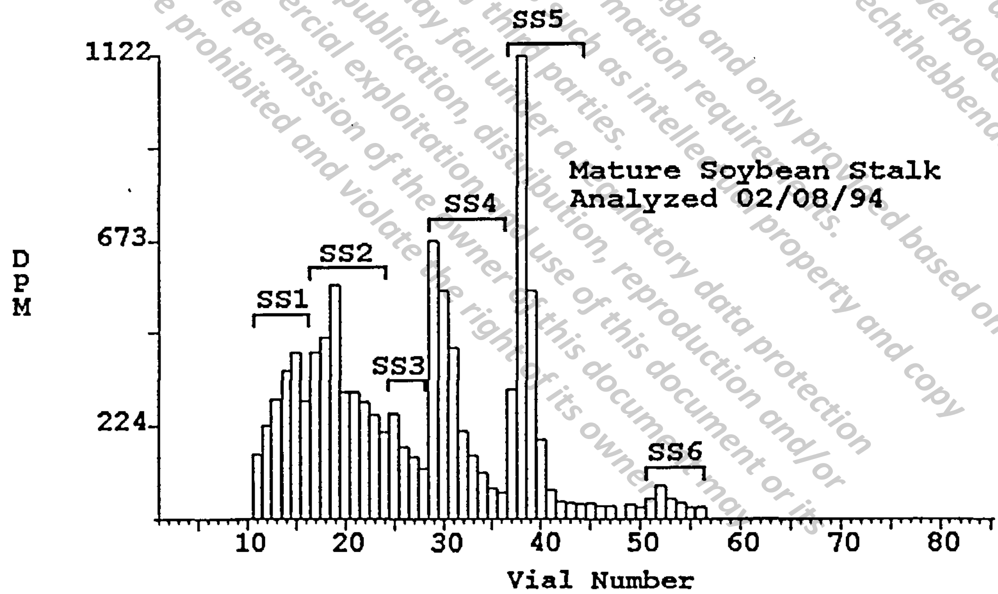
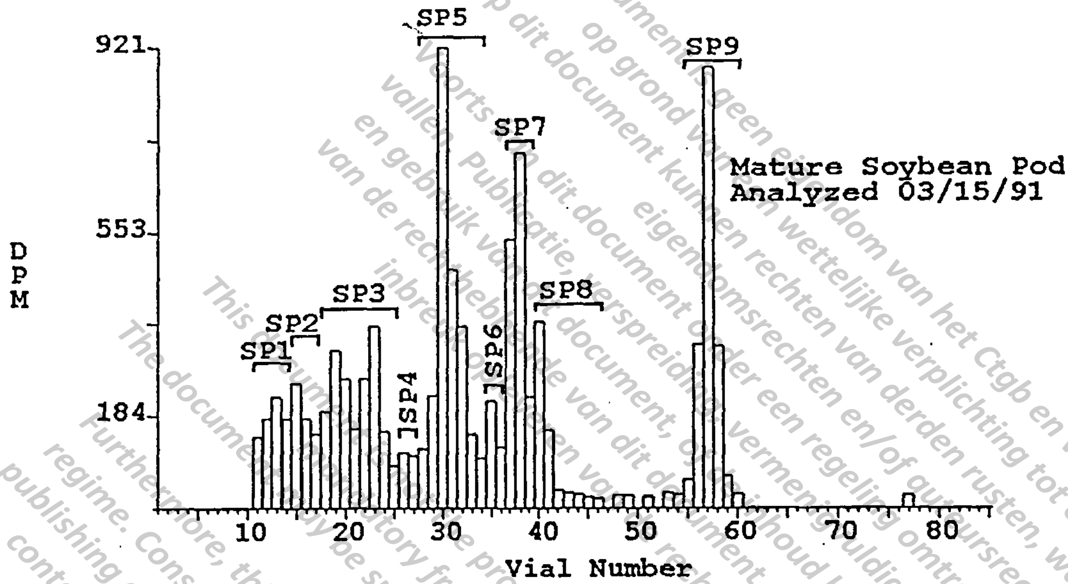


FIGURE 6. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN POD



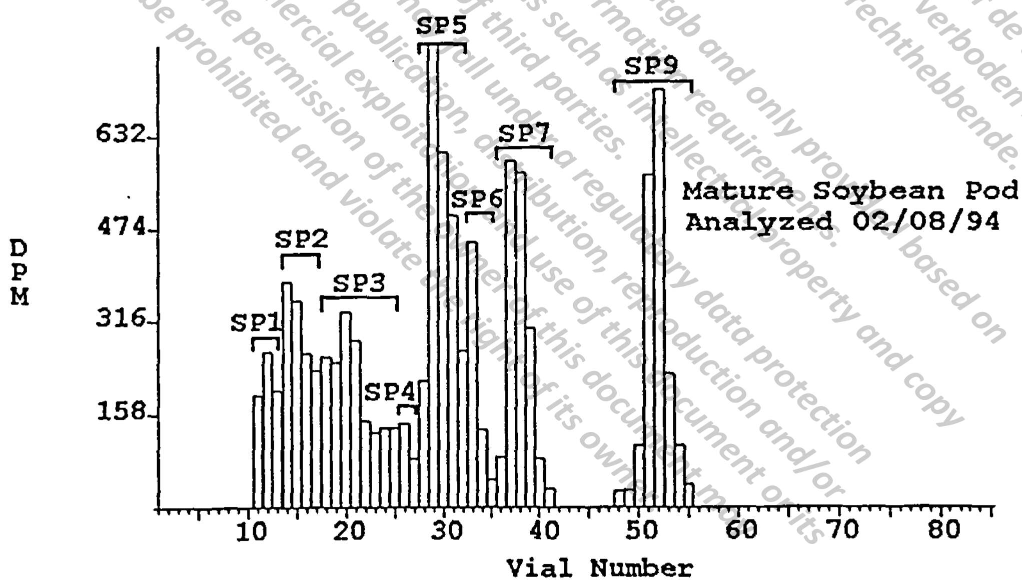
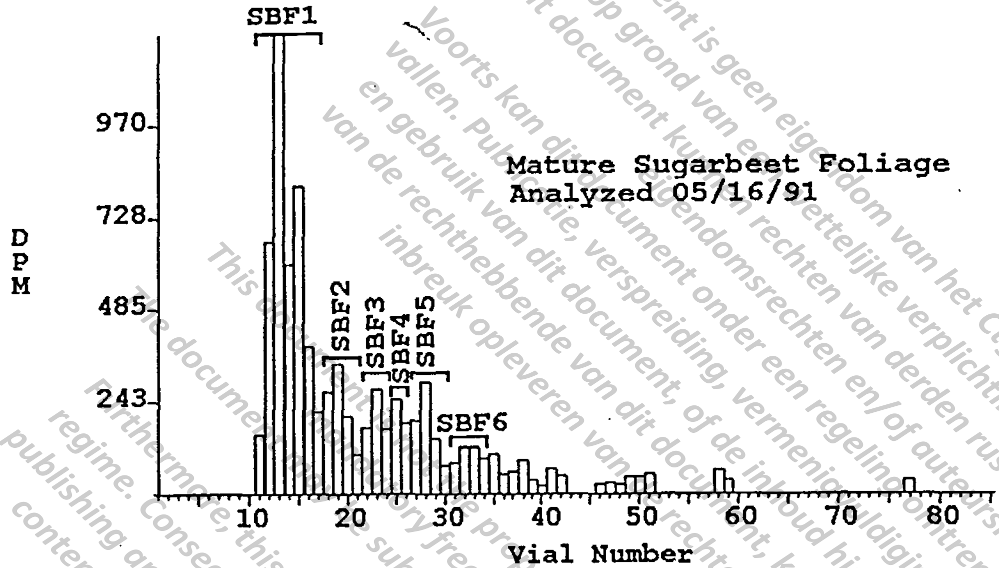


FIGURE 7. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SUGARBEET FOLIAGE



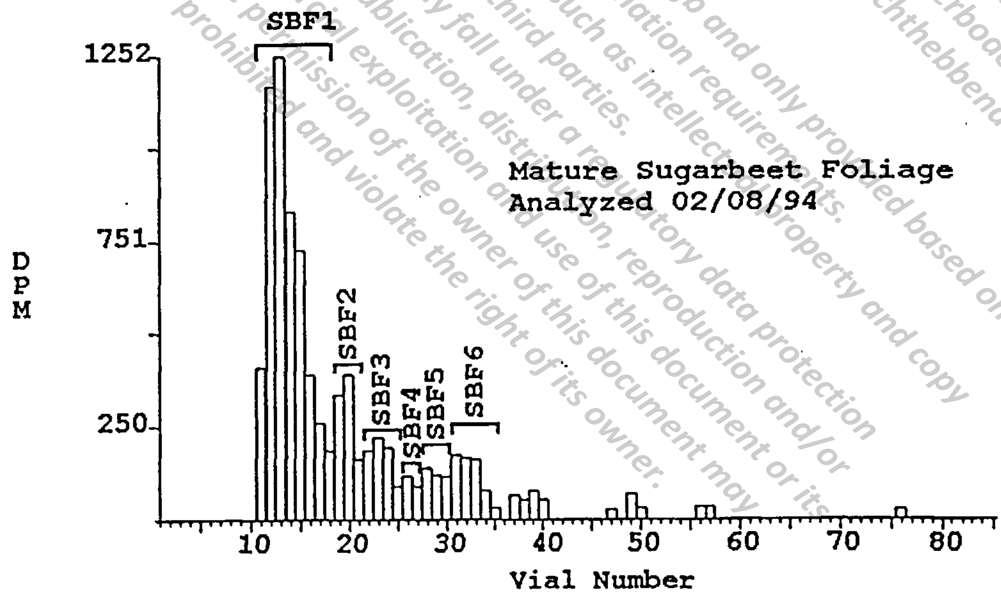
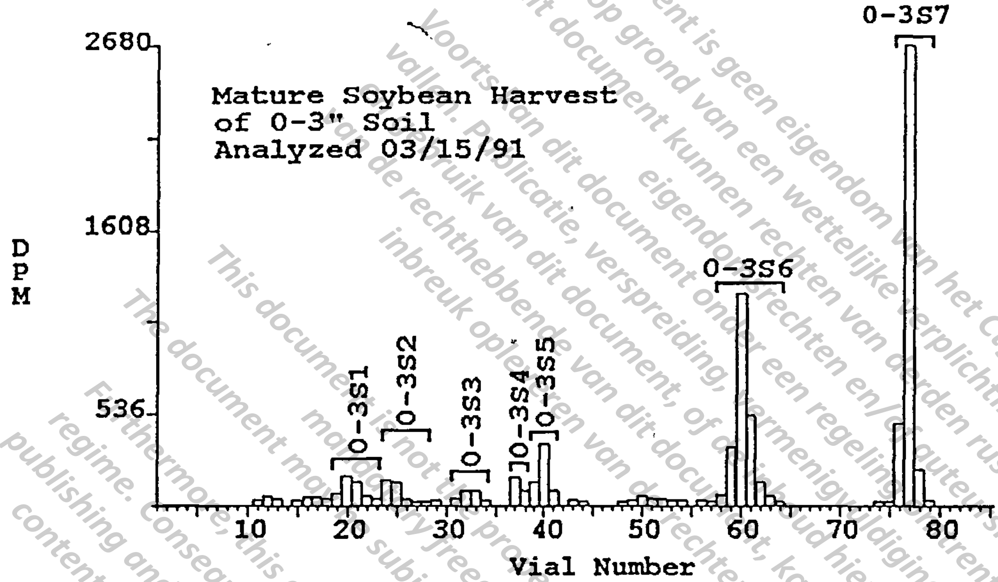


FIGURE 8. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN HARVEST 0-3" SOIL



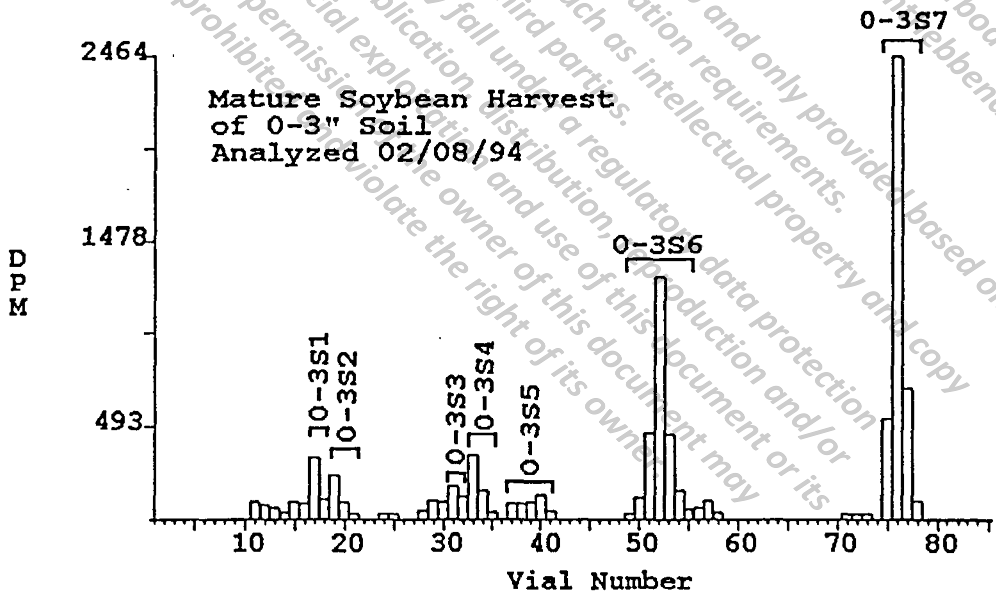
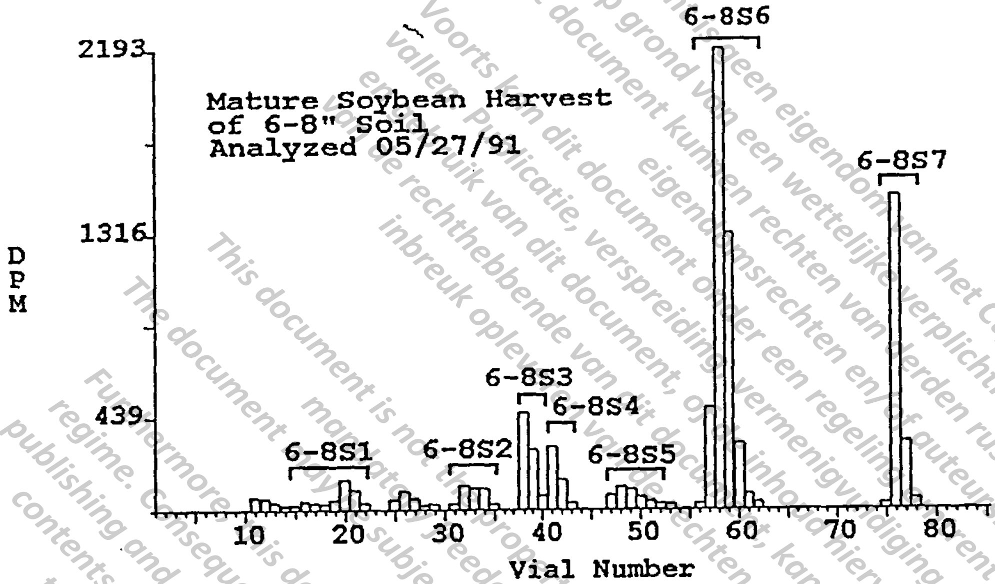


FIGURE 9. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN HARVEST 6-8" SOIL



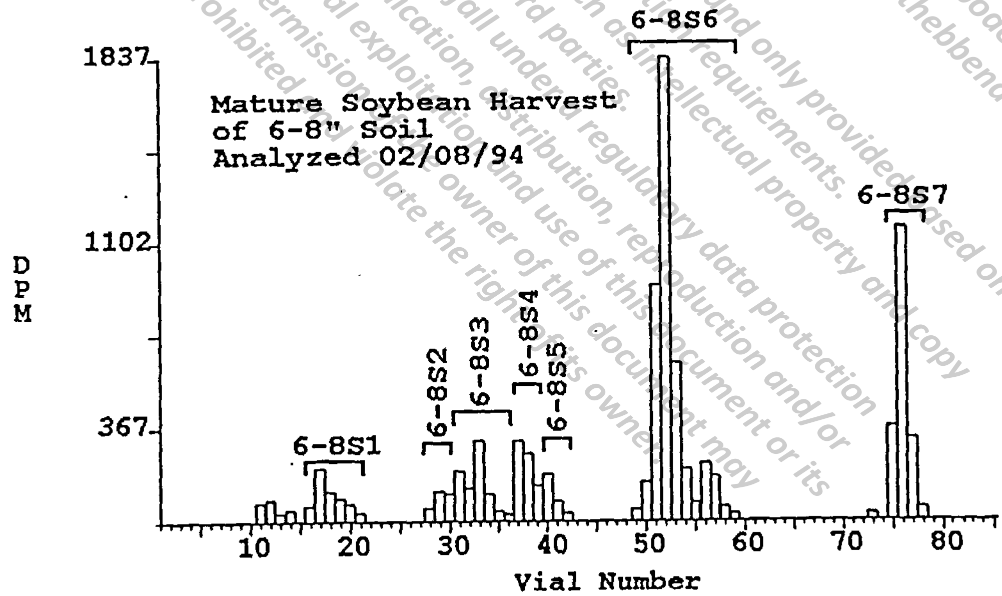
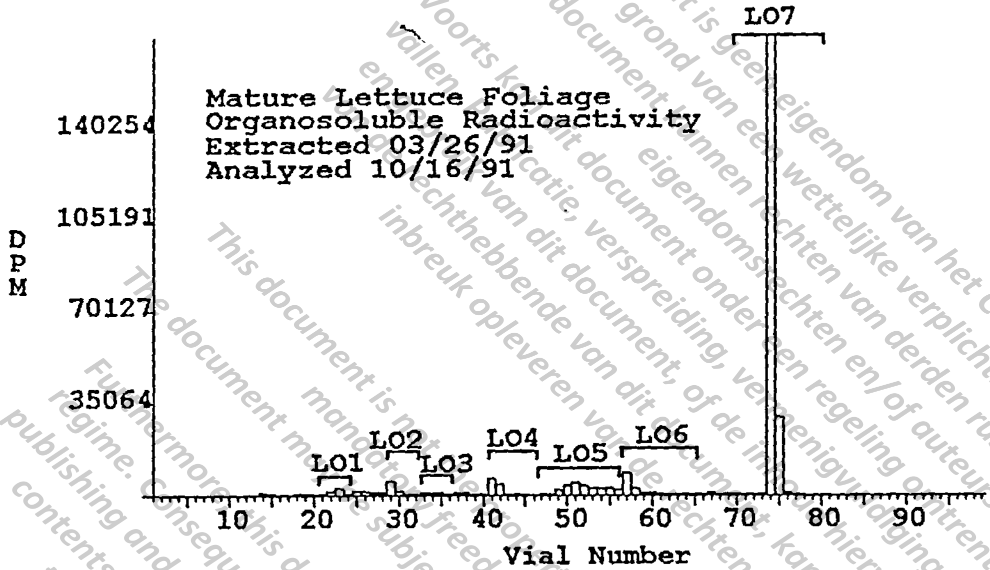


FIGURE 10. STORAGE STABILITY ANALYSIS BY HPLC OF THE ORGANOSOLUBLE RADIOACTIVITY IN TWO SEPARATE EXTRACTIONS OF PHENYL-^{14}C-METALAXYL 50% MATURE LETTUCE FOLIAGE



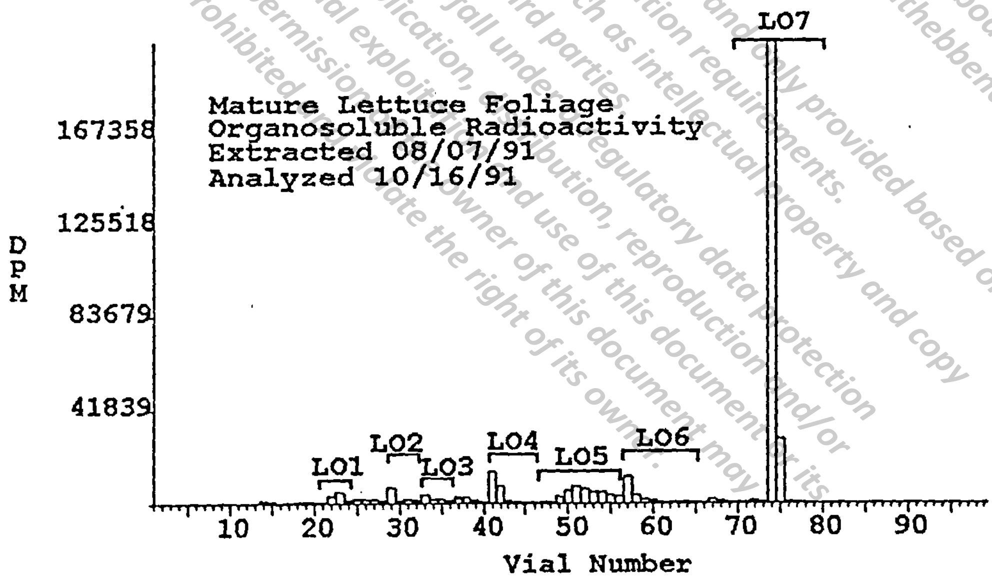
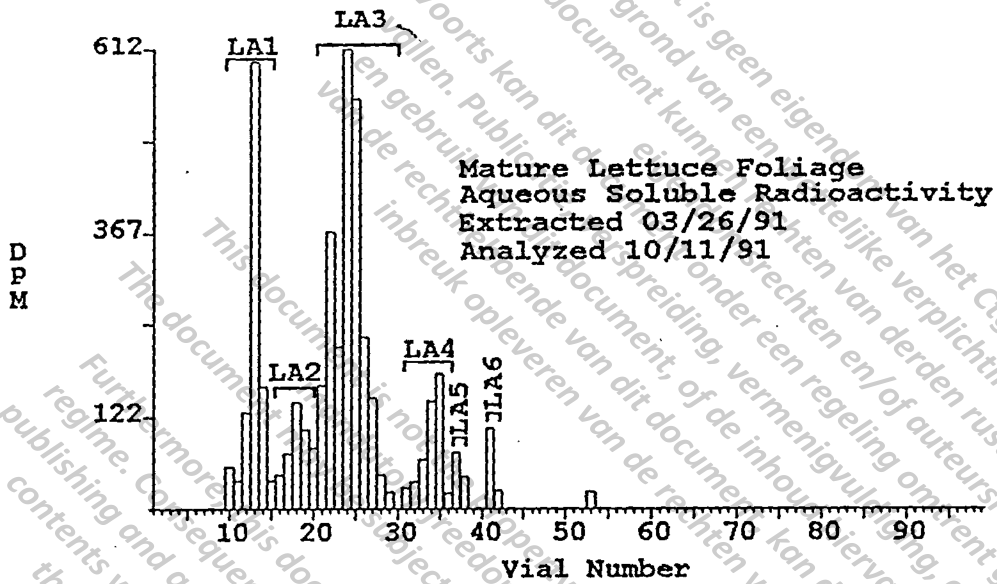


FIGURE 11. STORAGE STABILITY ANALYSIS BY HPLC OF THE AQUEOUS SOLUBLE RADIOACTIVITY IN TWO SEPARATE EXTRACTIONS OF PHENYL-^{14}C-METALAXYL 50% MATURE LETTUCE FOLIAGE



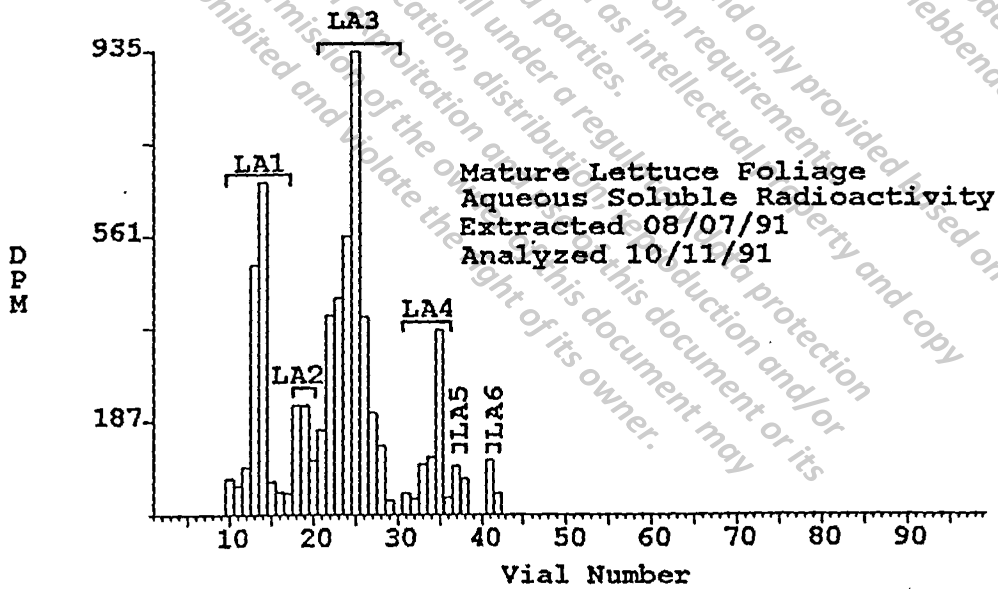
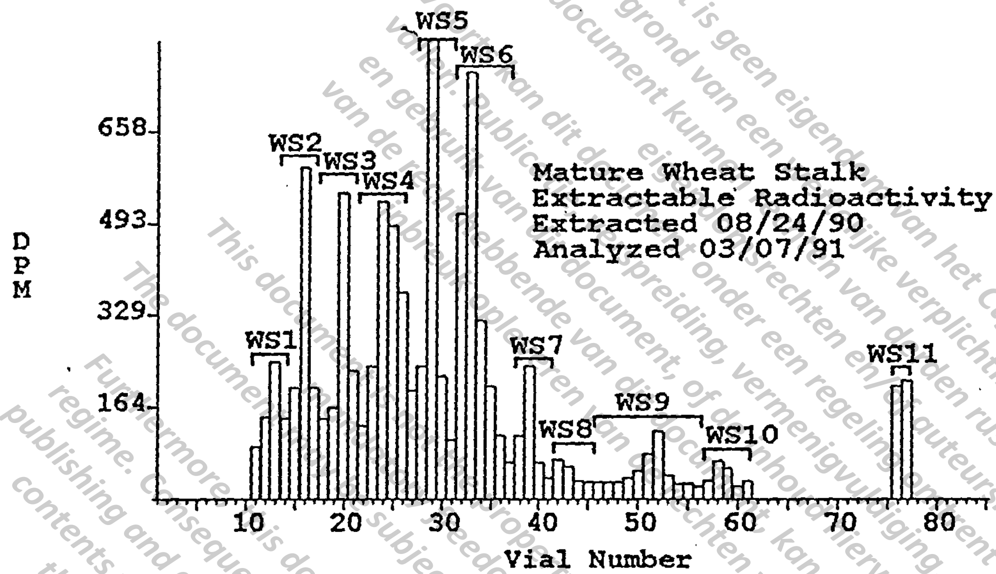


FIGURE 12. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN TWO SEPARATE EXTRACTIONS OF PHENYL-^{14}C-METALAXYL MATURE WHEAT STALK



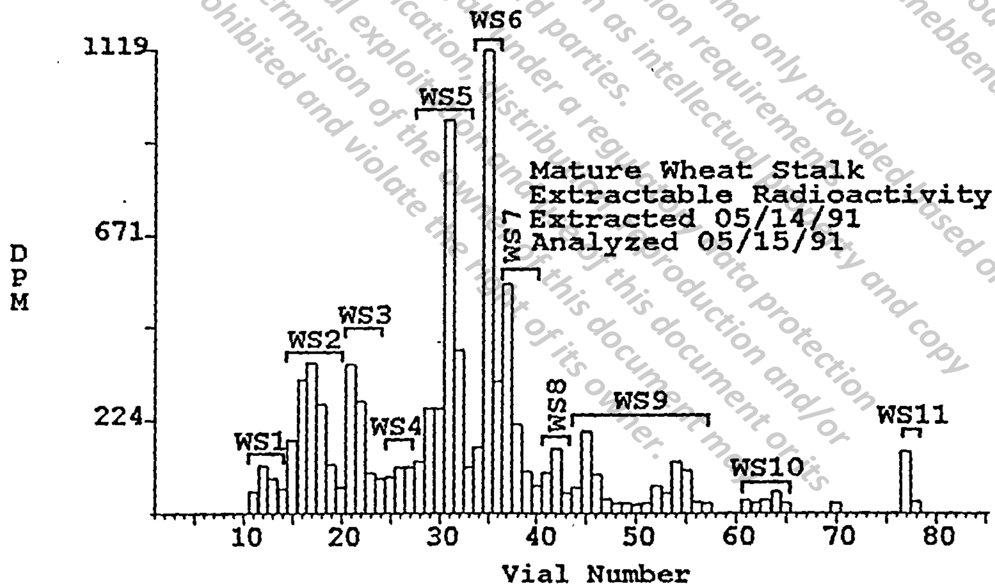
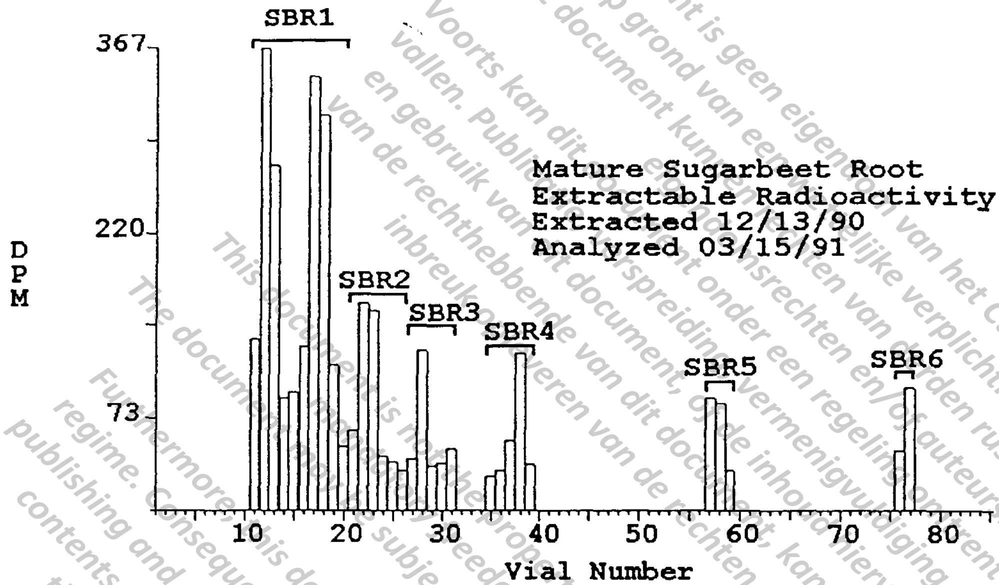
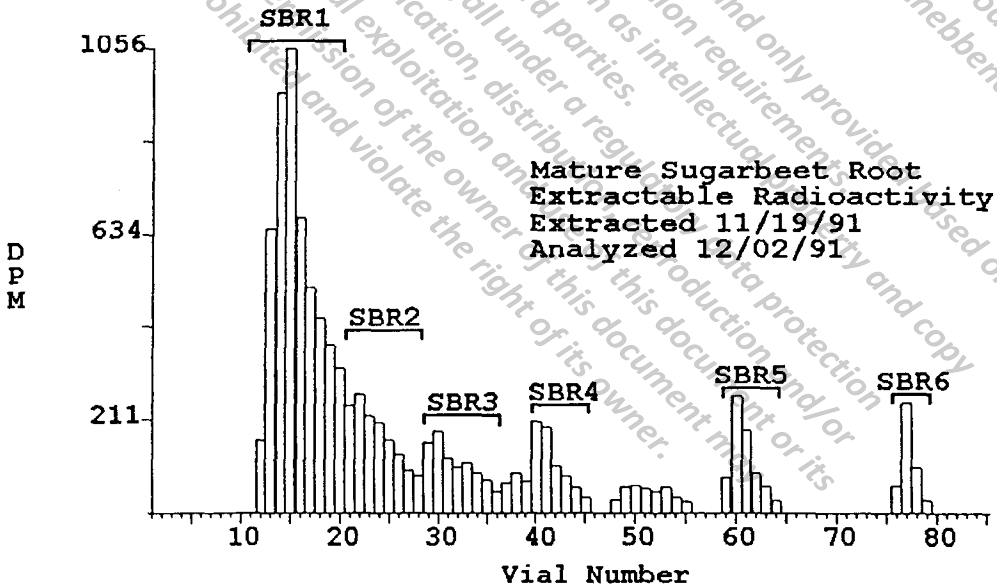


FIGURE 13. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN TWO SEPARATE EXTRACTIONS OF PHENYL-^{14}C-METALAXYL MATURE SUGARBEET ROOT





VIII. REFERENCES

1. [redacted], "Uptake and Metabolism of ^{14}C-CGA-48988 as a Preemergence Treatment on Greenhouse Grown Tobacco," with Amendments; "Protocol 203-89 Part A, Uptake and Metabolism of ^{14}C-CGA-48988 in Greenhouse Rotational Crops Following a Tobacco Target Crop in Soil Previously Treated With ^{14}C-CGA-48988," with Amendments; and "Protocol 203-89 Part B Development of Methods for the Determination of Nonextractable Residues in Greenhouse Grown Target Tobacco and Rotational Crops Following Soil Treatment with Phenyl-^{14}C-CGA-48988," with Amendments.

2. ABR-91084, [redacted] "Uptake and Metabolism of Metalaxyl in Greenhouse Rotational Crops Following Target Tobacco Grown in Soil Treated With [Phenyl-^{14}C]-Metalaxyl," MRID #42196501.

3. Ciba-Geigy Corporation, Ridomil® 2E Fungicide Label, 1994, EPA Reg. No. 100-607.

4. BIOL-90016, [redacted], "Biological Report for Greenhouse Rotational Crops Grown After Tobacco in Soil Treated With ^{14}C-CGA-48988," MRID #42196501.

5. BIOL-90017, [redacted], "Biological Report for Greenhouse Tobacco Grown in Soil Treated With ^{14}C-CGA-48988," MRID #42196501.

VOLUME 3 OF 3 OF SUBMISSION

METALAXYL

REPORT AMENDMENT 1

STUDY TITLE

FINAL REPORT AMENDMENT ON THE UPTAKE AND METABOLISM OF METALAXYL IN GREENHOUSE ROTATIONAL CROPS FOLLOWING TARGET TOBACCO GROWN IN SOIL TREATED WITH [PHENYL-14C]-METALAXYL

DATA REQUIREMENT

EPA GUIDELINE NUMBER 165-1

AUTHOR

[redacted]

STUDY COMPLETED ON

JANUARY 29, 1992

EPA MRID 42196501

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 49

Ciba Crop Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27419

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA section 10(d) (1) (A), (B), or (C).

Company: Ciba-Geigy Corporation

Company Representative: 5.1.2.e Woo

Title: Regulatory Manager

Signature: 5.1.2.e Woo Date: 7/25/94

These data are the property of Ciba-Geigy Corporation and, as such, are considered to be confidential for all purposes other than compliance with FIFRA section 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Because this volume contains an amendment to a previously submitted study, EPA MRID 42196501, the Good Laboratory Practice Statement appearing in the original study covers this amendment as well.

The Good Laboratory Practice Compliance Statement as defined by 40 CFR Part 160, found on page 10 of this volume, and signed by the study director is truthful and accurate.

5.1.2.e Woo 7/25/94

Signature of Representative of Submitter/Sponsor Date

5.1.2.e Woo

Manager, Metabolism
Biochemistry Department

Submitter/Sponsor: Ciba Crop Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27419

3599

Biochemistry Department
Ciba Plant Protection
Ciba-Geigy Corporation
Greensboro, North Carolina

FINAL REPORT AMENDMENT ON THE UPTAKE AND METABOLISM OF METALAXYL IN GREENHOUSE ROTATIONAL CROPS FOLLOWING TARGET TOBACCO GROWN IN SOIL TREATED WITH [PHENYL-^{14}C]-METALAXYL

AMENDMENT 1

Registration Category: Pesticide Assessment Guidelines
Subdivision N
Environmental Fate
EPA Guideline No. 165-1
Section 158.290
Confined Accumulation Studies on Rotation Crops

Report No.: ABR-91084 **Project No.:** 409925

Study Director: [redacted] **Approved By:** [redacted]

Title: Research Specialist **Title:** Manager, Metabolism

Signature: [redacted] **Signature:** [redacted]

Date: 7/21/94 **Date:** 7/21/94

Submitted by: [redacted]

Sponsor: Biochemistry Department
Ciba Plant Protection
Ciba-Geigy Corporation
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

Study Initiation Date: September 11, 1989

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The supplemental portion of this study was conducted in accordance with the applicable EPA Good Laboratory Practice Standards (40 CFR Part 160) with the following exception:

1. Standards CGA-62826 and CGA-67869 were qualitative standards used to aid calibration of the HPLC instrument and were not used for quantitative or qualitative purposes.

5.1.2.e Woo ______________________ 7-21-94
Study Director Date

5.1.2.e Woo ______________________ 7-21-94
Manager, Metabolism Date
Representative of Submitter/Sponsor

Submitter/Sponsor: Biochemistry Department
Ciba Plant Protection
Ciba-Geigy Corporation
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

QUALITY ASSURANCE STATEMENT

CIBA-GEIGY
CIBA CROP PROTECTION
QUALITY ASSURANCE UNIT

QUALITY ASSURANCE STATEMENT

Study Title: Final Report Amendment On The Uptake And Metabolism of Metalaxyl in Greenhouse Rotational Crops Following Target Tobacco Grown In Soil Treated with [Phenyl-^{14}C]-Metalaxyl: Amendment 1

Study Director: [redacted] 5.1.2.e Woo

Final Report Number: ABR-91084

Study Number: 203-89 and Amendments

Pursuant to Good Laboratory Practice Regulations, this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

INSPECTION/AUDIT TYPE	INSPECTION/AUDIT DATES	REPORTING DATE
In-Progress Inspection	02/08/94	02/08/94
Final Report Audit	07/1,5,6/94	07/07/94

Prepared By: [redacted] 5.1.2.e Woo Date: 7/07/94

GENERAL INFORMATION

Protocol Number: 203-89[1] with Amendment Lists (Tobacco), 203-89-Part A with Amendment Lists (Rotational Crops), and 203-89-Part B with Amendment Lists (Non-extractable Residues)

Guidelines: This study is being conducted to support the requirements outlined in: Pesticide Assessment Guidelines, Subdivision N, Environmental Fate EPA Guideline No. 165-1, Section 158.290, Confined Accumulation Studies on Rotational Crops.

Sponsor:
Ciba Plant Protection
Ciba-Geigy Corporation
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

Test Substance:
[Phenyl-^{14}C]-Metalaxyl
Code: ^{14}C-CGA-48988
Common Name: Metalaxyl
Trade Name: Ridomil®
Specific Activity: 29.8 µCi/mg
Lot No.: CL-XX-34
Radiochemical Purity: 98.4%
Chemical Purity: 99.7%

Carrier: Acetone - HPLC Grade
Fisher Scientific Lot No.: 891019

Experiment Numbers:
M90-409-001P (Lettuce)
M90-409-002P (Spring Wheat)
M90-409-003P (Soybeans)
M90-409-004P (Sugar Beets)
M90-409-005S (Soils)

Study Director:
5.1.2.e Woo
Biochemistry Department
Ciba Plant Protection
Ciba-Geigy Corporation
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

Study Coordinator:
5.1.2.e Woo

Study Participants:
5.1.2.e Woo

Testing Facility:
Biochemistry Department
Ciba Plant Protection
Ciba-Geigy Corporation
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

Supplier of the Test Substance and Reference Materials:
5.1.2.e Woo
Chemical Synthesis Group
Ciba Plant Protection
Ciba-Geigy Corporation
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

Testing Periods:

Tobacco Biology Phase (Target Crop)

Protocol Approval - 09/11/89
Soil Treatment - 09/13/89
Tobacco Transplant - 09/13/89
Mature Tobacco Harvest - 04/27/90

Rotational Crop Biology Phase

Protocol Approval - 05/03/90
Crop Planting - 05/03/90
Final Harvest - 11/19/90

Supplemental Analytical Phase

Start Date - 01/21/94
Termination Date - 05/10/94

Archives:

The protocol, raw data, biology reports, final report, final report amendment and other pertinent records and reports will be filed and archived at:

Biochemistry Group Archives
Ciba Crop Protection
Ciba-Geigy Corporation
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

Specimens, if not depleted during the study will be retained at Ciba-Geigy Corporation, Greensboro, NC for as long as the quality of the preparation affords evaluation, at which time the specimens will be discarded after Quality Assurance verification.

I. ABSTRACT

A. Chemical

Metalaxyl, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester, the active ingredient in Ridomil®, Apron® and Subdue® fungicides, is used in numerous food and non-food crops for the control of diseases, such as late blight, downy mildew and seedling diseases caused by oomycetes.

The structure of metalaxyl is included in Figure 1. As reported in ABR-91084[2], the uptake and metabolism of [phenyl-^{14}C]-metalaxyl was investigated in four rotational crops grown in soil previously used for growing tobacco. The tobacco target crop was treated with one soil application of [phenyl-^{14}C]-metalaxyl at the rate of 3 lbs. a.i./A which is the maximum treatment rate used in tobacco[3]. The active ingredient was mixed with acetone prior to incorporation into the soil.

B. Maintenance of Treatment Area

Details concerning the biological phase of this study are documented in ABR-91084[2], BIOL-90016 (rotational crops)[4] and BIOL-90017 (target tobacco)[5].

C. Residues

The uptake and metabolism of [phenyl-^{14}C]-metalaxyl in leaf lettuce, spring wheat, soybeans and sugarbeets planted 232 days after soil treatment is given in ABR-91084[2].

Storage stability data for homogenates and extracts is presented in this final report amendment, showing that metalaxyl and its metabolites are stable under frozen storage conditions. These results support the metabolic pathways of metalaxyl as reported in ABR-91084[2].

D. Problems

No problems occurred that would adversely affect the results of the study.

E. Contact Person

5.1.2.e Woo

II. INTRODUCTION

This study was initiated to support the reregistration of metalaxyl. Metalaxyl, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester, the active ingredient in Ridomil®, Apron® and Subdue® fungicides, is used to control specific fungi in several crops. The objectives of this study were to investigate the uptake and metabolism of [phenyl-^{14}C]-metalaxyl in rotational crops to support the data requirements in the Environmental Fate Chemistry Section 158.290, Guideline No. 165-1 (Confined Accumulation Studies in Rotational Crops).

Earlier, a final report, ABR-91084[2], was submitted to support the reregistration of metalaxyl according to Metabolism Protocol Number 203-89 (203-89, 203-89 Part A and 203-89 Part B). The objectives were to investigate the uptake and metabolism of [phenyl-^{14}C]-metalaxyl in lettuce, spring wheat, soybean and sugarbeet rotational crops grown in the greenhouse following a target crop of tobacco. The tobacco was grown in soil treated with a 3.0 lbs. a.i./A preemergent application. Data on the uptake and characterization of metabolites in the tobacco target crop treated prior to the planting of the greenhouse rotational crops were also included in ABR-91084[2]. Details on the biological phase are documented in biological report numbers BIOL-90016 (rotational crops)[4] and BIOL-90017 (target tobacco)[5].

The objective of the present final report amendment is to give storage stability data for rotational crop plant and soil homogenates and extracts. Stability data is given here because it was omitted in the final report, ABR-91084[2], and was cited as a deficiency in the Environmental Fate and Ground Water Branch (EFGWB) review of the original study. The EFGWB reviewer (Richard J. Mahler) concluded that this study provides acceptable data that shows metalaxyl residues are present in rotational crops planted 232 days after treatment. A storage stability test needs to be performed in order to determine whether metalaxyl residues in the samples degraded during handling and storage, since no data was presented relating to freezer stability during storage.

III. MATERIALS AND METHODS

Methods were as previously described in ABR-91084[2]. In this final report amendment, the following additions were made:

A. Reference Materials

In addition to the reference standards listed in Table I from ABR-91084[2], Table I in this final report amendment presents information on standards used after the submission of ABR-91084[2]. Radiolabeled and non-radiolabeled structures of metalaxyl and possible metalaxyl metabolites are shown in Figure 1. Standard stock solution concentrations, storage conditions and usage are described in ABR-91084[2].

B. Data Evaluation

Data reported as dpm, dpm/g, dpm/ml, histograms and other quantitative data calculated from dpm values were processed by the VAX Metabolism Data Base System (VAX) for the original study and by the Talisman System for the supplemental work presented. The calculations used were the same for both systems. The only difference is the definition of quantifiable DPMs.

Quantifiable DPMs as defined by the VAX Metabolism Data Base System are the DPMs that are greater than $[2 \times DPM_{bkg}]$. Quantifiable DPMs as defined by the Talisman System are the DPMs that are greater than Minimum Quantifiable Amount (MQA). MQA is defined as:

$$MQA = \{(14.1)SQRT[(avg\ DPM_{bkg})(avg\ count\ time)]\}/(avg\ count\ time)$$

For this final report amendment, VAX histogram data from ABR-91084[2] has been converted to the Talisman histogram format. The new figures were generated by using the dpm values collected by the VAX System and regraphing the data through the Talisman System. This was done to facilitate comparison of histograms from both systems.

C. Storage Stability

All of the sample homogenates and extracts were maintained in a freezer at approximately -20°C.

Extract stability was addressed by comparing the initial HPLC profiles of selected mature extracts which were analyzed within 8 months of harvest to HPLC profiles performed 3 to 4 years later.

Homogenate stability was addressed by extracting two subsamples each of 50% mature lettuce foliage, mature wheat stalk and mature sugarbeet root homogenates and comparing HPLC profiles of the extracts. Time in frozen freezer conditions between extractions ranged from 4.5 to 11.5 months.

IV. RESULTS AND DISCUSSION

A. Extract Stability

All mature plant samples and the last soil sampled were extracted and analyzed within 5 months of harvest. Harvest, extraction and analysis dates for each of these samples are given in Table II. Initial 2D-TLC analyses of the extracts were completed within approximately 1 to 5 months of harvest. The gradient for HPLC analysis was not developed until a later date. Initial HPLC analyses of the mature plant extracts were completed within approximately 3.5 to 8 months of harvest (Table II). Comparison of the HPLC analyses presented in ABR-91084[2] and HPLC analyses performed 3 to 4 years later show that metalaxyl and its metabolites are stable under frozen storage conditions in plant and soil extracts (Tables III-X and Figures 2-9).

B. Plant Homogenate Stability

Several plant samples were reextracted for identification purposes. Comparisons of HPLC profiles from these extracts were used to show that metalaxyl and its metabolites are stable under frozen storage conditions in plant homogenates.

Subsamples of 50% mature lettuce foliage were extracted 298 and 432 days after harvest. The amount extractable was 92.6% and 90.8%, respectively. Each extract was partitioned with ethyl acetate. The organic and aqueous fractions from both extracts were analyzed by HPLC. Profiles of the organic and aqueous fractions are qualitatively and quantitatively similar, thus showing stability of metalaxyl and its metabolites in frozen lettuce homogenates (Figures 10 and 11 and Table XI).

Subsamples of mature wheat stalks were extracted 22 and 285 days after harvest. The amount extractable was 79.2% and 74.5%, respectively. The extracts were analyzed by HPLC. Comparison of the HPLC quantitation shows quantitative variances in composition, particularly for peaks WS1, WS4, WS5 and WS7. (Figure 12 and Table XII). However, it can be concluded that the qualitative nature of the metalaxyl residues were preserved during the freezer storage conditions employed in this study.

Subsamples of mature sugarbeet roots were extracted 45 and 386 days after harvest. The amount extractable was 62.5% and 61.5%, respectively. The extracts were analyzed by HPLC. Comparison of the HPLC quantitation shows peak shifting and differing peak resolution, probably due to the use of different HPLC instrument systems (Figure 13 and Table XIII). However, the quantitation is similar for corresponding regions; thus, it is concluded that metalaxyl and its metabolites are stable under frozen storage conditions in sugarbeet homogenates.

V. CONCLUSION

Comparison of HPLC profiles of extractable radioactivity from various rotational crop plant and soil samples with the profiles of extracts stored in the freezer for approximately 3 to 4 years showed that the metalaxyl metabolites in rotational crops are stable during frozen storage.

TABLE I. SYNTHESIS REFERENCE NUMBERS AND PURITY INFORMATION FOR METALAXYL STANDARDS

STANDARD	RECEIPT DATE	SYNTHESIS REFERENCE NUMBER	PURITY	INFORMATION REANALYSIS DATE
CGA-78532	02/02/94	JAK-IV-15-1	>99.9	01/96
CGA-68124	02/02/94	BPM-X-31	>99.9	01/96
CGA-108906	02/02/94	WFH-VII-96	>99.9	01/96
CGA-119857	02/02/94	GB-XLV-3	>99.9	06/94
CGA-62826	02/02/94	BPM-I-4B	>99.9	03/94
CGA-37734	02/02/94	BPM-I-8	96.9	02/96
CGA-67867	02/02/94	RAF-IX-58	>99.9	01/96
CGA-100255	02/02/94	WFH-VII-86	99.7	01/96
CGA-94689A/B	02/02/94	JAK-II-21	96.5	06/94
CGA-67868	02/02/94	BPM-X-26	>99.9	01/96
CGA-67869	02/02/94	DAH-XV-35	>99.9	04/94
CGA-48988	02/02/94	BPM-XIII-58	>99.9	01/96
CGA-67866	02/02/94	RAF-IX-91	99.4	01/96
CGA-107955	02/02/94	GB-XLVI-7	>99.9	02/95
CGA-79353	02/02/94	JAK-IV-14-1	99.6	01/96
CGA-108905	02/02/94	GB-XLIV-78	95.5	07/94

TABLE II. HARVEST, EXTRACTION AND ANALYSIS DATES FOR MATURE PLANT AND SOIL SAMPLES

	Harvest Date	First Extraction Date	Days After Harvest	TLC Analysis Date	Days After Harvest	First HPLC Analysis Date	Days After Harvest	Second HPLC Analysis Date	Days After Harvest
Lettuce									
Mature Foliage	07/02/90	09/14/90	74	09/18/90	78	03/07/91	248	02/08/94	1317
Spring Wheat									
Mature Stalk	08/02/90	08/24/90	22	08/24/90	22	03/07/91	217	--	--
Mature Grain	08/02/90	09/07/90	36	09/18/90	47	03/15/91	225	05/10/94	1377
Mature Hull	08/02/90	08/28/90	26	09/07/90	36	02/28/91	210	02/08/94	1286
Soybean									
Mature Stalk	11/17/90	01/11/91	55	01/11/91	55	03/07/91	110	02/08/94	1179
Mature Pod	11/17/90	01/15/91	59	01/16/91	60	03/15/91	118	02/08/94	1179
Mature Bean	11/17/90	01/17/91	61	--	--	--	--	--	--
Sugarbeet									
Mature Foliage	10/29/90	03/27/91	149	04/03/91	156	05/16/91	199	02/08/94	1198
Mature Root	10/29/90	12/13/90	45	12/19/90	51	03/15/91	137	--	--
Mature Soybean Soil									
0-3"	11/17/90	01/18/91	62	01/23/91	67	03/15/91	118	02/08/94	1179
6-8"	11/17/90	04/05/91	139	04/10/91	144	05/27/91	191	02/08/94	1179

TABLE III. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE LETTUCE FOLIAGE

Harvest Date	07/02/90
Extraction Date	09/14/90
% Extractable	90.8

Peak[1]	Injection #1 03/07/91 % TRR	Injection #2 02/08/94 % TRR
L1	20.4	21.1
L2	5.0	5.0
L3	37.1	37.8
L4	13.1	12.0
L5	6.3	5.2
L6	3.0	4.5
L7	5.0	4.8

[1]See Figure 2 for corresponding peaks.

TABLE IV. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SPRING WHEAT GRAIN

Harvest Date	08/02/90
Extraction Date	09/07/90
% Extractable	22.0

Peak[1]	Injection #1 03/15/91 % TRR	Injection #2 05/10/94 % TRR
WG1	2.0	2.4
WG2	6.0	6.3
WG3	1.6	1.6
WG4	1.6	2.6
WG5	2.6	1.2
WG6	2.7	2.4
WG7	5.6	5.6

[1]See Figure 3 for corresponding peaks.

TABLE V. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SPRING WHEAT HULL

Harvest Date	08/02/90
Extraction Date	08/28/90
% Extractable	60.8

Peak[1]	Injection #1 02/28/91 % TRR	Injection #2 02/08/94 % TRR
WH1	3.1	2.3
WH2	7.8	8.3
WH3	5.5	6.1
WH4	7.1	5.5
WH5	10.7	10.8
WH6	16.8	16.3
WH7	2.8	1.9
WH8	1.9	2.0

[1]See Figure 4 for corresponding peaks.

TABLE VI. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN STALK

Harvest Date 11/17/90
Extraction Date 01/11/91
% Extractable 88.2

Peak[1]	Injection #1 03/07/91 % TRR	Injection #2 02/08/94 % TRR
SS1	14.3	14.8
SS2	24.5	23.8
SS3	5.2	6.0
SS4	19.6	19.4
SS5	20.6	20.5
SS6	2.1	2.3

[1]See Figure 5 for corresponding peaks.

TABLE VII. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN POD

Harvest Date	11/17/90
Extraction Date	01/15/91
% Extractable	81.6

Peak[1]	Injection #1 03/15/91 % TRR	Injection #2 02/08/94 % TRR
SP1	5.9	5.1
SP2	4.7	9.8
SP3	14.7	13.2
SP4	1.7	1.8
SP5	19.3	18.9
SP6	2.8	5.1
SP7	12.1	13.3
SP8	5.5	---
SP9	13.8	14.4

[1]See Figure 6 for corresponding peaks.

TABLE VIII. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SUGARBEET FOLIAGE

Harvest Date	10/29/90
Extraction Date	03/27/91
% Extractable	88.1

Peak[1]	Injection #1 05/16/91 % TRR	Injection #2 02/08/94 % TRR
SBF1	44.6	54.4
SBF2	10.1	9.3
SBF3	6.9	7.1
SBF4	4.8	2.1
SBF5	7.8	3.9
SBF6	4.7	6.3

[1]See Figure 7 for corresponding peaks.

TABLE IX. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN HARVEST 0-3" SOIL

Harvest Date	11/17/90
Extraction Date	01/18/91
% Extractable	29.6

Peak[1]	Injection #1 03/15/91 % TRR	Injection #2 02/08/94 % TRR
0-3S1	1.7	1.4
0-3S2	1.3	1.1
0-3S3	0.9	0.9
0-3S4	0.9	1.7
0-3S5	2.1	1.4
0-3S6	8.3	8.0
0-3S7	11.8	11.9

[1]See Figure 8 for corresponding peaks.

TABLE X. STABILITY OF EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN HARVEST 6-8" SOIL

Harvest Date	11/17/90
Extraction Date	04/05/91
% Extractable	20.7

Peak[1]	Injection #1 05/27/91 % TRR	Injection #2 02/08/94 % TRR
6-8S1	1.0	1.3
6-8S2	0.8	0.6
6-8S3	1.8	1.8
6-8S4	1.1	1.6
6-8S5	0.9	0.7
6-8S6	9.9	9.8
6-8S7	4.2	4.3

[1]See Figure 9 for corresponding peaks.

TABLE XI. COMPARISON OF HPLC QUANTITATION FROM TWO SEPARATE EXTRACTIONS OF 50% MATURE LETTUCE FOLIAGE

	50% Mature Lettuce Foliage	
	Extraction #1	Extraction #2
Date Harvested	06/01/90	06/01/90
Date Extracted	03/26/91	08/07/91
Days Post Harvest	298	432
PPM	0.877	0.877
% Extractable	92.6	90.8
% Organic	24.9	23.1
% Aqueous	75.1	76.9

Peak[3]	% TRR[1] #1	% TRR[1] #2	Peak[4]	% TRR[2] #1	% TRR[2] #2
LO1	0.4	0.5	LA1	15.5	17.0
LO2	0.6	0.6	LA2	6.9	6.0
LO3	0.3	0.5	LA3	35.9	35.8
LO4	1.0	1.4	LA4	7.2	7.6
LO5	1.9	2.3	LA5	1.9	1.9
LO6	1.2	1.2	LA6	2.0	1.6
LO7	16.6	13.3			

[1]The organic fractions from both extracts were analyzed 502 days after harvest.
[2]The aqueous fractions from both extracts were analyzed 497 days after harvest.
[3]See Figure 10 for corresponding peaks.
[4]See Figure 11 for corresponding peaks.

TABLE XII. COMPARISON OF HPLC QUANTITATION FROM TWO SEPARATE EXTRACTIONS OF MATURE SPRING WHEAT STALK

	Mature Spring Wheat Stalk	
	Extraction #1	Extraction #2
Date Harvested	08/02/90	08/02/90
Date Extracted	08/24/90	05/14/91
Days Post Harvest	22	285
PPM	7.171	7.171
% Extractable	79.2	74.5

	% TRR[1]	
Peak[2]	#1	#2
WS1	5.1	2.6
WS2	9.2	11.0
WS3	8.7	6.8
WS4	14.7	2.6
WS5	11.2	17.5
WS6	15.9	13.4
WS7	3.7	7.8
WS8	1.6	2.5
WS9	4.0	7.3
WS10	1.7	1.3
WS11	3.4	1.5

[1]The extractable fractions from both extracts were analyzed 217 and 286 days after harvest, respectively.
[2]See Figure 12 for corresponding peaks.

TABLE XIII. COMPARISON OF HPLC QUANTITATION FROM TWO SEPARATE EXTRACTIONS OF MATURE SUGARBEET ROOT

	Mature Sugarbeet Root	
	Extraction #1	Extraction #2
Date Harvested	10/29/90	10/29/90
Date Extracted	12/13/90	11/19/91
Days Post Harvest	45	386
PPM	0.275	0.275
% Extractable	62.5	61.5

	% TRR[1]	
Peak[2]	#1	#2
SBR1	35.9	31.9
SBR2	9.4	8.8
SBR3	5.4	5.5
SBR4	5.2	4.2
SBR5	3.9	4.4
SBR6	2.8	2.8

[1]The extractable fractions from both extracts were analyzed 137 and 399 days after harvest, respectively.
[2]See Figure 13 for corresponding peaks.

FIGURE 1. CHEMICAL NAMES AND STRUCTURES

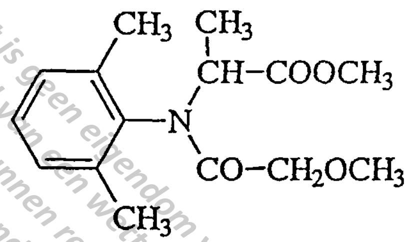
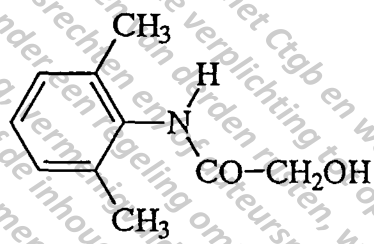
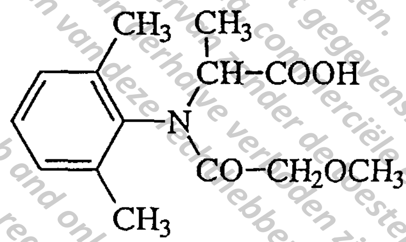
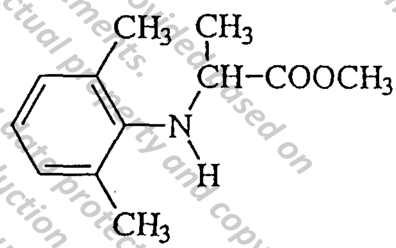
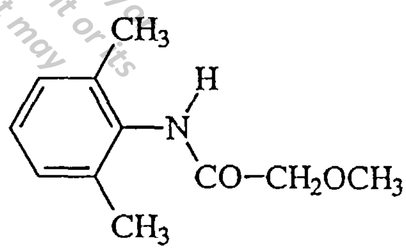
Company Code Chemical Name	Structure
CGA-48988 N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester	
CGA-37734 N-(2,6-dimethylphenyl)-2-hydroxyacetamide	
CGA-62826 N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine	
CGA-67866 N-(2,6-dimethyphenyl)-alanine methyl ester	
CGA-67868 N-(2,6-dimethylphenyl)-2-methoxyacetamide	

FIGURE 1. CHEMICAL NAMES AND STRUCTURES (Continued)

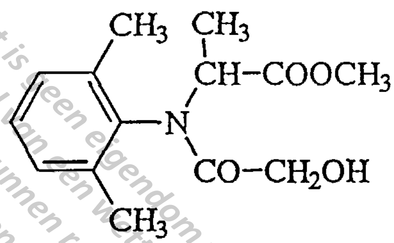
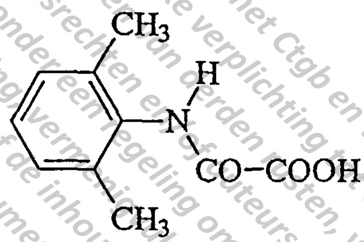
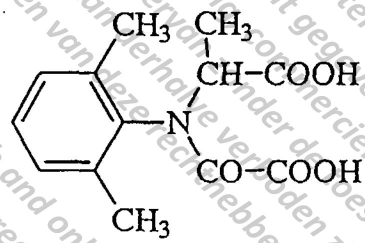
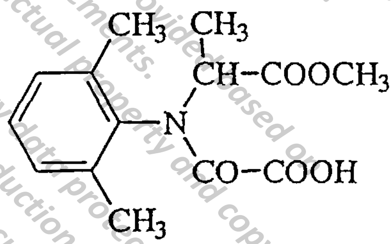
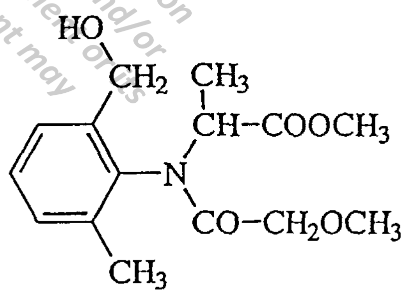
Company Code Chemical Name	Structure
CGA-67869 N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-alanine methyl ester	
CGA-68124 [(2,6-dimethylphenyl)-amino]oxoacetic acid	
CGA-78532 N-(carboxycarbonyl)-N-(2,6-dimethylphenyl) alanine	
CGA-79353 N-(carboxycarbonyl)-N-(2,6-dimethylphenyl) alanine methyl ester	
CGA-94689 N-[(2-hydroxymethyl)-6-methylphenyl]-N-(methoxy acetyl)-alanine methyl ester	

FIGURE 1. <u>CHEMICAL NAMES AND STRUCTURES</u> (Continued)

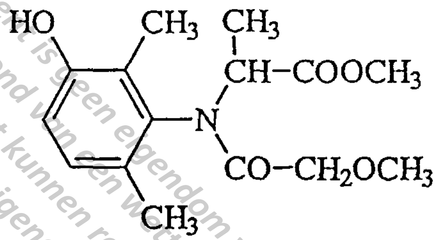
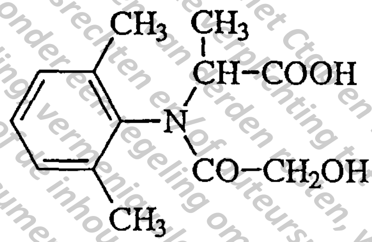
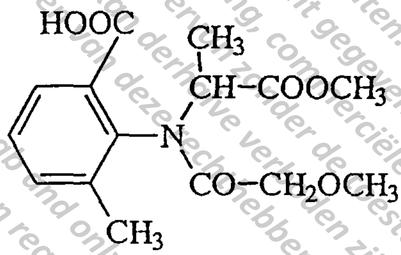
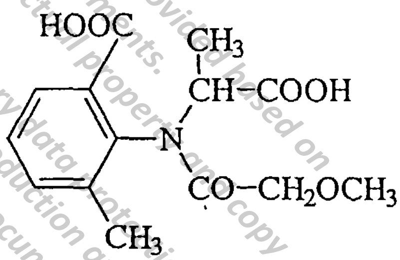
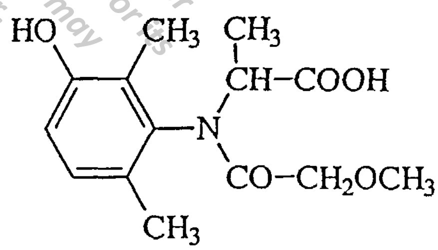
Company Code Chemical Name	Structure
CGA-100255 N-(3-hydroxy-2,6-dimethyl phenyl)-N-(methoxyacetyl) alanine methyl ester	
CGA-107955 N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-alanine	
CGA-108905 2-[(methoxyacetyl)(2-methoxy-1-methyl-2-oxoethyl)amino]-3-methylbenzoic acid	
CGA-108906 2-[(1-carboxyethyl)(methoxy acetyl)amino]-3-methyl benzoic acid	
CGA-119857 N-(3-hydroxy-2,6-dimethyl phenyl)-N-(methoxyacetyl)-alanine	

FIGURE 1. CHEMICAL NAMES AND STRUCTURES (Continued)

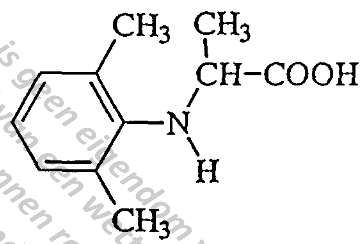
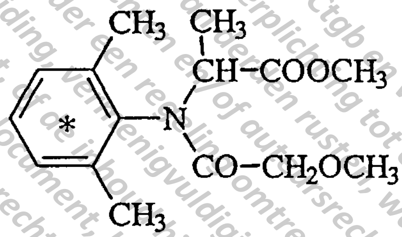
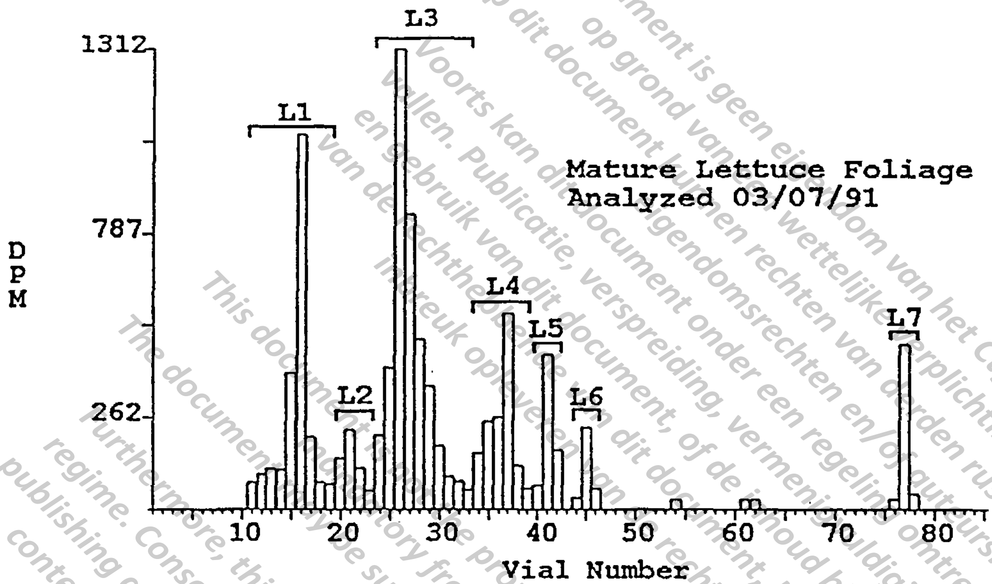
Company Code Chemical Name	Structure
CGA-67867 N-(2,6-dimethylphenyl)-alanine	
[phenyl-^{14}C]-metalaxyl	 * indicates position of radiolabeled atom

FIGURE 2. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE LETTUCE FOLIAGE



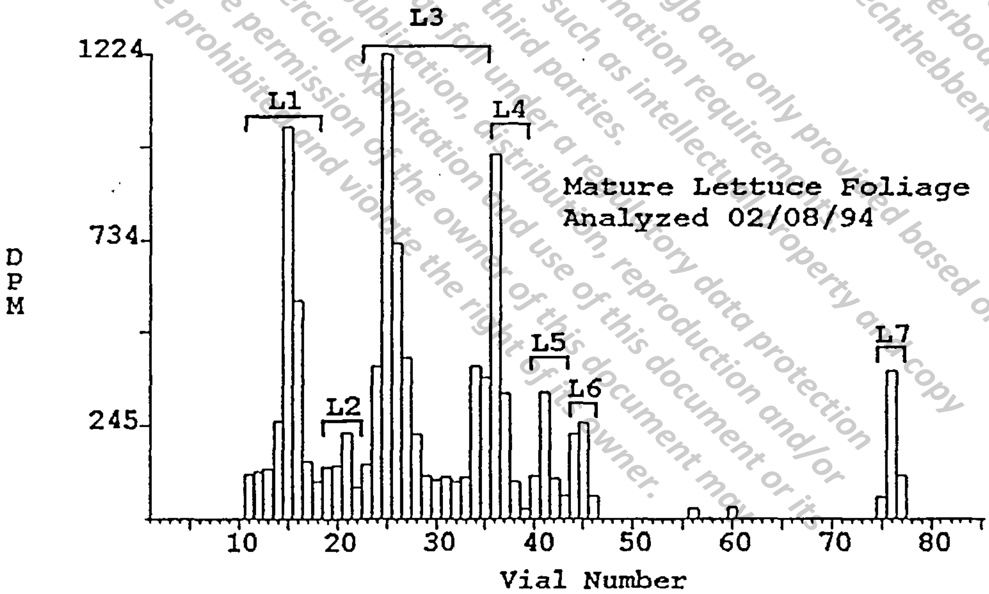
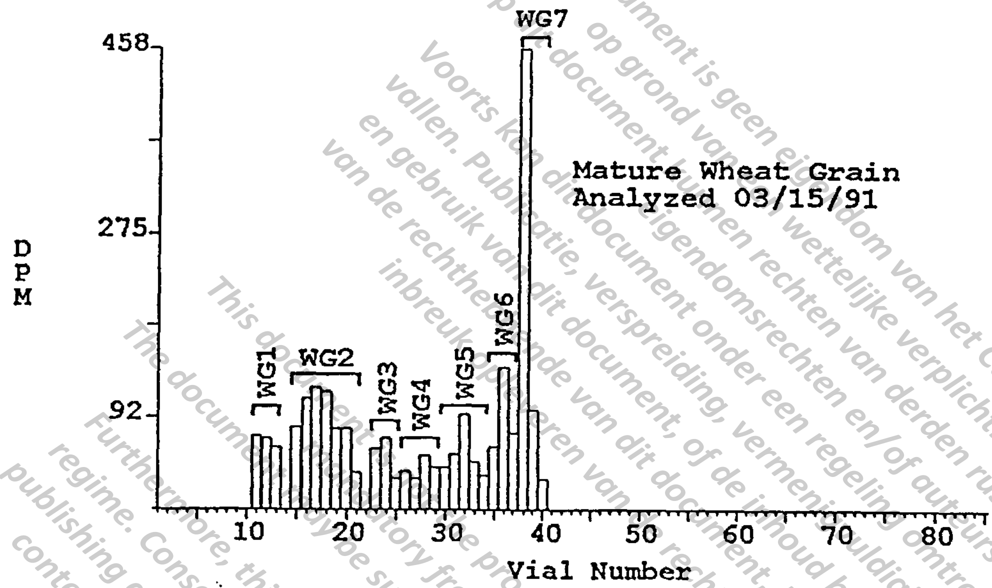


FIGURE 3. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SPRING WHEAT GRAIN



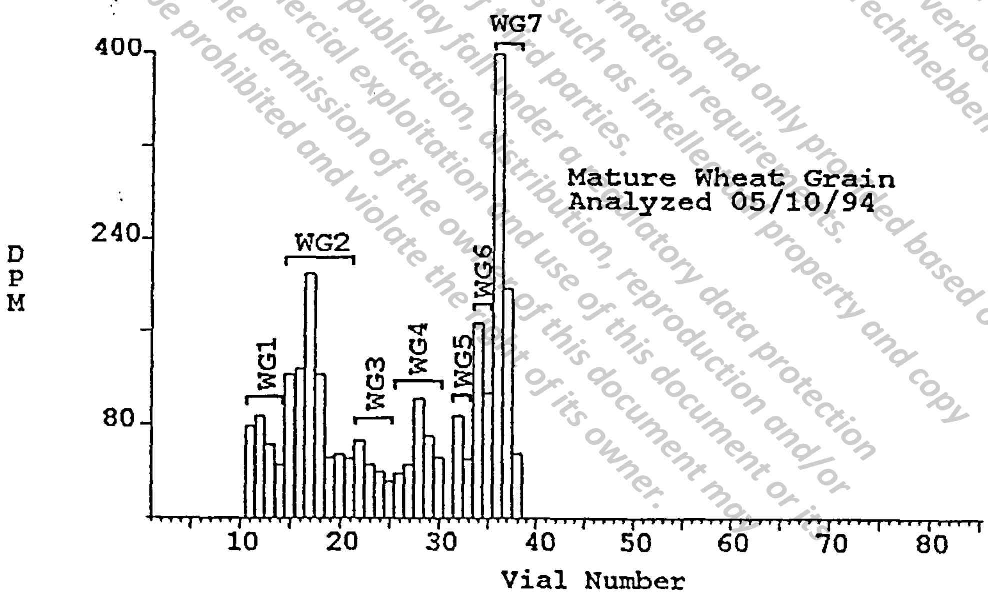


FIGURE 4. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SPRING WHEAT HULL

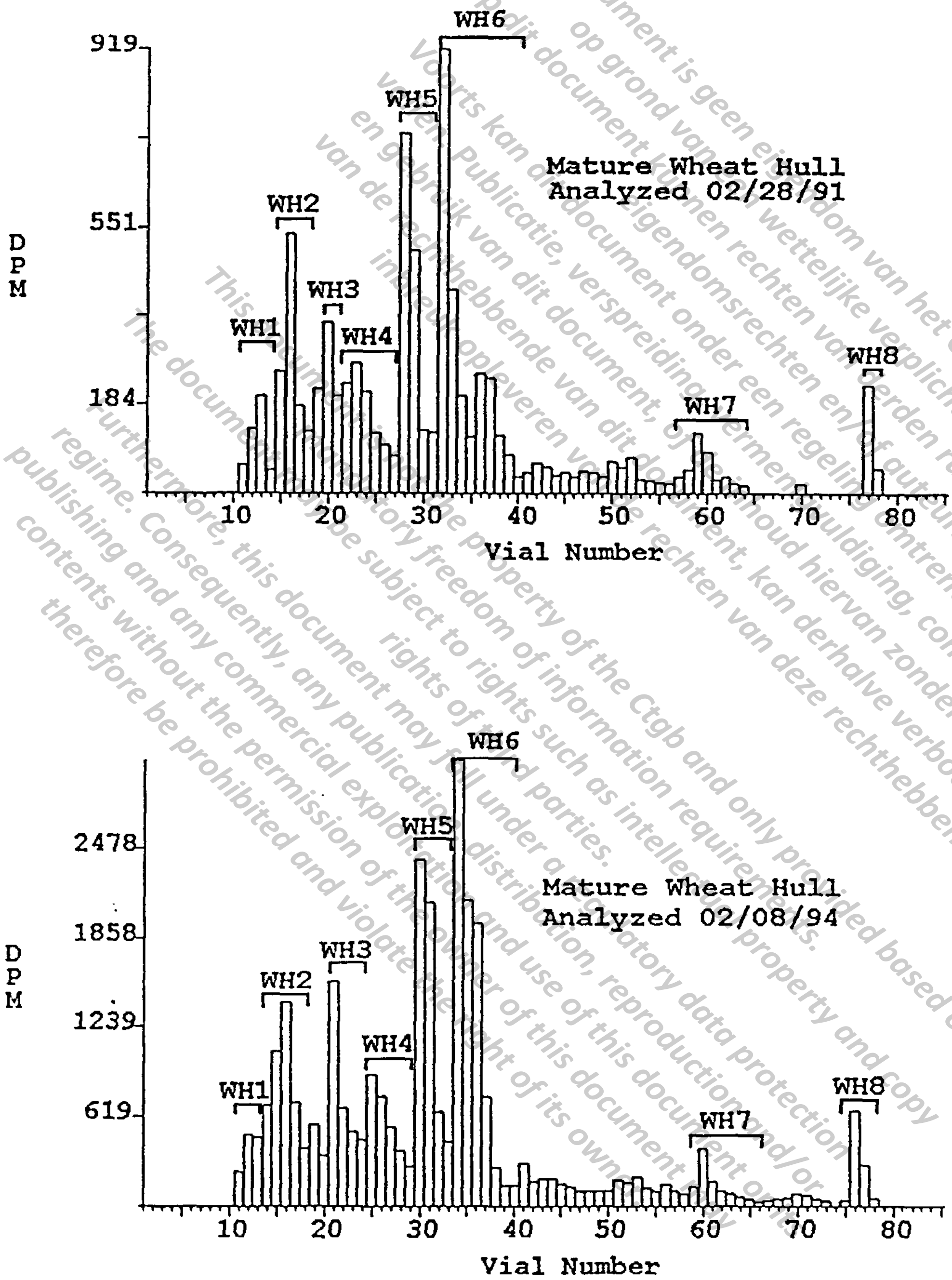
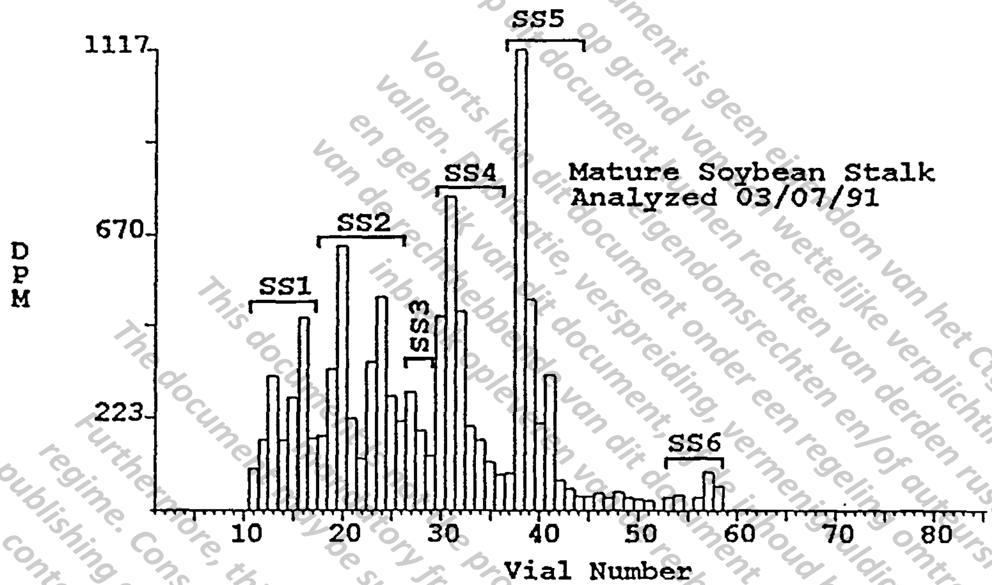


FIGURE 5. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN STALK



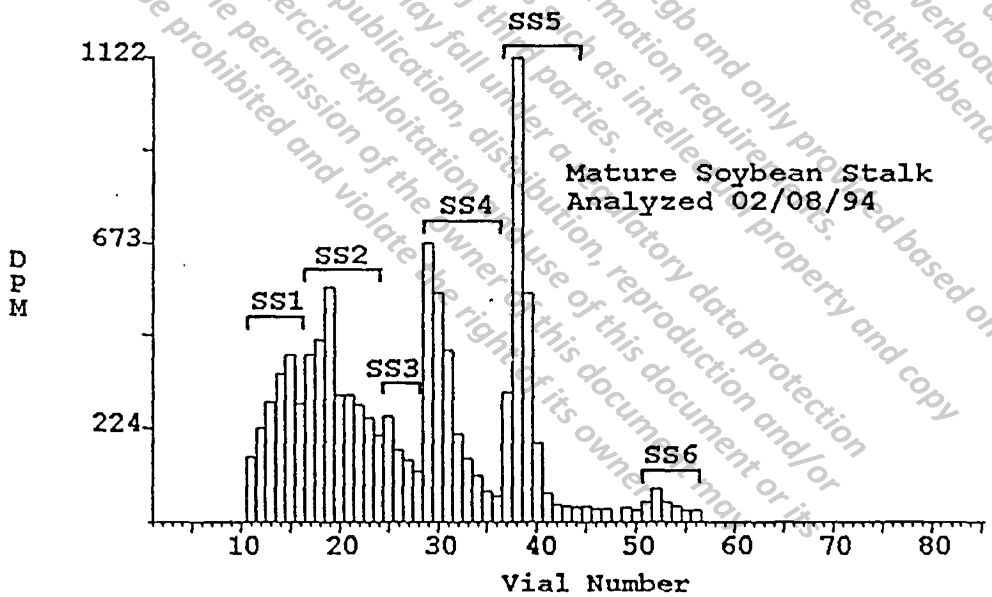
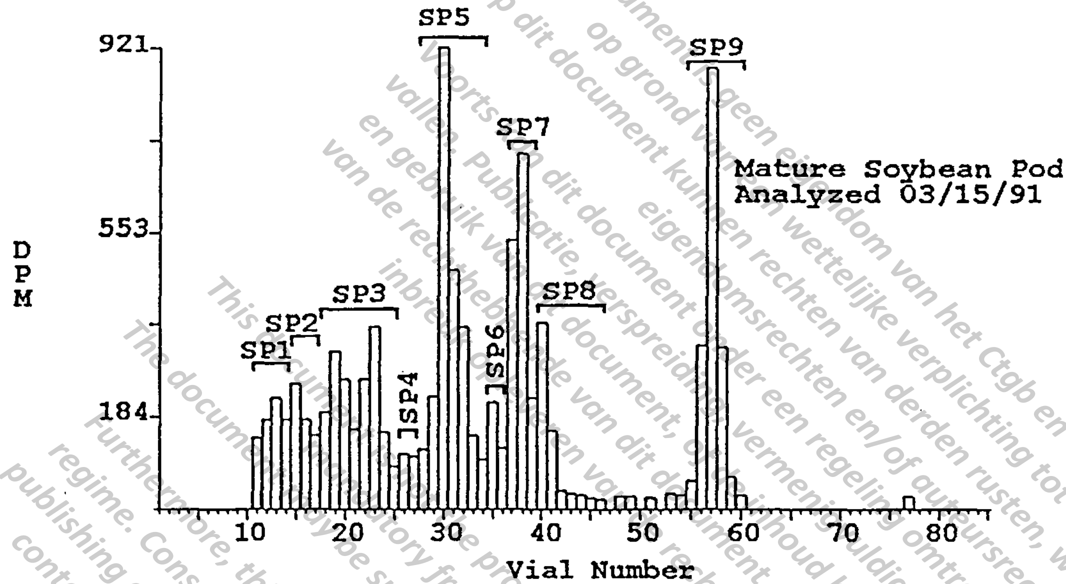


FIGURE 6. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN POD



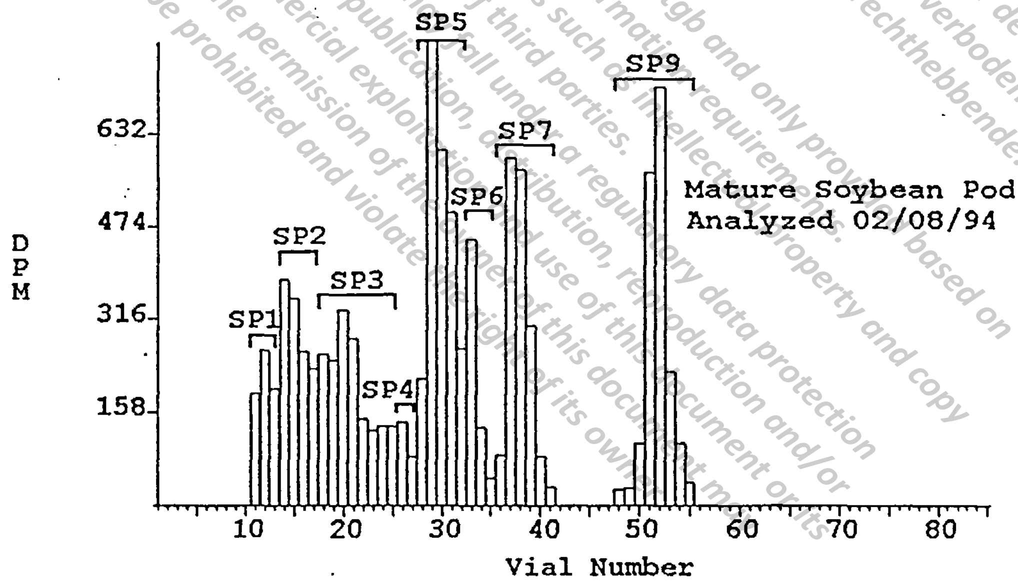


FIGURE 7. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SUGARBEET FOLIAGE

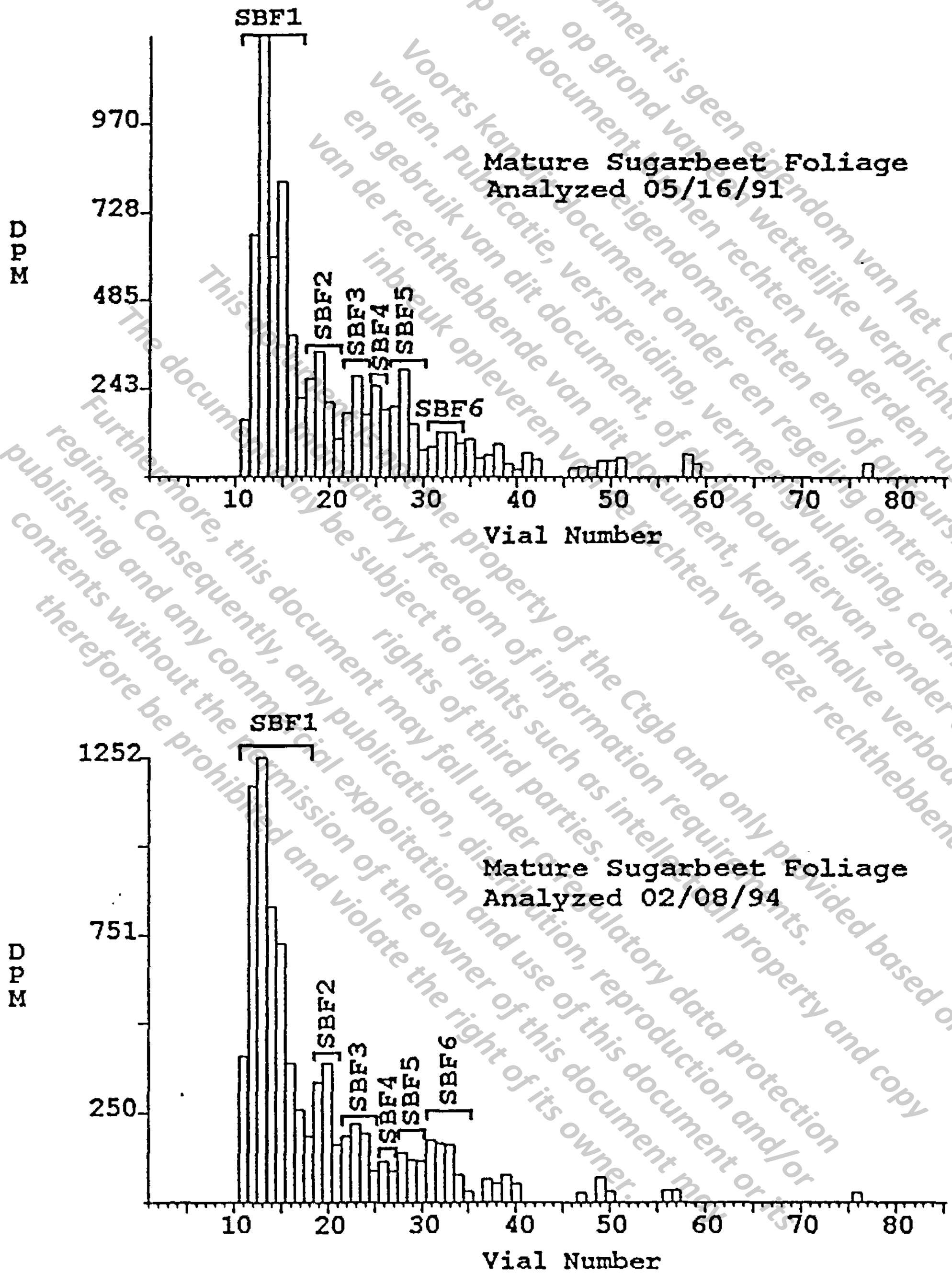
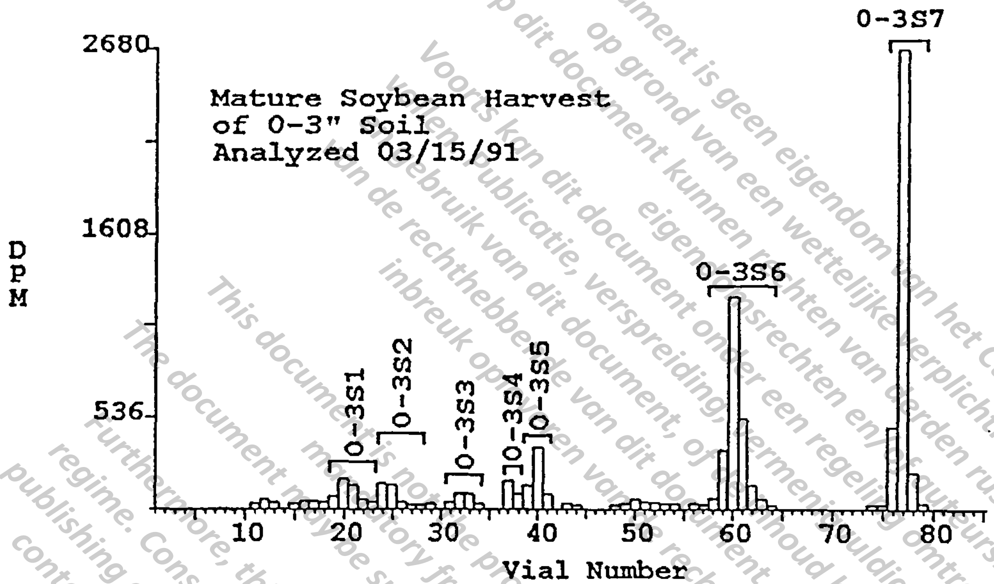


FIGURE 8. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN HARVEST 0-3″ SOIL



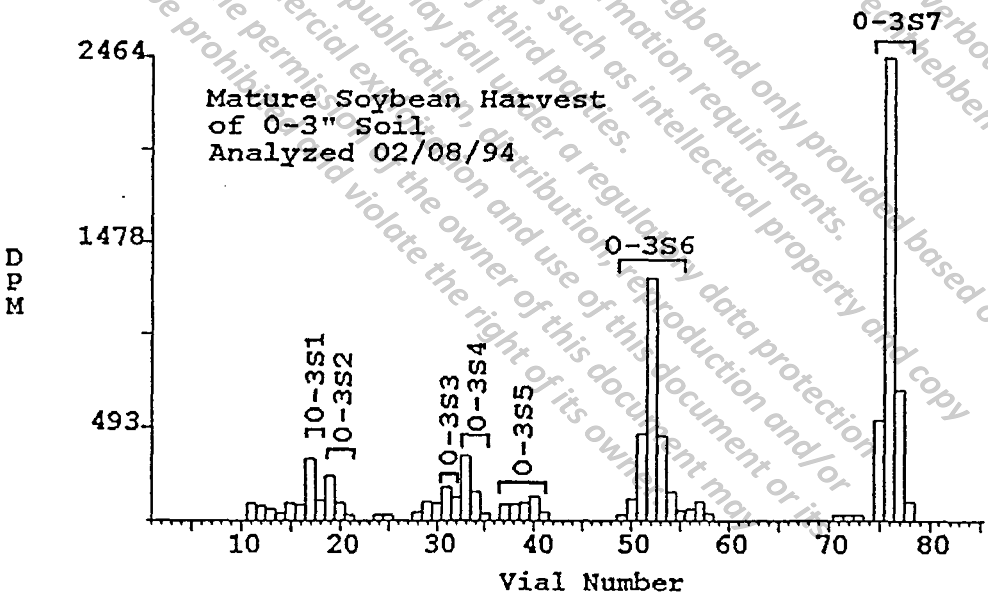


FIGURE 9. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN PHENYL-^{14}C-METALAXYL MATURE SOYBEAN HARVEST 6-8" SOIL

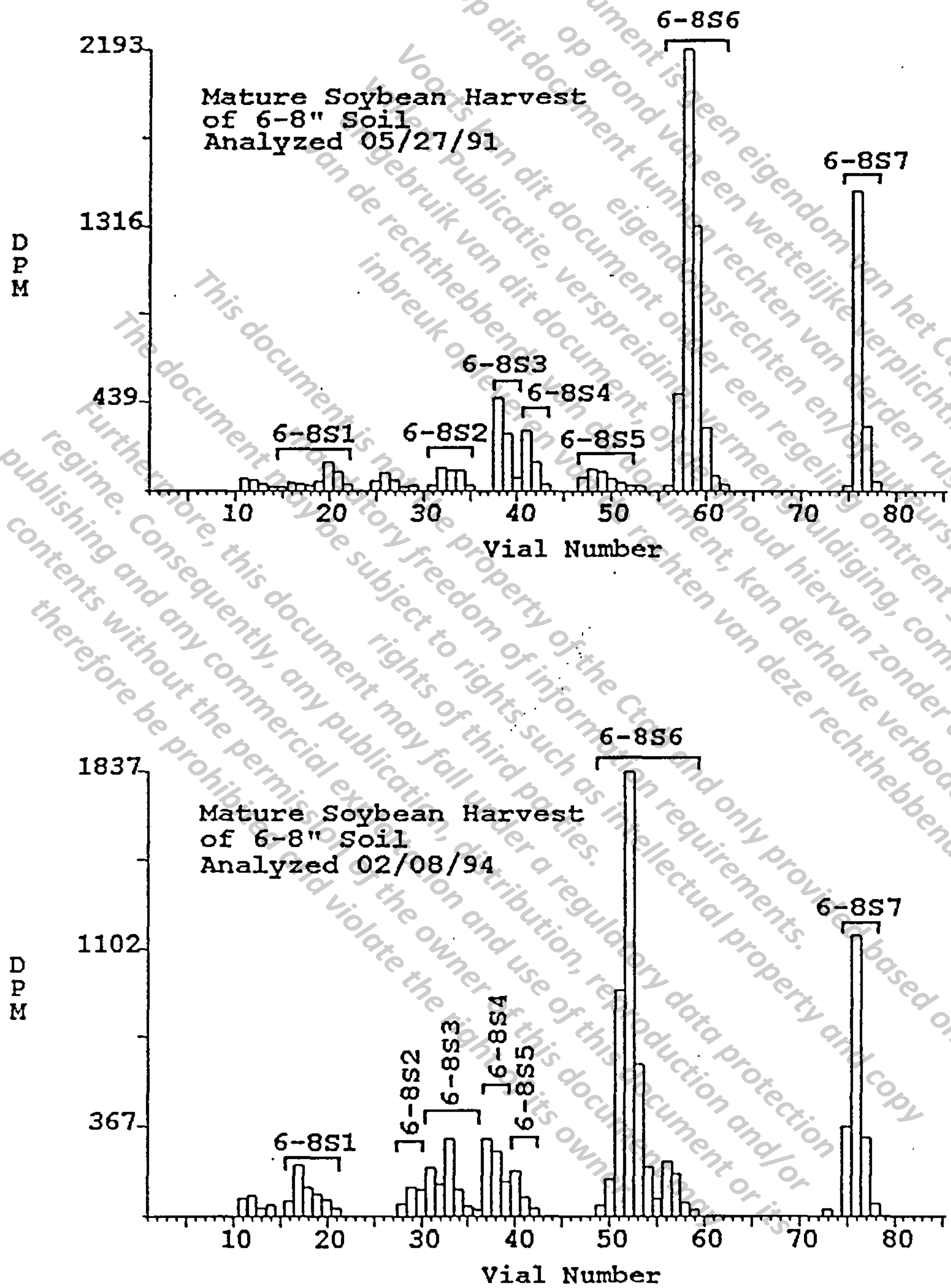
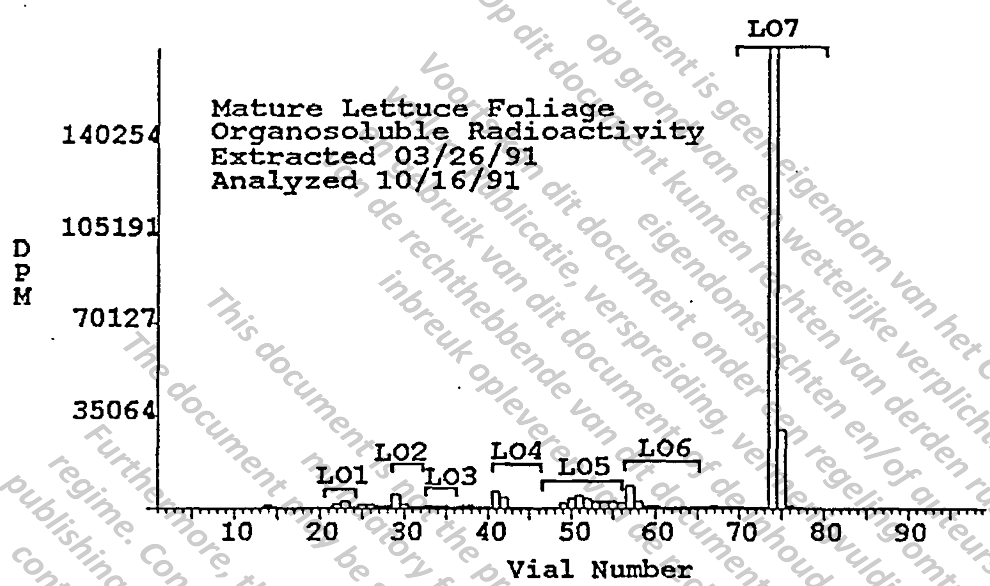


FIGURE 10. STORAGE STABILITY ANALYSIS BY HPLC OF THE ORGANOSOLUBLE RADIOACTIVITY IN TWO SEPARATE EXTRACTIONS OF PHENYL-^{14}C-METALAXYL 50% MATURE LETTUCE FOLIAGE



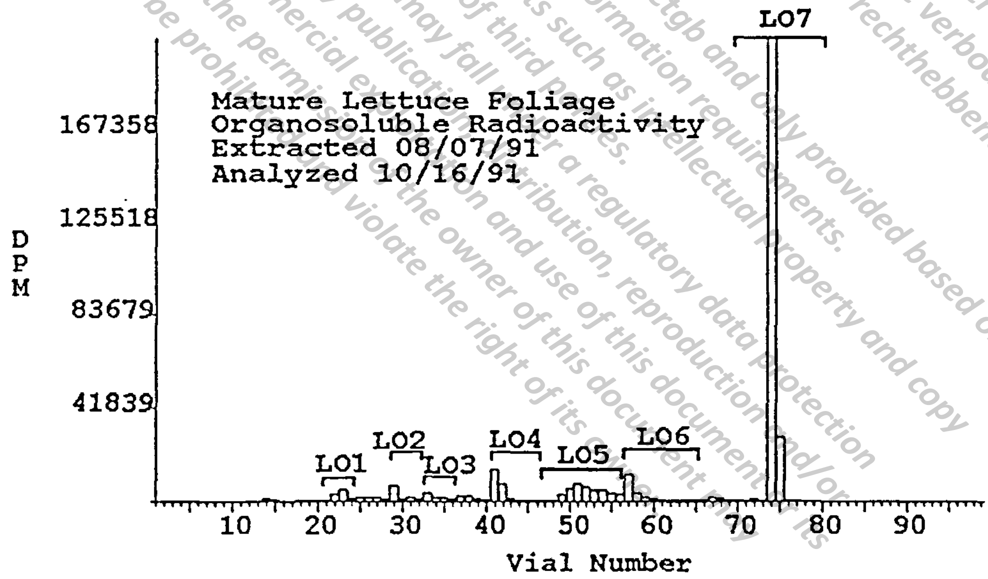
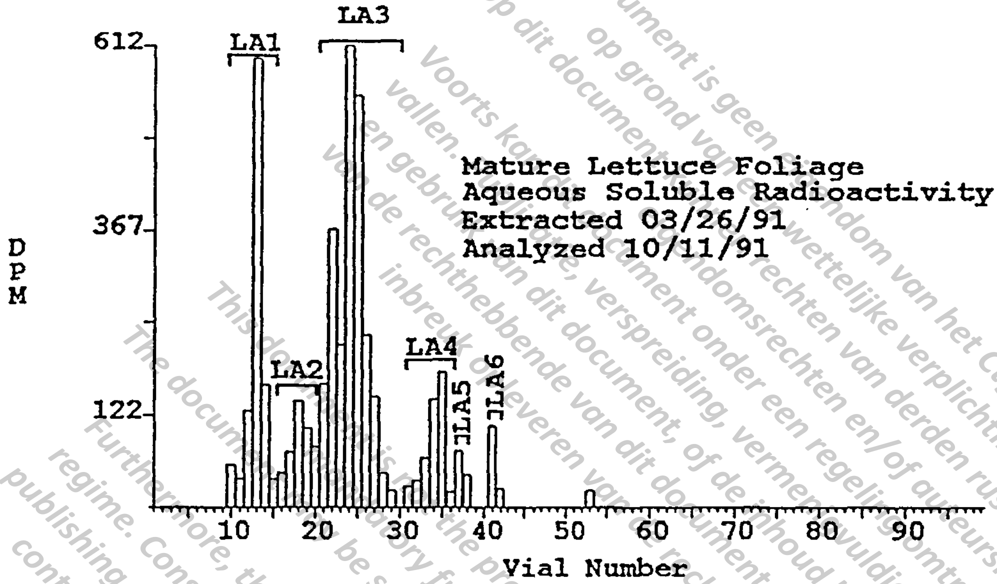


FIGURE 11. STORAGE STABILITY ANALYSIS BY HPLC OF THE AQUEOUS SOLUBLE RADIOACTIVITY IN TWO SEPARATE EXTRACTIONS OF PHENYL-^{14}C-METALAXYL 50% MATURE LETTUCE FOLIAGE



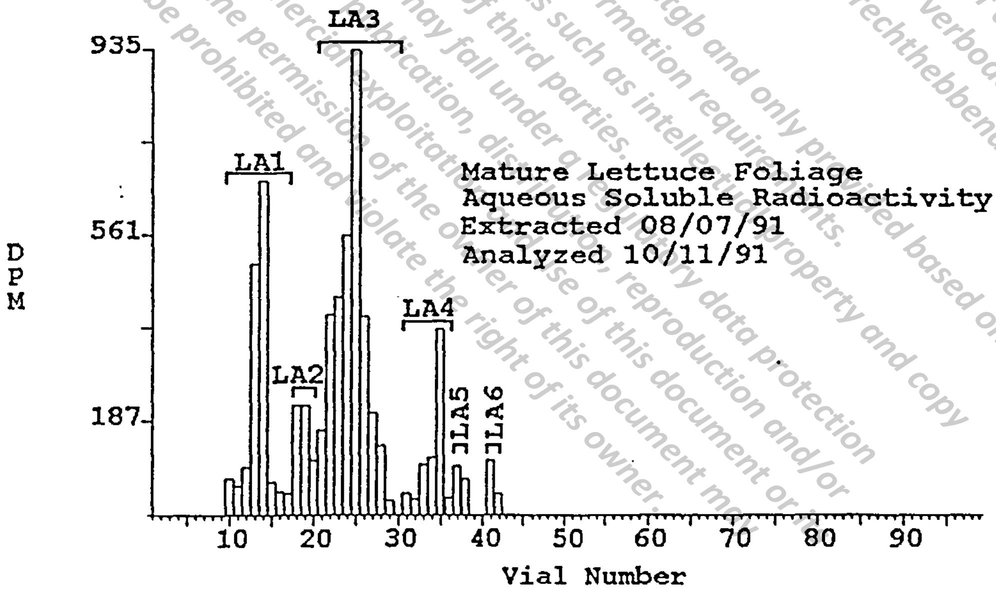
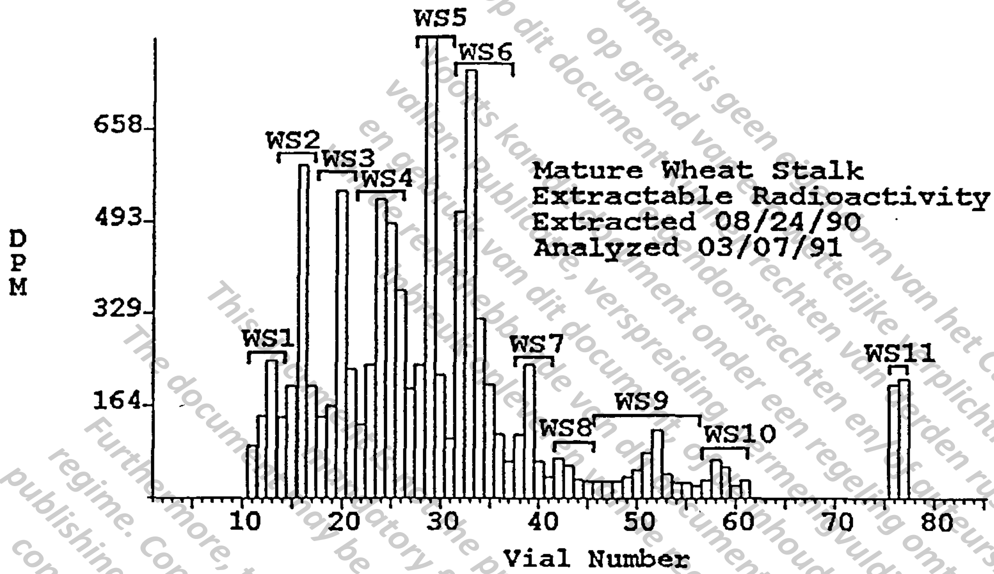


FIGURE 12. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN TWO SEPARATE EXTRACTIONS OF PHENYL-^{14}C-METALAXYL MATURE WHEAT STALK



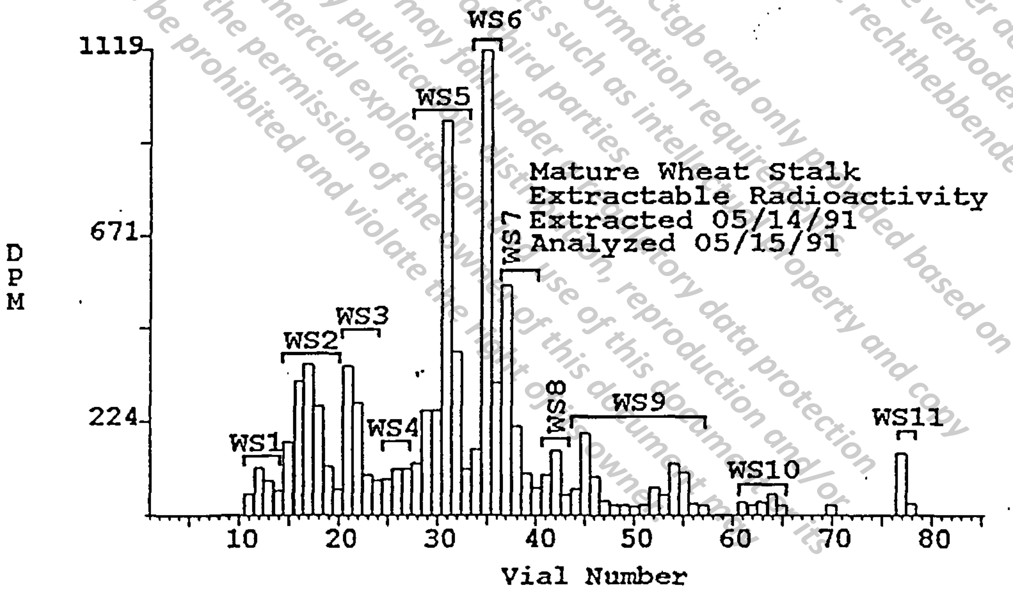
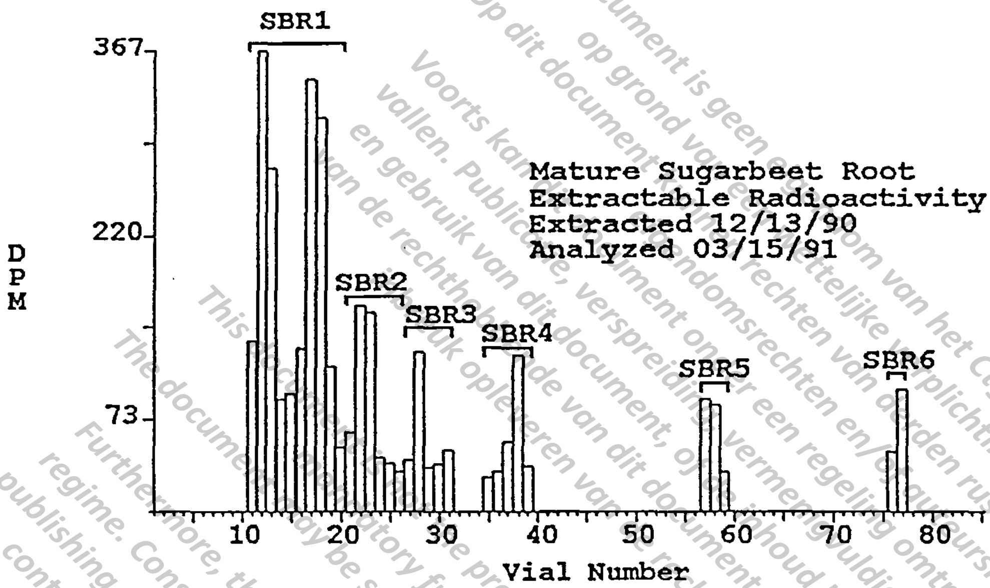
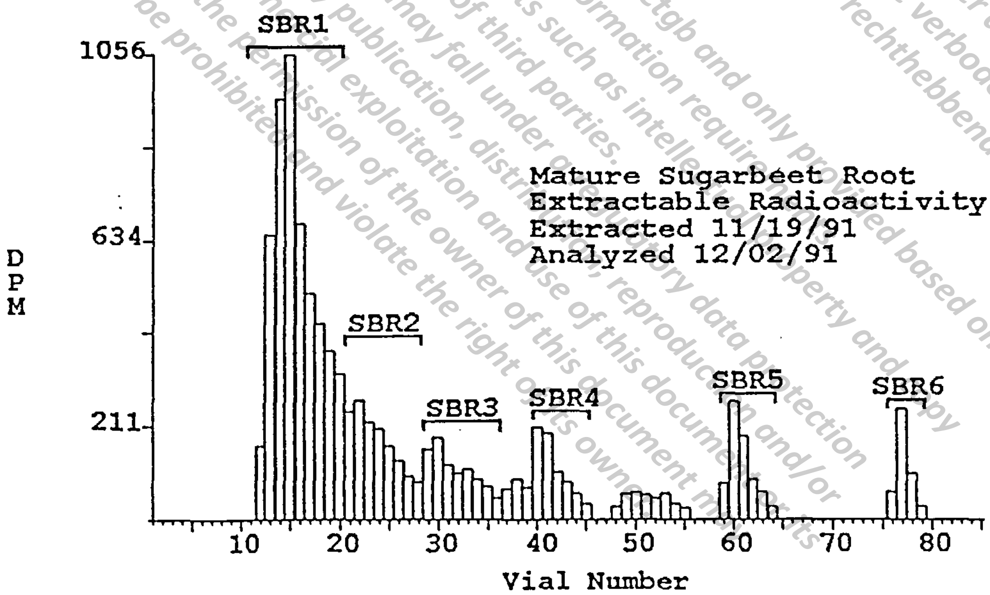


FIGURE 13. STORAGE STABILITY ANALYSIS BY HPLC OF THE EXTRACTABLE RADIOACTIVITY IN TWO SEPARATE EXTRACTIONS OF PHENYL-^{14}C-METALAXYL MATURE SUGARBEET ROOT





VIII. REFERENCES

1. [redacted] "Uptake and Metabolism of ^{14}C-CGA-48988 as a Preemergence Treatment on Greenhouse Grown Tobacco," with Amendments; "Protocol 203-89 Part A, Uptake and Metabolism of ^{14}C-CGA-48988 in Greenhouse Rotational Crops Following a Tobacco Target Crop in Soil Previously Treated With ^{14}C-CGA-48988," with Amendments; and "Protocol 203-89 Part B Development of Methods for the Determination of Nonextractable Residues in Greenhouse Grown Target Tobacco and Rotational Crops Following Soil Treatment with Phenyl-^{14}C-CGA-48988," with Amendments.

2. ABR-91084, [redacted], [redacted] "Uptake and Metabolism of Metalaxyl in Greenhouse Rotational Crops Following Target Tobacco Grown in Soil Treated With [Phenyl-^{14}C]-Metalaxyl," MRID #42196501.

3. Ciba-Geigy Corporation, Ridomil® 2E Fungicide Label, 1994, EPA Reg. No. 100-607.

4. BIOL-90016, [redacted], "Biological Report for Greenhouse Rotational Crops Grown After Tobacco in Soil Treated With ^{14}C-CGA-48988," MRID #42196501.

5. BIOL-90017, [redacted], "Biological Report for Greenhouse Tobacco Grown in Soil Treated With ^{14}C-CGA-48988," MRID #42196501.