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Study title

DEGRADATION AND LEACHING OF ^{14}C-METALAXYL IN TWO SAND LYSIMETERS UNDER OUTDOOR CONDITIONS AFTER APPLICATION TO POTATOES

Code of the study

CIB02

Data Requirement

Guidelines contained in BBA-Richtlinien Teil IV, 4-3 *

* BBA = Federal Biological Institute for Agriculture and Forestry (Braunschweig, FRG)

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Sponsor

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Plant Protection Div.
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STATEMENT OF COMPLIANCE

To the best of my knowledge and belief, this study was conducted in compliance with the Good Laboratory Practice Regulations; Act on Protection against Hazardous Substances, Appendix 1 of August 1, 1990, FRG (Chemikaliengesetz, Anhang 1 vom 01.08.1990).
No unforeseen circumstances were noticed which may have affected the quality or intregrity of the study.

Study director: _____ _____
(Date)

STUDY APPROVAL OF TEST FACILITY

Study director : _____ (Date) _____ (Dr. 5.1.2.e Woo)

Management (Deputy) : _____ (Date) _____ (Dr. 5.1.2.e Woo)

Statement of the Quality Assurance Unit

The protocol, the conduction and the final report of the study CIB02 were inspected/audited by the Quality Assurance Unit.

The dates on which inspections/audits were made and the dates on which the findings were reported to the Study director and to the Management are given below.

Inspections		Information	
Date	Phase	Study director	Head of test. fac.
05/19/92	Protocol	05/20/92	05/20/92
07/02/92	Conduction	07/10/92	07/13/92
07/14/92	Conduction	08/07/92	08/07/92
09/03/92	Conduction	09/22/92	09/22/92
09/17/92	Conduction	09/24/92	09/24/92
10/28/92	Conduction	10/30/92	10/30/92
05/18/94	Conduction	05/26/94	05/26/94
07/01/94	Conduction	07/01/94	04/05/94
09/08/94	Conduction	02/01/95	02/03/95
11/11/94	Conduction	11/14/94	11/17/94
02/06/95	Conduction	02/09/95	02/09/95
02/10/95	Conduction	02/14/95	02/14/95
02/14/95	Report	02/21/95	02/24/95
02/17/95	Report	02/21/95	02/24/95
02/24/95	Report	02/24/95	02/24/95

February 24, 95 5.1.2.e Woo
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February 1995
date (5.1.2.e Woo , QAU)

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I. ZUSAMMENFASSUNG

Das Verlagerungsverhalten von [Phenyl-^{14}C]Metalaxyl (Methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alaninat) wurde nach Applikation zu Kartoffeln in zwei Lysimetern (Lys. 19 und Lys. 20), die einen ungestörten Sandboden enthielten, unter Freilandbedingungen über einen Zeitraum von 2 Jahren untersucht. In Übereinstimmung mit dem empfohlenen Einsatz wurden zwei praxisgerechte Behandlungen (Metalaxyl in formulierter Form) mit ca. 200 g A.I./ha durchgeführt. Die erste Behandlung wurde in geteilter Spritzung am 29. Juni 92 und 14. Juli 92 und die zweite Behandlung wurde am 27. Juli 92 durchgeführt.

Im Verlauf der zweijährigen Untersuchungen wurden folgende Ergebnisse erzielt:

Sickerwasser

Lysimeter 19:

Nach dem 1. Versuchsjahr wurden in 310,3 l Perkolat 6,29 % der applizierten Radioaktivität gefunden (5,31 µg Wirkstoffäquivalente/l). Nach dem 2. Versuchsjahr wurden in 322,5 l Perkolat 2,00 % der applizierten Radioaktivität gefunden (1,62 µg Wirkstoffäquivalente/l). Einzelne ^{14}C-Metalaxylbefunde führten zu einer Konzentration von 0,05 µg/l im zweijährigen Mittel. Neben dem Wirkstoff wurden auch die Metaboliten CGA 62826 (2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionsäure) und CGA 108906 (2-[(1-carboxy-ethyl)-methoxyacetylamino]-3-methyl benzoesäure) in mittleren Konzentrationen von 2,44 µg/l und 0,61 µg/l bestimmt (HPLC, DC, Massenspektroskopie).

Lysimeter 20:

Nach dem 1. Versuchsjahr wurden in 284,5 l Perkolat 4,24 % der applizierten Radioaktivität gefunden (4,23 µg Wirkstoffäquivalente/l). Nach dem 2. Versuchsjahr wurden in 320,0 l Perkolat 1,25 % der applizierten Radioaktivität gefunden (1,11 µg Wirkstoffäquivalente/l). Jeweils ein ^{14}C-Metalaxybefund im ersten und zweiten Versuchsjahr führte zu einer Konzentration von < 0,01 ug/l im zweijährigen Mittel. Daneben wurden die Metaboliten CGA 62826 und CGA 108906 in mittleren Konzentrationen von 1,30 µg/l und 0,80 µg/l bestimmt (HPLC, DC, Massenspektroskopie).

Pflanzen und Boden

38 Tage nach der 1. Applikation wurden in den behandelten Kartoffelpflanzen 19,5 (Lys. 19) und 21,3 % (Lys. 20) der insgesamt applizierten Radioaktivität, überwiegend in den Blättern konzentriert, gefunden. Die ^{14}C-Gehalte in den Folgekulturen Winterweizen und Wintergerste waren vernachlässigbar.
91 Tage nach der 1. Applikation wurden 18,8 % (Lys. 19) und 19,5 % (Lys. 20) der insgesamt applizierten Radioaktivität in den oberen 10 cm des Bodens gefunden. Nach 373 Tagen betrug dieser Wert 3,4 % (Lys. 19) und 6,6 % (Lys. 20).

Bei Versuchsende nach 731 Tagen wurden im gesamten Bodenprofil 13,5 % (Lys. 19) und 15,5 % (Lys. 20) gefunden. Diese Radioaktivität repräsentierte in keinem Fall ^{14}C-Metalaxyl oder einen der bekannten Metaboliten.

Die folgende Tabelle faßt die wichtigsten Ergebnisse der Sickerwasseruntersuchungen beider Lysimeter zusammen:

I. SUMMARY

The leaching behaviour of [Phenyl-^{14}C]Metalaxyl, i.e. methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate, applied to potatoes was studied under outdoor conditions, using two lysimeters (19 and 20) containing an undisturbed sandy soil. According to the recommended use, two treatments were carried out, at rates corresponding to 200 g A.I./ha. The 1st treatment was carried out in a devided spray application on June 29, 1992 and July 14, 1992. On July 27, 1992 the 2nd treatment was performed.

During the experimental period of 2 years the following results were obtained:

Percolate

Lysimeter 19:

After the 1st test year, 6.29 % of the ^{14}C applied were determined in 310.3 l percolate (5.31 ug A.I. equivalents/l). After the 2nd test year, 2.00 % of the ^{14}C applied were determined in 322.5 l percolate (1.62 µg A.I. equivalents/l). Single ^{14}C- Metalaxyl findings led to a mean concentration of 0.05 µg/l in both test years. Besides the A.I. also the metabolites CGA 62826, i.e. 2-[(2,6-dimethyl-phenyl)-methoxyacetylamino]-propionic acid, and CGA 108906, i.e. 2-[(1-carboxyethyl)-methoxyacetylamino]-3-methyl benzoic acid were determined in mean concentrations of 2.44 µg/l and 0.61 µg/l (HPLC, TLC, mass-spectroscopy).

Lysimeter 20:

After the 1st test year 4.24 % of the ^{14}C applied were determined in 284.5 l percolate (4.23 µg A.I. equivalents/l). After the 2nd test year 1.25 % of the ^{14}C applied were determined in 320.0 l percolate (1.11 µg A.I. equivalents/l). One ^{14}C-Metalaxyl finding in each test year led to a mean concentration of < 0.01 µg/l in both test years. Besides the A.I. also the metabolites CGA 62826, and CGA 108906 were determined in mean concentrations of 1.30 µg/l and 0.80 µg/l (HPLC, TLC, mass-spectroscopy).

Plants and Soil

38 days after the 1st application 19.5 % (lys. 19) and 21.3 % (lys. 20) of the total ^{14}C applied were determined in the treated potatoeplants. This radioactivity was mainly concentrated in the leaves. The radioactivity in the following crops winter wheat and winter barley was negligible.

91 days after the 1st application 18.8 % (lys. 19) and 19.5 % (lys. 20) of the total ^{14}C applied were determined in the 0 - 10 cm soil layer. 3.4 % (lys. 19) and 6.6 % (lys. 20) were determined after 373 days.
After the end of the experiment after 731 days, 13.5 % (lys. 19) and 15.5 % (lys. 20) were measured in the whole soil profile. This radioactivity represented in no case ^{14}C-Metalaxyl or any of the known metabolites.

The following table summarizes important results of the leachate investigation of both lysimeters:

Lysimeter investigation, 1st and 2nd year
percolate

lysimeter	L 19	L 20
date of application 1st a) b) 2nd	June 29, 92 July 14, 92 July 27, 92	June 29, 92 July 14, 92 July 27, 92
radioactivity applied [kBq]	52645.4	57087.8
spec. radioactivity [kBq/mg]	2010.0	2010.0
radioactivity percolated [kBq]	4364.2	3136.1
^{14}C-carbonate in the percolate [kBq]	41.7	178.2
radioactivity percolated without ^{14}C-carbonate [kBq]	4322.5	2957.9
amount of percolate [l]	632.8	604.5
radioactivity of A.I. in the percolate [kBq]	61.3	4.1
radioactivity in the percolate without A.I. and ^{14}C-carbonate [kBq]	4261.2	2953.8
radioactivity percolated (% of the amount applied)	8.29	5.49
radioactivity percolated without ^{14}C-carbonate (% of the amount applied)	8.21	5.18
concentration of A.I. in the percolate [μg/l]	0.05	< 0.01
concentration of the metabolites [μg/l]		
CGA 108906	0.61	0.80
CGA 62826	2.44	1.30

II. ORGANISATION AND PERSONNEL

1.1 Sponsor

CIBA GEIGY AG
Plant Protection Div.
Safety Evaluation
Postfach
CH-4002 BASEL

Study Monitor: Mr. [redacted]

1.2 Test Facility

Fachbereich Phytomedizin
SLFA Neustadt/Weinstraße
Breitenweg 71
67435 Neustadt/Weinstraße

Management	: Dr. [redacted] Dr. [redacted] (deputy)
Study Director	: Dr. [redacted] : Dr. [redacted] (deputy)
Technical staff	: [redacted]
Quality Assurance	: Dr. [redacted] : [redacted]

1.3 GLP-Certificate

See the last three pages of this final report. The name of the test facility in the certificate and in this final report is not exactly the same.
Reason: The **LLFA Neustadt** was renamed in **SLFA Neustadt** (July 01, 1993).
At the same date the **Abteilung Phytomedizin** was renamed in **Fachbereich Phytomedizin**.

1.4 Archivement and Retention of Records and Material

1.4.1 Written Documents

The following written documents:

- study plan and amendment (original)
- all raw data (originals)
- final report (one of three originals)
- correspondence with sponsor
- data concerning test- and reference substances
- the QAU-documents (reports of insprections and audits)

will be stored in the GLP-archives of the test facility (address cf. point 1.2) at least for the period of time specified in the GLP regulations.

1.4.2 Samples and Specimen

Specimen:

The test substance was completely worked up. One specimen of the reference substances will be stored at least for the period of time specified in the GLP regulations.

Samples:

Samples taken during the experiments were completely worked up.

1.5 Dates

Beginning of the study (signature of study monitor in study plan)	: June 05, 1992
Beginning of the experimental phase (1st application)	: June 29, 1992
End of the experimental phase	: Nov. 28, 1994
End of the study	: Signatures in this report

III. INTRODUCTION

Experiments with ^{14}C-labelled pesticides under laboratory conditions provide detailed information about their behaviour under controlled conditions. The ability to establish mass balances of the applied radioactivity, and the quantification of the non-extracted ^{14}C are fundamental advantages of this technique. On the other hand, results from laboratory experiments can not be transferred to the practical field situation without certain restrictions, because changing temperature and moisture conditions, precipitation distribution and plants have an effective influence on the fate of the applied compounds.
Experiments in outdoor lysimeters under field conditions combine the advantages of the tracer technique and the validity of field experiments. Therefore, they are an important tool for the assessment of pesticides in ecosystems.
To investigate the fate of Metalaxyl, a two year study was conducted using two lysimeters, filled with a sandy soil. During the 1st year, the lysimeters were planted with potatoes followed by rape (intermediate crop) and winter wheat, rape and winter barley in the 2nd year.

IV INVESTIGATION

1. Test substance

The test substance (active ingredient) used was:

[Phenyl-^{14}C] Metalaxyl

formulated in an EC 240 formulation.
A copy of the certificate of the formulated ^{14}C-Metalaxyl is given in app. 01a.

1.1 Radiochemical purity

The radiochemical purity of the test substance [Phenyl-^{14}C] Metalaxyl, dates of certificate see app. 01a) used for the application in the 1st experimental year was:

97 %.

Repeated measurements in the course of the study (for TLC and HPLC conditions see app. 10b) confirmed the radiochemical purity of 97 %.

1.2 Labelled position

Cf. appendix 01a.

1.3 Specific radioactivity

The specific radioactivity of the test substance [Phenyl-^{14}C] Metalaxyl used for the applications during the 1st experimental year was:

2010 kBq/mg.

1.4 Reference substances

The following reference substances were used for characterization of unknown radioactivity in the samples.

CGA 42447 (not labelled)	CGA 67868 (not labelled)
CGA 48988 (not labelled)	CGA 67867 (not labelled)
CGA 37734 (not labelled)	CGA 62826 (not labelled)
CGA 119857(not labelled)	CGA 68125 (not labelled)
CGA 67869 (not labelled)	CGA 68124 (not labelled)
CGA 67866 (not labelled)	CGA 108905(not labelled)
CGA 108906(not labelled)	

For details concerning the reference substances see appendix 01b - 1f.

2. Lysimeters

2.1 Test soil

As specified in BBA-guideline IV, 1990 (1) a sandy soil was used for the experiments.
Important chemical and physical data are given in appendix 02. The sampling area was Birkenheide, Rhineland Palatinate, Germany.
Date of sampling was December 20, 1990.

2.2 Size of lysimeters

The lysimeters (appendix 03) used for the study were circular vessels made of V-steel. An inner container was filled with an undisturbed soil core (cf. 2.3) with a surface area of 0.8 m^2. A sieved bottom was attached to allow for free drainage of the percolate, which was collected in an outer container from which it could be drawn off (app.03).

2.3 Depth of soil core

The undisturbed soil cores were 130 cm deep.

2.4 Position of the lysimeters in the lysimeter field

To ensure practical conditions, the lysimeters were placed outdoors. The position of the two lysimeters in the lysimeter field is shown in appendix 04. The distance between lysimeter L19 and L20 was 110 cm.
The surroundings of the two lysimeters were used as a control area (app. 04) and were planted with the same plants as used in the lysimeters.

2.5 Position of the lysimeter

The lysimeters were inserted into the ground, i.e. the surface of the lysimeter was at the same level as the surface of the control area.

3. Application of the test substance

3.1 Date of application

The first application was devided into two treatments because the ready-to-use formulation received from the sponsor was half-concentrated.
For both lysimeters the dates of applications in the 1st experimental year were:

1st application (divided):	a)	June 29, 92
	b)	July 14, 92
2nd application :		July 27, 92

3.2 Amount (mg/m^2 and g/ha)

The planned application rate of the test substance was:

1st application: 16 mg A.I./lysimeter = 200 g A.I./ha
2nd application: 16 mg A.I./lysimeter = 200 g A.I./ha

The quantity of test substance required for application was calculated for the surface area of the lysimeters (0.8 m^2).
For details concerning the amounts used for the application see appendix 05a, b.

3.2.1 Total amount of the radioactivity applied

After deduction of the application losses (the amount not reaching soil or plants), the total amount of radioactivity applied was determined.
For details concerning the amounts applied see appendix 05a, b.

3.3 Kind of formulation

The radioactive metalaxyl was received from Ciba-Geigy preformulated (EC 240, formulation code A-7192 B, 24% A.I.). For details see app. 01a.

3.4 Application technique

A superstructure made of connected metal hoops was used for the spraying operations. It was 0.5 m high and ran flush with the edge of the lysimeter. A tube of plastic foil was fitted round the superstructure and closed at the top. For application, a hole was cut in the foil and the radioactive solution was sprayed through it at a pressure of 2.0 bar with a hollow-cone nozzle. A glass tube, closed at the bottom, served as a storage vessel for the spray mixture. This tube was placed in a plastic container screwed tightly to the spraying equipment and to the compressed air flasks used for obtaining the required pressure. A figure of the spray equipment is given in appendix 05c.

3.5 Application losses

The spray losses consisted in ^{14}C residues in the storage container, the spraying equipment and on the plastics foil. In order to quantify these losses, the storage container was rinsed with methanol and the spraying equipment was rinsed with methanol after each application, and aliquots were taken to determine the radioactivity. Each of the plastics foils was laid in approx. 10 l acetone for several days in order to dissolve all adhering radioactivity. Aliquots of the acetone were then measured (all data cf. app. 05a, b).

4. Cultivation of the lysimeters

4.1 Plants

1st year

Potatoes: The application of the test substance was carried out to potatoes.
Rape : After harvesting the potatoes, rape was sown as intermediate crop.

The control area (app. 04) was planted like the lysimeters.
For details see app. 06a.

2nd year

Winter wheat : After soil tillage winter wheat was sown.
Rape : After harvesting the winter wheat, rape was sown as intermediate crop.
Winter barley: After soil tilage winter barley was sown. The control area (app. 04) was planted like the lysimeters.
For details see app. 19a.

4.1.1 Seed time

1st year

Potatoes: April 07, 1992
Rape : Aug. 14, 1992

2nd year

Winter wheat: Oct. 19, 1992
Rape : Aug. 12, 1993
Winter barl.: Oct. 08, 1993

4.2 Fertilization

The details concerning the fertilization are given in appendix 06b (1st year) and appendix 19b (2nd year).

4.3 Plant protection treatment

E 605 Forte was sprayed on July 13, 92 (see app. 06b).
No plant protection was neccessary in the 2nd year.

4.4 Further cultivation treatment

The weeds were eliminated manually and remained on the lysimeters.

4.5 Harvest time

1st year

Potatoes: Aug. 06, 1992 (app. 06a)
Rape: Sep. 28, 1992 (app. 06a)

2nd year

Winter wheat: July 20, 1993 (app. 19a)
Rape: Oct. 08, 1993 (app. 19a)
Winter barl.: June 29, 1994 (app. 19a)

4.6 Crop yield

1st year (app. 06a)

Potatoes were fractinated in potatoes and leaves.
The intermediate crop rape was not investigated.

2nd year (app. 19a)

Winter wheat was fractionated in grain chaff and straw.
The intermediate crop rape was not investigated and the following crop winter barley was investigated without fractionation.

5. Experimental conditions

5.1 Air temperatures

The air temperatures were continuously measured in the lysimeter field. The monthly mean values are given in appendix 07a (1st year) and appendix 20a (2nd year).

5.2 Soil temperatures

Continuous measurements of the soil temperatures (10 and 30 cm) were conducted in a control lysimeter. The monthly mean values are given in appendix 07a (1st year) and appendix 20a (2nd year).

5.3 Precipitation

Precipitation in the lysimeter field was continuously measured in the lysimeter field. The monthly precipitation data are listed in appendix 07b and 07c (1st year) and 20b and 20c (2nd year).

5.4 Irrigation

In order to ensure a minimum of 800 mm of total precipitation per year, the lysimeters were irrigated after natural rainfall. For details see appendix 07b, 07c and appendix 09 (1st year) and appendix 20b, 20c and app. 22 (2nd year).

6. Percolate sampling

The percolate from each of the two lysimeters was collected using a suction pump and the exact volume was determined. For intervals of the samplings see app. 08 and app. 21.

6.1 Amount of percolate and sampling date

Cf. appendix 08 and appendix 09 (1st year) and appendix 21 and 22 (2nd year).

6.2 Total amount of percolate

<u>1st year</u> (see app. 08)

Lysimeter 19: 310.3 l
Lysimeter 20: 284.5 l

<u>2nd year</u> (see app. 21)

Lysimeter 19: 322.5 l
Lysimeter 20: 320.0 l

Total of both test years (see app. 23d)

Lysimeter 19: 632.8 l
Lysimeter 20: 604.5 l

6.3 Percolate storage

After determination of the total radioactivity (^{14}C-balance) and the $^{14}CO_2$ aliquots of the percolate, samples were stored at 2 °C until extraction. Extracts were stored at -20 °C until chromatographic investigation.

7. Results

7.1 Percolate

The method used for extracting the test substance from water and the results of this extraction are given in appendix 10a. A.I. characterization as well as characterization of CGA 62826 and CGA 108906 were carried out by co-TLC and co-HPLC. These results were confirmed by mass spectroscopic analysis of representative samples (for details see attached Spectroscopy report from June 11, 1993)[2]. TLC and HPLC conditions are given in app. 10b. Chromatograms (TLC) of the A.I. at the determination limit (0.05 µg/l) and the detection limit (0.01 µg/l) are given in app. 10c. The corresponding HPLC chromatograms are given in app. 10d.
In the 1st experimental year, the following total amount of radioactivity was detected in the percolate:

1st year (app. 11c)

Lysimeter 19: 3311.4 kBq = 6.29 % of the ^{14}C applied

Lysimeter 20: 2419.8 kBq = 4.24 % of the ^{14}C applied

2nd year (app. 23c)

Lysimeter 19: 1052.8 kBq = 2.00 % of the ^{14}C applied

Lysimeter 20: 716.3 kBq = 1.25 % of the ^{14}C applied

Both test years (app. 32d)

Lysimeter 19: 4364.2 kBq = 8.29 % of the ^{14}C applied

Lysimeter 20: 3136.1 kBq = 5.49 % of the ^{14}C applied

7.1.1 Active ingredient (^{14}C-Metalaxyl) in individual leachates of the 1st and 2nd year

The percolate of the 1st and 2nd sampling date contained no radioactivity (app. 11a, b).

1st year

^{14}C-Metalaxyl was determined in the percolate of the following sampling dates:

<u>Lysimeter L19:</u>

The percolate collected in September, October and November 1992 (app. 11a).

<u>Lysimeter L20:</u>

The percolate collected in October 1992 (app. 11b).
Examples of the chromatographic analysis are given in appendices 12a, b.

<u>2nd year</u>

^{14}C-Metalaxyl was determined in the percolate of the following sampling dates:

<u>Lysimeter L19:</u>

The percolate collected from October 1993 to December 1993 (app. 23a).

<u>Lysimeter L20:</u>

The percolate collected on October 01, 1993 (app. 23b).
Examples of the chromatographic analysis are given in appendix 24a, b.

7.1.2 Metabolites in individual leachates of the 1st and 2nd year

<u>1st year:</u>

$^{14}CO_2$:
0.02 to 0.14 µg/l A.I. equivalents were determined as $^{14}CO_2$ in most of the percolate samples of lysimeter 19 (app. 11a).
0.02 to 0.51 µg/l A.I. equivalents were determined as $^{14}CO_2$ in all percolate samples of lysimeter 20 (app. 11b).

Metabolite CGA 108906:
This metabolite appeared in all percolate samples of both lysimeters in concentrations from < 0.01 to 3.69 µg A.I. equivalents/l (app. 11a, b).

Metabolite CGA 62826:
This metabolite appeared in all percolate samples of both lysimeters in concentrations from < 0.01 to 9.05 µg A.I. equivalents/l (app. 11a, b).

<u>2nd year:</u>

$^{14}CO_2$:
0.01 to 0.06 µg/l A.I. equivalents were determined as $^{14}CO_2$ in most of the percolate samples of lysimeter 19 (app. 23a).
0.08 to 0.23 µg/l A.I. equivalents were determined as $^{14}CO_2$ in all percolate samples of lysimeter 20 (app. 23b).

Metabolite CGA 108906:
This metabolite appeared in all percolate samples of both lysimeters in concentrations from 0.20 to 1.22 µg A.I. equivalents/l (app. 23a, b).

Metabolite CGA 62826:
This metabolite appeared in all percolate samples of both lysimeters (with the exception of the last sampling date of lysimeter 20) in concentrations from 0.06 to 2.77 µg A.I. equivalents/l (app. 23a, b).

7.1.3 Unidentified radioactivity in individual leachates of the 1st an 2nd year

1st year

Lysimeter 19: The amount of radioactivity which could not be identified because of loss or unextractability ranged between no unidentified radioactivity and 1.05 µg A.I. equivalents/l (app. 11a).

Lysimeter 20: The amount of radioactivity which could not be identified because of loss or unextractability ranged between no unidentified radioactivity and 2.09 µg A.I. equivalents/l (app. 11b).

2nd year

Lysimeter 19: The amount of radioactivity which could not be identified because of loss or unextractability ranged between 0.08 and 0.33 µg A.I. equivalents/l (app. 23a).

Lysimeter 20: The amount of radioactivity which could not be identified because of loss or unextractability ranged between 0.11 and 0.30 µg A.I. equivalents/l (app. 23b).

7.1.4 Average concentration of active ingredient (^{14}C-Metalaxyl) in the total leachate of the 1st and 2nd year

1st year

Average concentration of the A.I. was 0.05 µg/l (lys. 19) and 0.01 µg/l (lys. 20; app. 11c).

2nd year

Average concentration of the A.I. was 0.05 µg/l (lys. 19) and < 0.01 µg/l (lys. 20; app. 23c).

Both test years

Average concentration of the A.I. was 0.05 µg/l (lys. 19) and < 0.01 µg/l (lys. 20; app. 23d).

7.1.5 Average concentration of metabolites in the total leachate of the 1st and 2nd year

1st year

$^{14}CO_2$:
0.02 (lys. 19) and 0.17 (lys. 20) µg A.I. equivalents/l (app. 11c).

CGA 108906:
0.71 (lys. 19) and 1.11 (lys. 20) µg A.I. equivalents/l (app. 11c).

CGA 62826:
4.12 (lys. 19) and 2.48 (lys. 20) µg A.I. equivalents/l (app. 11c).

<u>2nd year</u>

$^{14}CO_2$:
0.04 (lys. 19) and 0.12 (lys. 20) µg A.I. equivalents/l (app. 23c).

CGA 108906:
0.52 (lys. 19) and 0.52 (lys. 20) µg A.I. equivalents/l (app. 23c).

CGA 62826:
0.82 (lys. 19) and 0.25 (lys. 20) µg A.I. equivalents/l (app. 23c).

<u>Both test years</u>

$^{14}CO_2$:
0.03 (lys. 19) and 0.15 (lys. 20) µg A.I. equivalents/l (app. 23d).

CGA 108906:
0.61 (lys. 19) and 0.80 (lys. 20) µg A.I. equivalents/l (app. 23d).

CGA 62826:
2.44 (lys. 19) and 1.30 (lys. 20) µg A.I. equivalents/l (app. 23d).

7.1.6 Average concentration of unidentified radioactivity in the total leachate of the 1st and 2nd year

<u>1st year</u>

The amount of non-extracted (not determined polar) radioactivity or lost during extraction procedure was 0.41 µg/l (lysimeter 19) and 0.47 µg/l (lysimeter 20), (app. 11c).

<u>2nd year</u>

The amount of non-extracted (not determined polar) radioactivity or lost during extraction procedure was 0.17 µg/l (lysimeter 19) and 0.19 µg/l (lysimeter 20), (app. 23c).

<u>Both test years</u>

The amount of non-extracted (not determined polar) radioactivity or lost during extraction procedure was 0.29 µg/l (lysimeter 19) and 0.32 µg/l (lysimeter 20), (app. 23d).

7.2 Soil

7.2.1 Sampling dates

In the first experimental year, soil samples were taken 91 days after the first application (app. 13).
2nd sampling date was 373 days (app. 25a) after the 1st application and last sampling date was at the end of the experiment after 731 days (app. 35b).

7.2.2 Depth of soil sampling

The sampling depth was 10 cm for the 1st and 2nd sampling date and the whole soil profile (1-130 cm) was investigated in steps of 10 cm at the end of the experiment (3rd sampling date).

7.2.3 Extraction method

The method used for extracting the test substance from soil and the result of this extraction are given in appendix 14a. TLC and HPLC analysis for determination and detection limit are given in app. 14b, c. The extraction method for the last sampling date is given in app. 28.

7.2.4 Total radioactivity in soil

<u>1st sampling date:</u>

91 days after the first application, 18.8 % (lysimeter 19) and 19.5 % (lysimeter 20) of the total radioactivity applied were detected in the 0 - 10 cm soil layer (app. 13).

<u>2nd sampling date:</u>

373 days after the first application, 3.4 % (lysimeter 19) and 6.6 % (lysimeter 20) of the total radioactivity applied were detected in the 0 - 10 cm soil layer (app. 25a).

<u>3rd (last) sampling date:</u>

731 days after the first application, 13.5 % (lysimeter 19) and 15.5 % (lysimeter 20) of the total radioactivity applied were detected in the whole soil profile most of it being concentrated in the plough layer (app. 25b).

7.2.5 Extraction results (0 - 10 cm soil layer)

<u>1st sampling date:</u>

^{14}C-Metalaxyl was determined in concentrations of 9.15/10.65 µg/kg (lys. 19; app. 15a). 5.67/7.56 µg/kg were determined in the soil samples from lysimeter 20 (app. 15 b).

Also the metabolites CGA 108906 and CGA 62826 were determined (app. 15a, b).
Examples of chromatographic analyses are given in app. 16a, b.

2nd sampling date:

^{14}C-Metalaxyl concentration was < the detection limit of 0.5 µg/kg in lysimeter 19 and lysimeter 20 (app 26a, b).
Also the metabolites CGA 108906 and CGA 62826 could not be de- tected (app. 26a, b).
Examples of chromatographic analyses are given in app. 27a, b.

3rd (last) sampling date

The A.I. equivalents in the organic phases were below the detection limit. Most of the radioactivity was not extractable. For details see app. 29 a, b and app. 30 a, b.

7.3. Plants

For details concerning the cultivation of potatoes in the lysimeters see appendix 6a, b (1st year) and for the 2nd year see app. 19a, b.

7.3.1 Total radioactivity in plants

1st year

The total activity in potatoes at harvest time was 19.50 % (lysimeter 19) and 21.33 % (lysimeter 20) respectively of the radioactivity applied (app. 17). Most of these amounts were detected in the leaves.

2nd year

The total radioactivity in the winter wheat was 0.02 and 0.03 % of the radioactivity applied (app. 31a). The total radioactivity in the winter barley at the end of the study was < 0.01 % (app. 31b).

8. Total radioactivity

The total radioactivity (sum of percolate, plants and soil) present in the lysimeters after the 1st experimental year is given in appendix 18. The results for the whole experimental time are given in app. 32.

9. Analysis conditions

Determination of ^{14}C:

Four to six aliquots of 0.5 g air-dried and homogenized material were mixed with cellulose and combusted (Sample Oxidizer 406, Packard Tec.). The $^{14}CO_2$ released was trapped in a mixture of Carbosorb (Packard Tec.) and Permaflour (Packard Tec.) (9:12, vol/vol). The "dpm" values of the radioactivity of the samples were determined in a liquid scintillation spectrometer (1219-Rackbeta, LKB) with automatic quench correction.
Aqueous samples were mixed with ready-to-use scintillator (Instagel,

Packard Tec.), afterwards the radioactivity was measured as described above.

The determination limit of ^{14}C was the threefold background value (100 dpm). The corresponding detection limit was the half of the determination limit (50 dpm).

Thin layer chromatography:

The extracts were applied to the TLC-plates by using an automatic applicator (Linomat IV, Camag). The reference substances were spiked with a pipette onto the extract bands or alternatively onto either end of the plate. For evaluation, radio-TL chromatograms were scannded with a Linear Analyser (LB 283 Berthold) and evaluated with the help of a data processing system (1-Dimensonial Chroma version 7.25, Berthold on Compaq 386). For TLC-conditions see appendix 10b.

HPLC:

For HPLC a LiChrospher 100 RP-18 column and the eluents ammoniumbicarbonate and acetonitrile were used in combination with a LB 506 C detector (Berthold). Further details are given in app. 10b.

Extraction of percolate samples:

1000 ml of percolate were extracted as described in app. 10a. The determination limit was 0.05 µg/l. The detection limit was 0.01 µg/l. For A.I. concentrations between determination and detection limit the base for further calculations was the half of the determination limit (0.025 µg/l). The recovery rate of this method was 100 and 110% for the A.I.
Percolate samples were sent to the sponsor for additional analysis (mass-spectroscopy).

Extraction of soil samples:

The soil was extracted according to the method described in appendix 14a. The recovery rates obtained by this method were determined as follows: defined quantities of ^{14}C labelled active ingredient were added to 50 g aliquots (d.w.) of the test soil twice and were extracted as described in app. 15. The resulting ^{14}C-balance sheet yielded recovery rates of 110 %. The determination limit was 1.0 µg/kg. The detection limit was 0.5 µg/kg.
Soil samples were sent to the sponsor for additional analysis.

IV DISCUSSION

1. Percolate

No major differences between the two lysimeters (app. 23d) were observed with respect to the total amount of percolate. Also in both lysimeters mean A.I. concentrations were ≤ 0.05 µg/l. These findings were partly due to the appearance of the A.I. in the first leachates after application indicating that preferential flow of small amounts of percolate was partly responsible for this results.
The metabolites CGA 108906 and CGA 62826 were found in nearly each percolate sample of both lysimeters in mean concentrations significantly above 0.1 µg/l. These metabolites, also determined by mass-spectroscopy investigation of the sponsor (see report attached), must be classified as leachable compounds.
Parts of the total radioactivity in the percolate were very polar and could not be identified (0.7 % of the total ^{14}C applied). Also $^{14}CO_2$ was determined in the percolate indicating the mineralization of the phenylring in the soil.

2. Soil and plants

At days 91 and 373 only soil from the 0 - 10 cm soil layer was sampled. Therefore, only a part of the radioactivity remaining in the soil could be investigated. At the end of the study 13.50 and 15.49 % of the ^{14}C applied were measured in the whole soil profile 3.59 % and 1.79 % being localized below 60 cm of depth. The extraction results obtained at the end of the study showed that this radioactivity mainly represented radioactivity neither extractable with organic solvents nor with $CaCl_2$. The A.I. equivalents in the organic phases of this soil samples were below the detection limit of ^{14}C-Metalaxyl (0.5 µg/kg).
Because of the application to potatoes at the beginning of the study the radioactivity in the potatoe leaves was rather high but was negligible for all following crops investigated indicating that uptake of soil residues by plants did not play an important role.

V LITERATURE CITED

1) Richtlinien für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren, Teil IV 4-3 (Februar 1990). Lysimeteruntersuchungen zur Verlagerung von Pflanzenschutzmitteln in den Untergrund

2) 5.1.2.e Woo (1993). Metalaxyl, Structure Elucidation of Metabolites. Internal Spectroscopy Report of Ciba-Geigy Ltd., Basel, Switzerland

app. 01a

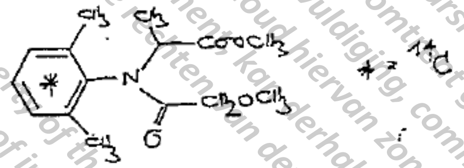
Test substance
(A.I. = ^{14}C-Metalaxyl)

CIBA-GEIGY
DIVISION AGRO
F + E Pflanzenschutz
Fachbereich Chemie
Isotopenlabor
PP 2.234
1. W...

Delivery Date: 15. Juni 1992
Delivered to :
Prof. Dr. 5.1.2.e Woo
Landes- Lehr- & Forschungsanstalt
f. Landwirtschaft, Wein- & Gartenbau
Breitenweg 91
D-6730 Neustadt / Weinstrasse

RADIOCHEMICAL SPECIFICATIONS

Compound : [^{14}C]CGA 48'988
D,L-N-(2,6-dimethyl-[^{14}C(U)]phenyl)-N-(2'-methoxyacetyl)-alanin-methylester



Batch-No. : GAN-XVII-45/II-2

Spec. activity : 2.01 MBq/mg

Activity : 134.7 MBq

Amount : 67 mg

Radiochemical purity : 97 %

Analytical methods : DC: Kieselgel MERCK 60 F 254
Essigester / Hexan 1:1
Linear-Analyzer LB 282/511

Special informations : Lieferung als EC-240 Formulierung in
Leerformulierung A-7192 B
Lagerung bei -20 °C
Exp. Date: 30. 9. 92

Analysed : 10. Juni 1992 Signature : 5.1.2.e Woo

IMPORTANT : Reanalysis before use is compulsory, because the stability of radiolabelled compounds may be different from the stability of non-labelled reference substances.

WICHTIG : Die Stabilität von radioaktiv markierten Präparaten kann sich stark von derjenigen der nicht markierten Vergleichssubstanzen unterscheiden. Das Präparat muss vor Gebrauch bezüglich der radiochemischen Reinheit überprüft werden.

app. 01b

List of metabolites

code : **CGA 42447**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: LU 440
storage : 0 - 5 °C
exp. date: no declaration

storage : 4 °C

code : **CGA 48988**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 18, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: AMS 175/104
storage : 0 - 5 °C
exp. date: Jan. 1994

storage : 4 °C

code : **CGA 37734**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: LU.646
storage : 0 - 5 °C
exp. date: no declaration

storage : 4 °C

app. 01c

List of metabolites

code : **CGA 119857**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: LU-1208
storage : 0 - 5 °C
exp. date: no declaration

storage : 4 °C

code : **CGA 67869**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: RU-1912/1
storage : 0 - 5 °C
exp. date: no declaration

storage : 4 °C

code : **CGA 67868**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: RU-2228/2
storage : 0 - 5 °C
exp. date: August 1995

storage : 4 °C

app. 01d

List of metabolites

code : **CGA 67867**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: LU-598
storage : 0 - 5 °C
exp. date: no declaration

storage : 4 °C

code : **CGA 62826**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 19, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: RU-1592/3
storage : 0 - 5 °C
exp. date: August 1998

storage : 4 °C

code : **CGA 68125**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: LU-603
storage : 0 - 5 °C
exp. date: no declaration

storage : 4 °C

app. 01e

List of metabolites

code : **CGA 68124**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: LU 602
storage : 0 - 5 °C
exp. date: no declaration

storage : 4 °C

code : **CGA 67866**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: 99844.8
storage : 0 - 5 °C
exp. date: February 1994

storage : 4 °C

code : **CGA 119857**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: LU-1208
storage : 0 - 5 °C
exp. date: no declaration

storage : 4 °C

app. 01f

List of metabolites

code : **CGA 108905**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: GB-XX-400
storage : 0 - 5 °C
exp. date: no declaration

storage : 4 °C

code : **CGA 108906**

characterisation: Bestellschein für unmarkierte Referenz- und Standardsubstanzen

- date : May 20, 1992
- address: Ciba Geigy AG, CH-4002 Basel
  Bereich R-1001A.2.14

batch-no.: GB-XIX-127B
storage : 0 - 5 °C
exp. date: no declaration

storage : 4 °C

app. 02

Data of test soil

soil parameter	0 - 30 cm	30 - 60 cm	60 - 120 cm
clay[1] [%] (0.0-0.002 mm)	3.6	4.6	5.6
silt[1] [%] (0.002-0.0635mm)	15.6	15.6	12.5
sand[1] [%] (0.063-2.0mm)	80.8	79.8	81.9
C_{org}[1] [%]	1.0	0.2	0.1

1) investigated by LUFA, Speyer, FRG

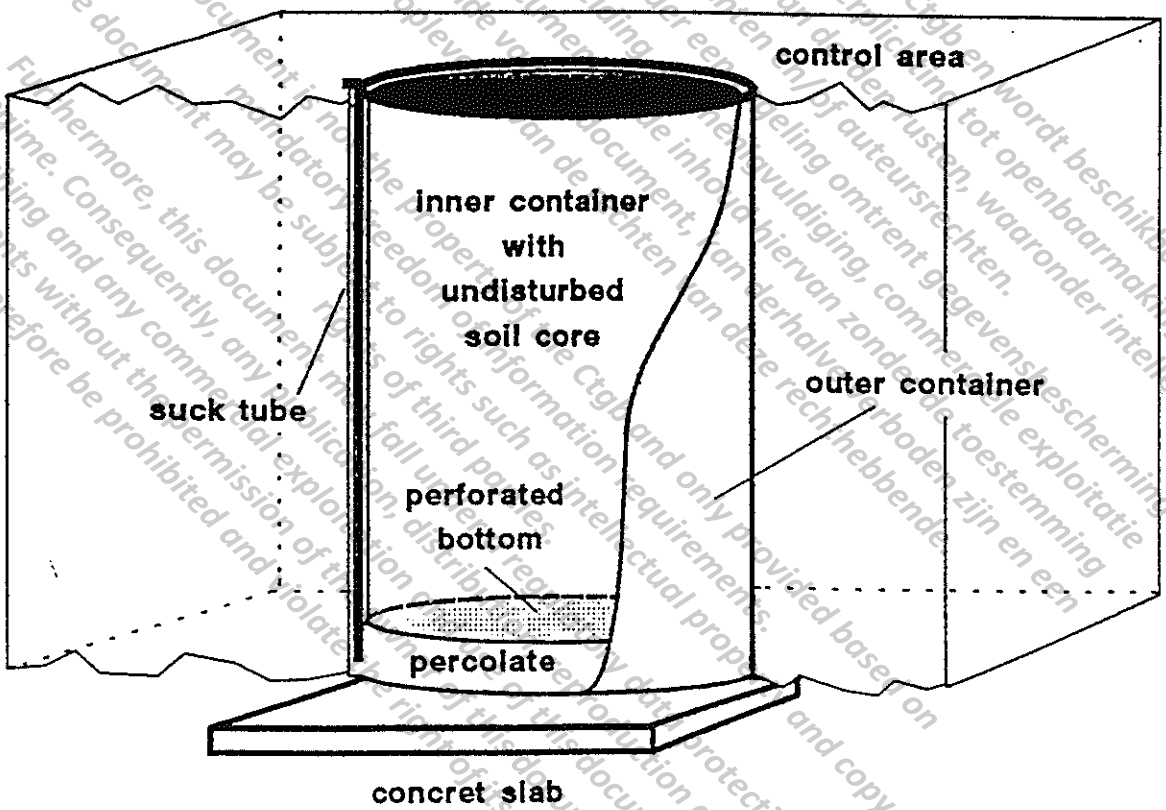
pH-values[2]

soil layer [cm]	lysimeter 19	lysimeter 20
0 - 10	6.6	7.1
10 - 20	6.5	7.2
20 - 30	6.2	7.1
30 - 40	5.4	7.1
40 - 50	6.4	7.2
50 - 60	6.2	7.3
60 - 70	6.2	7.1
70 - 80	6.4	7.2
80 - 90	6.5	7.1
90 - 100	6.6	6.8
100 - 110	6.7	6.9
110 - 120	6.6	6.7
120 - 130	5.5	6.2

2) results of the measurement of the soil samples of July 30, 1994

app. 03

Lysimeter with undisturbed soil profile

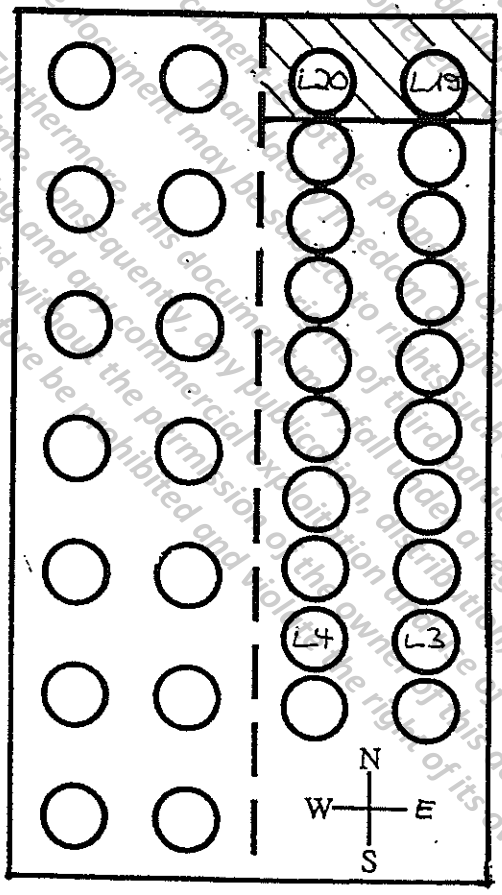


app. 04

Arrangement of lysimeters

Lysimeter field

SLFA Neustadt/Weinstr.
Fachbereich Phytomedizin
Referat Ökochemie



19,20 lysimeters for the study with CIB02

control area for the study with CIB02 (260 cm x 460 cm)

L 3 control-lysimeters for the
L 4 determination of climatic conditions

app. 05a

Application details, 1st year

1st treatment

lysimeter number	19	20
date of application	a) June 29,1992 b) July 14,1992	
amount A.I./ha	a)107.5 g b)112.5 g	a)105.0 g b)111.3 g
amount used (A.I./lys.)* and radioactivity used	a) 8.6 mg b) 9.0 mg a) 17253.0 kBq b) 18184.6 kBq	a) 8.4 mg b) 8.9 mg a) 16956.0 kBq b) 17937.4 kBq
kind of formulation	EC-240 (A-7192 B)	
kind of mixture 1) A.I. formulated* 2) amount of solution	1) a) 8.6 mg b) 9.0 mg 2) a)+b) 36 ml water/lys.= 450.0 l/ha	1) a) 8.4 mg b) 8.9 mg 2) a)+b) 36 ml water/lys.= 450.0 l/ha
application technique	sprayer AMTP 208 spray pressure 1.5 bar	
application losses radioactivity applied amount of active ingredient after deduction of application losses*	a) 19.3% 13916.0 kBq 6.9 mg A.I.	a) 10.5% 15177.1 kBq 7.6 mg A.I.
	b) 13.9% 15663.6 kBq 7.8 mg A.I.	b) 15.7% 15119.0 kBq 7.5 mg A.I.
spec. radioactivity	2.01 MBq/mg	

*) calculated on the base of the spec. radioactivity

app. 05b

Application details, 1st year

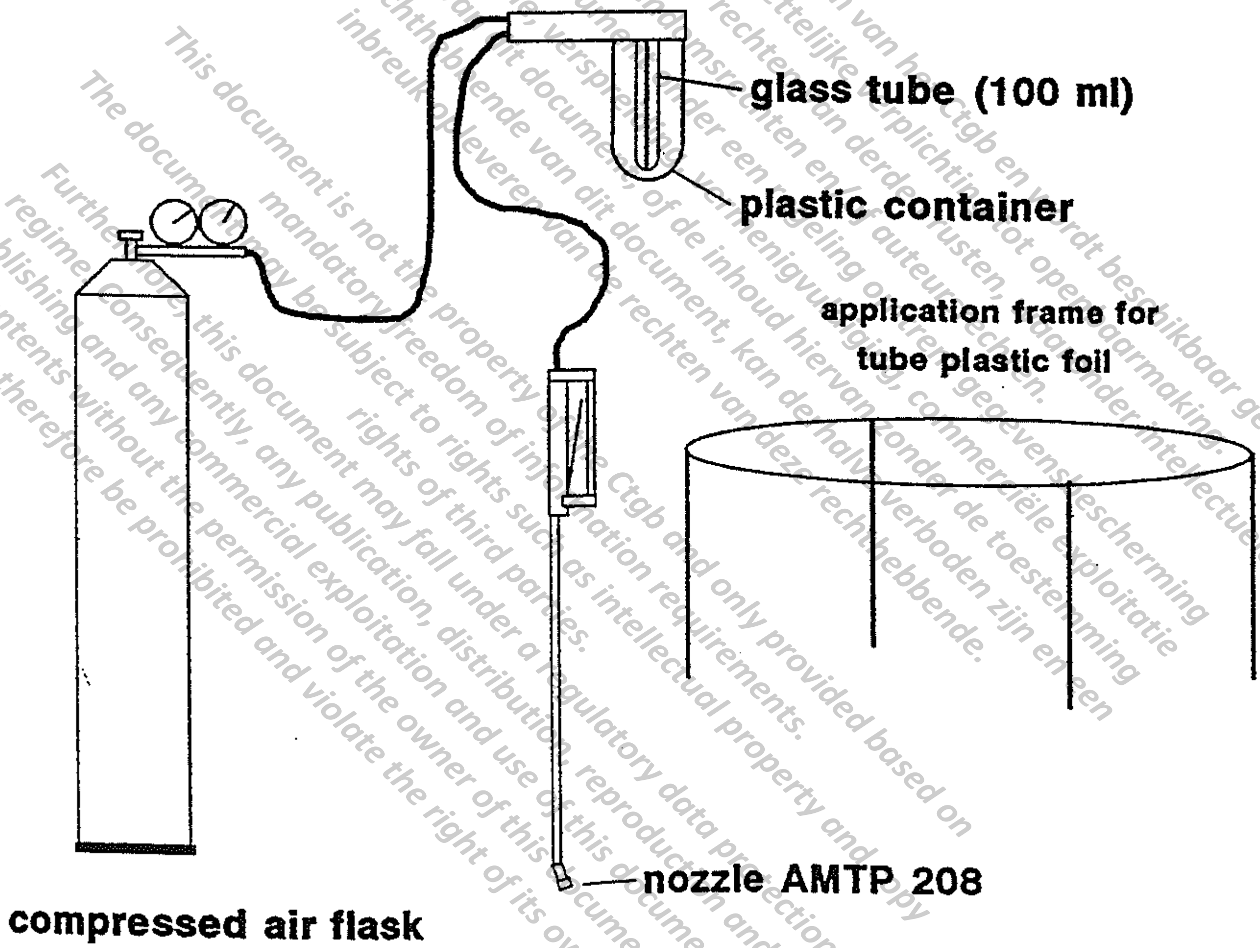
2nd treatment

lysimeter number	19	20
date of application	July 27, 92	
amount A.I./ha	175.0 g	200.0 g
amount used (A.I./lys)* and radioactivity used	14.0 mg 28183.7 kBq	16.0 mg 32200.6 kBq
kind of formulation	EC-240 (A-7192 B)	
kind of mixture 1) A.I. formulated* 2) amount of solution	1) 14.0 mg 2) 36 ml water/lys.= 450.0 l/ha	1) 16.0 mg 2) 36 ml water/lys.= 450.0 l/ha
application technique	sprayer AMTP 208 spray pressure 1.5 bar	
application losses radioactivity applied amount of active ingredient after deduction of application losses*	18.2% 23065.8 kBq 11.5 mg A.I.	16.8% 26791.7 kBq 13.3 mg A.I.
spec. radioactivity	2.01 MBq/mg	

*) calculated on the base of the spec. radioactivity

app. 05c

Figure of spraying system



app. 06a

Cultivation of the lysimeter, 1st year

1) Plants

plant		potatoes		rape	
lysimeter		19	20	19	20
seed time		Apr. 07, 1992		Aug. 14, 1992	
harvest time		Aug. 06, 1992[1)]		Sept. 28, 1992	
fresh weight					
potatoes	kg/lys	3.11	5.00	not investigated and left in the soil	
	dt/ha	388.75	625.00		
leaves	kg/lys	0.22	0.25		
	dt/ha	27.50	31.25		
total	kg/lys	3.33	5.25		
	dt/ha	416.25	656.25		

1) 38 days after 1st application

app. 06b

Cultivation of the lysimeter, 1st year

2) Fertilization

date of fertilizing	fertilizer	kg nutrient/ ha	g nutrient/ lysimeter
April 07,92	KAS (27 % N)	162.5 N	13.0 N
	superphosphate (18% P_2O_5)	143.8 P_2O_5	11.5 P_2O_5
	Kaliummagnesia (30/10)	360.0 K_2O 120.0 MgO	28.8 K_2O 9.6
March 08,93	ammoniumsulfate-saltpeter (25% N)	53.8 N	4.3 N
	superphosphate (18% P_2O_5)	90.0 P_2O_5	7.2 P_2O_5
	Kaliummagnesia (30/10)	180.0 K_2O 60.0 MgO	14.4 K_2O 4.8 MgO
May 07,93	ammoniumsulfate-saltpeter (25% N)	40.0 N	3.2 N

3) Plant protection

date	kind of pesticide	trade name	concentration
July 13,92	insecticide	E605 Forte	0.15%

app. 07a

Experimental conditions, 1st year

1) Temperatures

month	air [°C] 1)	soil 10 cm [°C] 1)	soil 30 cm [°C] 1)
July 92	20.5	18.3	17.6
August 92	21.5	19.5	19.0
September 92	15.2	12.9	13.1
October 92	8.1	6.9	7.5
November 92	7.2	4.2	4.6
December 92	2.2	0.8	1.5
January 93	4.8	1.0	0.8
February 93	0.3	-0.9	-0.6
March 93	6.2	3.0	2.9
April 93	12.6	8.9	8.4
May 93	16.2	13.4	13.1
June 93	18.5	15.4	15.1

1) monthly mean value

app. 07b

Experimental conditions, 1st year

2a) Precipitation and irrigation
July 1992 - February 1993

month	precipitation [mm]	irrigation total [mm]	irrigation single [mm]	irrigation date
July 92	42.0	-	-	
August 92	37.0	19.0	10.0 9.0	Aug. 3, 92 Aug. 4, 92
September 92	25.0	49.0	10.0 10.0 10.0 10.0 9.0	Sep. 21, 92 Sep. 22, 92 Sep. 23, 92 Sep. 24, 92 Sep. 25, 92
October 92	61.0	39.0	10.0 10.0 10.0 9.0	Oct. 21, 92 Oct. 23, 92 Oct. 26, 92 Oct. 31, 92
November 92	71.0	-	-	
December 92	38.0	-	-	
January 93	27.0	23.0	5.0 10.0 6.3 1.7	Jan. 13, 93 Jan. 15, 93 Jan. 26, 93 Jan. 29, 93
February 93	9.0	46.0	3.8 10.0 10.0 10.0 6.0 6.2	Feb. 03, 93 Feb. 19, 93 Feb. 22, 93 Feb. 24, 93 Feb. 25, 93 Feb. 26, 93
sum: July to February	310.0	176.0		total: 486.0 mm

app. 07c

Experimental conditions, 1st year

2b) Precipitation and irrigation
March 1993 - June 1993

month	precipitation [mm]	irrigation		
		total [mm]	single [mm]	date
March 93	4.0	54.0	3.8	March 02, 93
			6.2	March 08, 93
			6.3	March 09, 93
			5.0	March 10, 93
			2.5	March 11, 93
			5.0	March 17, 93
			6.2	March 23, 93
			10.0	March 24, 93
			9.0	March 25, 93
April 93	26.0	52.0	10.0	April 15, 93
			5.0	April 21, 93
			5.0	April 22, 93
			5.0	April 26, 93
			8.8	April 27, 93
			3.7	April 28, 93
			10.0	April 29, 93
			4.5	April 30, 93
May 93	60.0	41.0	7.5	May 12, 93
			5.0	May 24, 93
			10.0	May 25, 93
			10.0	May 27, 93
			8.5	May 28, 93
June 93	22.0	16.0	8.8	June 28, 93
			7.2	June 29, 93
sum:				
July to February	310.0	176.0		total: 486.0 mm
March to June	112.0	163.0		275.0 mm
total 1st year	422.0	339.0		761.0 mm

app. 08

Sampling of percolate, 1st year

date of sampling	19 amount [l]	20 amount [l]
July 02, 1992	19.5	10.3
Aug. 03, 1992	14.5	15.2
Sep. 03, 1992	3.7	-[1)]
Oct. 05, 1992	23.6	14.3
Nov. 02, 1992	49.5	36.9
Nov. 19, 1992	28.0	29.0
Dec. 08, 1992	33.6	30.3
Dec. 18, 1992	10.8	15.4
Jan. 04, 1993	7.7	15.0
Jan. 18, 1993	19.5	13.2
Feb. 01, 1993	12.8	15.0
Feb. 15, 1993	8.0	10.6
March 01, 1993	22.2	13.1
March 16, 1993	20.2	20.1
April 06, 1993	17.1	19.9
April 19, 1993	7.9	10.2
May 03, 1993	5.7	8.2
May 19, 1993	-[1)]	7.8
June 02, 1993	6.0	-[1)]
total	310.3	284.5

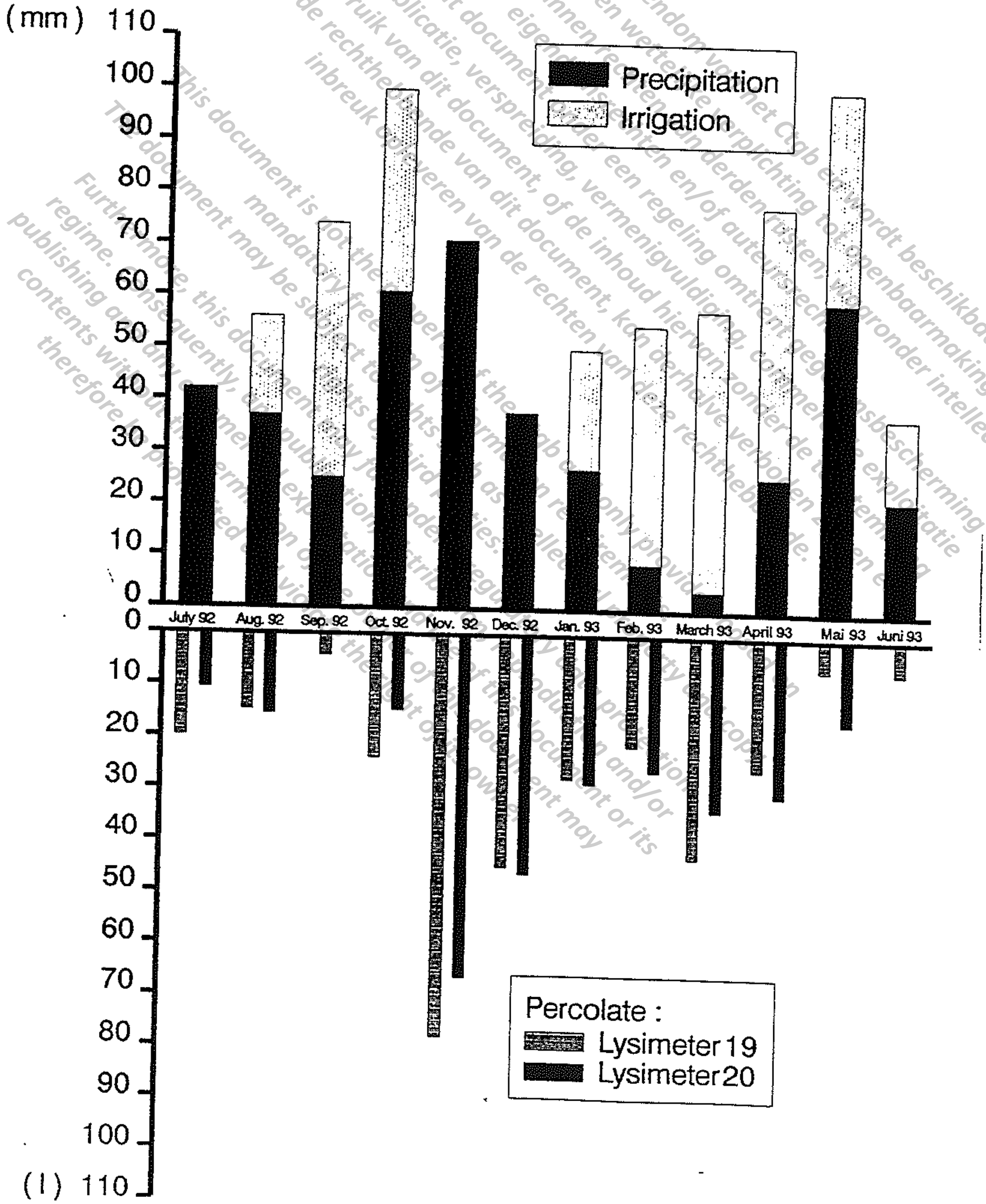
[1)] percolate volume < 3 liter

app. 09

Precipitation, irrigation and percolate

Lysimeter 19 and 20

(1st year)



app. 10a

Extraction scheme, percolate

Acidify 1000 ml percolate with 6 N H_2SO_4 to pH 2

*

Activate a C18-cartridge with methanol and water

*

Clean up the percolate over the cartridge

*

^{14}C-measurement from the eluated water

*

Desiccate the cartridge for 30 min with nitrogen

*

Eluate the cartridge with 4 * 500 µl methanol

*

Evaporate to 2 ml

*

TLC + HPLC analyses

concentration µg/l	radioactivity kBq	recovery rate kBq	%
10.00	A 10.7	10.8	100.9
	B 10.7	11.1	103.7
1.00	A 0.99	1.01	102.0
	B 0.99	1.01	102.0
0.05	A 0.10	0.10	100.0
	B 0.10	0.11	110.0

app. 10b

TLC conditions

Solvent	chloroform : tetrachloromethane : ethanol 3 : 1 : 1
Plate	silicagel $60F_{254}$
R_f-A.I.	0.80-0.92

HPLC conditions

Column :	MERCK 50983 LiChrospher 100 RP-18 5 µm, 250 x 4 mm
Eluent	A : ammoniumbicarbonate 0.1% B : acetonitrile
Gradient profile :	see below
Detector :	LB 506 C (Berthold),
R_t-A.I. :	18m38s-18m48s

Gradient profile:

time	flow (ml/min)	%B
0	1.5	0
5	1.5	5
10	1.5	10
21	1.5	90
30	1.5	0
35	1.5	0

R_t-Metabolites

Metabolite number	R_t-value
CGA 108906	1m36s-1m54s
CGA 62826	14m10s-14m28s

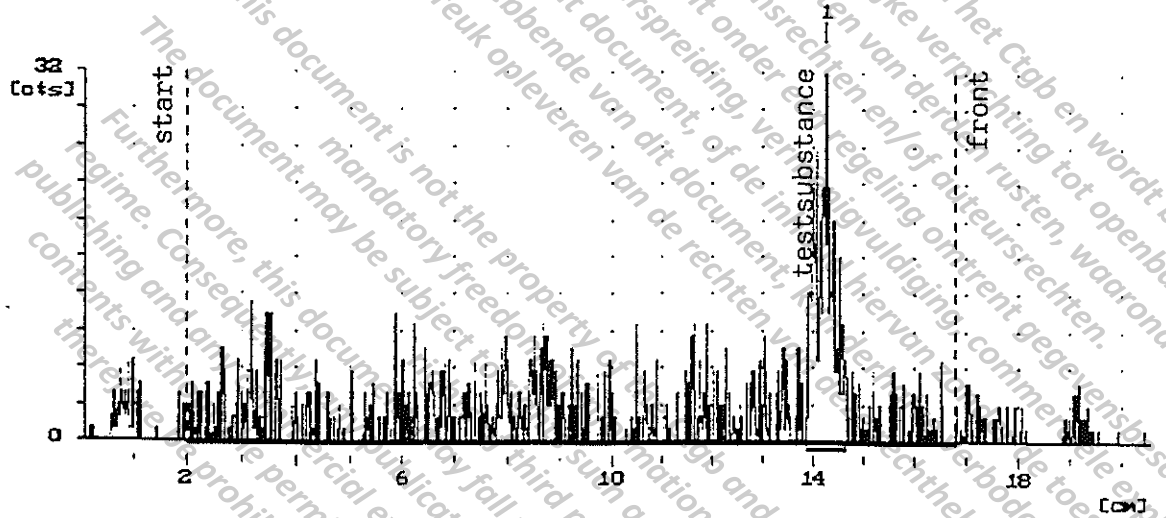
app. 10c

Determination and detection limits for the A.I.

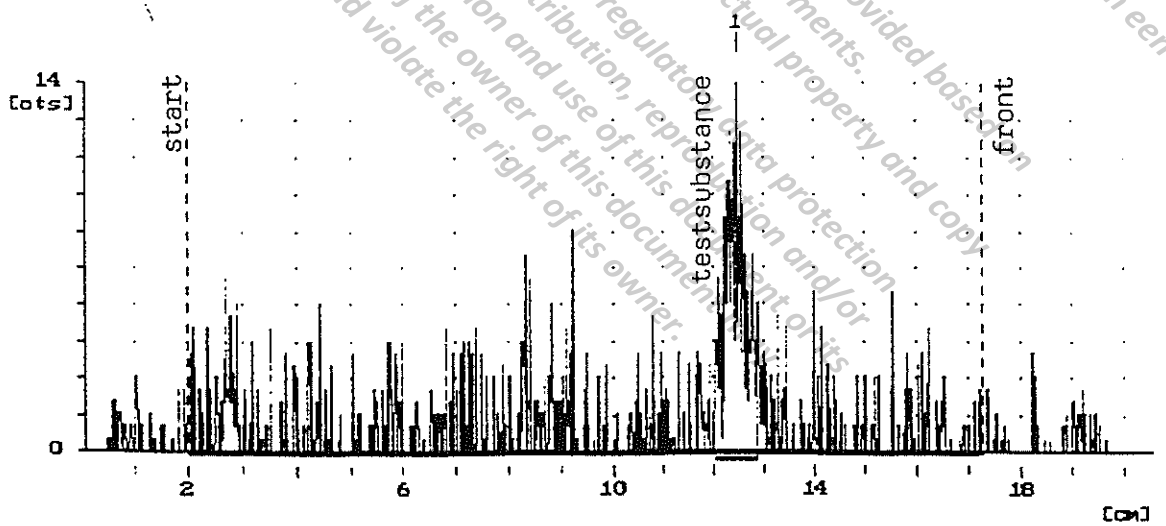
in percolate sampling by TLC

Solvent : chloroform : carbon tetrachloride : ethanol = 3:1:1
Plate : silicagel $60F_{254}$

a) Chromatogram of the determination limit (0.05 µg/l)



b) Chromatogram of the detection limit (0.01 µg/l)

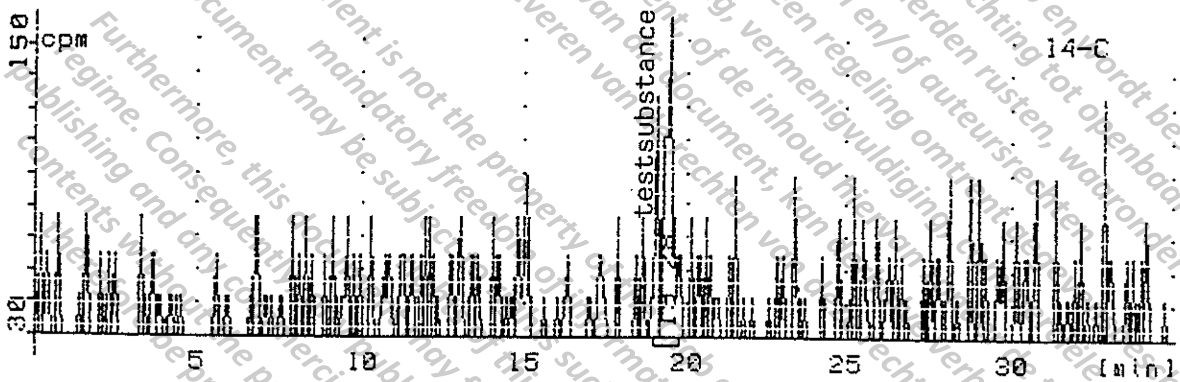


app. 10d

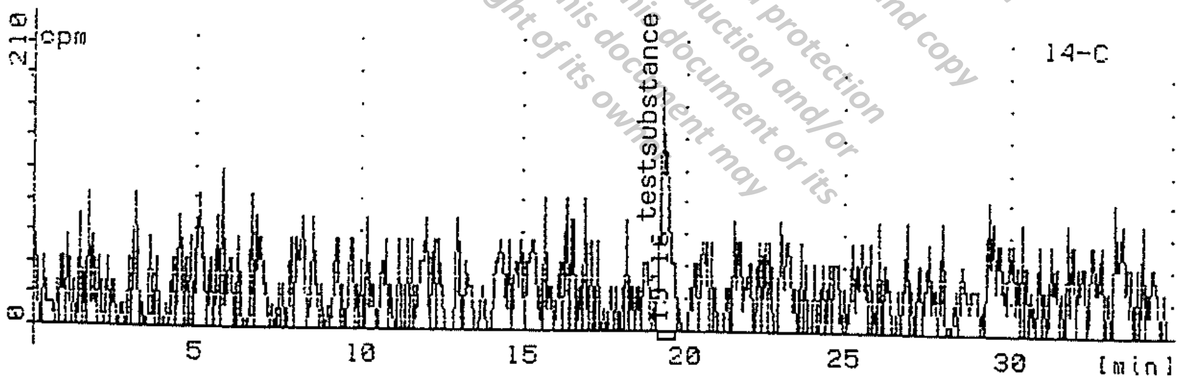
Determination and detection limits for the A.I.

in percolate sampling by HPLC

a) Chromatogram of the determination limit (0.05 µg/l)



b) Chromatogram of the detection limit (0.01 µg/l)



app. 11a

Percolate investigation, 1st year

Results of lysimeter 19

date of sampling	radioactivity in the percolate [l]	[kBq/l]	[µg/l] 1)	$^{14}CO_2$ [µg/l]	rep.	loss n.i. 2) [µg/l]	water phase 3) [µg/l]	extracted metabolites CGA 108906 [µg/l]	CGA 62826 [µg/l]	extracted active ingredient [µg/l]
July 02,92	19.5	–	–	–		–		–	–	–
Aug. 03,92	14.5	–	–	–		–		–	–	–
Sept. 03,92	3.7	0.60	0.30	–	a	0.01	–	0.01	0.02	0.26
					b	<0.01	–	–	0.02	0.27
Oct. 05,92	23.6	1.01	0.50	–	a	0.07	–	0.04	0.03	0.36
					b	0.07	–	0.04	0.03	0.35
Nov. 02,92	49.5	2.05	1.02	–	a	–	–	0.10	0.87	0.09
					b	–	–	0.20	0.79	0.07
Nov. 19,92	28.0	4.29	2.13	0.02	a	–	0.19	–	–	–
					b	0.09	0.18	0.28	1.67	0.07
Dec. 08,92	33.6	5.60	2.79	–	a	–	0.48	0.26	2.25	–
					b	–	0.48	0.21	2.37	–
Dec. 18,92	10.8	16.33	8.12	–	a	–	0.79	1.42	8.25	–
					b	–	0.55	1.52	8.40	–
Jan. 04,93	7.7	18.74	9.32	–	a	–	0.85	1.25	7.50	–
					b	–	0.91	0.85	7.94	–
Jan. 18,93	19.5	19.59	9.75	0.08	a	–	0.61	1.79	8.11	–
					b	–	0.60	1.88	7.93	–
Feb. 01,93	12.8	19.90	9.90	0.02	a	–	0.57	1.91	7.99	–
					b	–	0.55	2.00	8.06	–
Feb. 15,93	8.0	19.59	9.75	0.14	a	–	0.62	0.91	8.72	–
					b	–	0.59	1.00	8.66	–
March 01,93	22.2	20.69	10.29	0.06	a	–	0.56	1.39	9.05	–
					b	–	0.53	1.41	8.75	–
March 16,93	20.2	21.02	10.46	0.05	a	0.10	0.66	1.52	8.12	–
					b	0.37	0.68	1.33	8.02	–
April 06,93	17.1	19.51	9.71	0.04	a	–	1.05	1.06	8.13	–
					b	–	1.00	1.28	7.98	–
April 19,93	7.9	17.55	8.73	0.03	a	–	0.84	1.30	7.26	–
					b	–	0.84	1.34	7.32	–
May 03,93	5.7	16.30	8.11	0.03	a	–	0.81	0.99	7.09	–
June 02,93	6.0	15.62	7.77	0.04	a	–	1.07	1.11	6.28	–
					b	–	1.11	1.17	6.21	–

1) calculated as A.I. equivalents on the base of the spec. radioactivity (2.01 kBq/µg)
2) n.i. = not identified
3) not extracted and not identified
"-" below detection limit of 0.01 µg/l

app. 11b

Percolate investigation, 1st year

Results of lysimeter 20

date of sampling	radioactivity in the percolate			$^{14}CO_2$	rep.	loss n.i.2)	water phase3)	extracted metabolites CGA 108906	extracted metabolites CGA 62826	extracted active ingredient
	[l]	[kBq/l]	[µg/l]1)	[µg/l]		[µg/l]	[µg/l]	[µg/l]	[µg/l]	[µg/l]
July 02,92	10.3	-	-	-		-	-	-	-	-
Aug. 03,92	15.2	-	-	-		-	-	-	-	-
Oct. 05,92	14.3	0.37	0.18	0.02	a	-	-	0.02	0.05	0.10
					b	-	-	-	0.07	0.10
Nov. 02,92	36.9	0.85	0.42	0.05	a	-	-	0.10	0.30	-
					b	-	-	0.10	0.29	-
Nov. 19,92	29.0	2.43	1.21	0.04	a	0.03	-	0.41	0.83	-
					b	-		0.44	0.90	-
Dec. 08,92	30.3	4.31	2.14	0.05	a	-	0.20	0.95	1.05	-
					b	-	0.18	0.90	1.14	-
Dec. 18,92	15.4	13.60	6.77	0.14	a	-	0.58	3.14	3.43	-
					b	-	0.69	3.21	3.21	-
Jan. 04,93	15.0	16.87	8.39	0.19	a	-	0.76	3.69	3.97	-
					b	-	0.84	2.23	5.43	-
Jan. 18,93	13.2	16.21	8.06	0.24	a	-	0.64	2.24	5.43	-
					b	-	0.70	2.20	5.59	-
Feb. 01,93	15.0	17.06	8.49	0.23	a	-	0.65	2.39	5.35	-
					b	-	2.09	1.66	4.79	-
Feb. 15,93	10.6	16.91	8.41	0.24	a	-	0.74	2.23	5.53	-
					b	-	0.76	2.05	5.84	-
March 01,93	13.1	16.40	8.16	0.34	a	-	0.69	1.70	5.55	-
					b	-	0.72	1.66	5.78	-
March 16,93	20.1	14.35	7.14	0.28	a	0.07	0.97	1.24	4.58	-
					b	0.46	0.91	1.25	4.24	-
April 06,93	19.9	11.43	5.69	0.38	a	-	0.88	1.21	3.80	-
					b	-	0.72	1.40	4.00	-
April 19,93	10.2	9.36	4.66	0.43	a	-	0.63	1.11	2.87	-
					b	-	0.85	0.94	2.89	-
May 03,93	8.2	7.89	3.93	0.45	a	-	0.68	0.77	2.32	-
					b	-	0.67	0.84	2.32	-
May 19,93	7.8	6.77	3.37	0.51	a	-	0.57	0.73	1.91	-
					b	-	0.62	0.77	1.88	-

1) calculated as A.I. equivalents on the base of the spec. radioactivity (2.01 kBq/µg)
2) n.i. = not identified
3) not extracted and not identified
"-" below detection limit of 0.01 µg/l

app. 11c

Percolate analysis 1st year/total

	lysimeter 19			lysimeter 20		
radioactivity applied	52645.4 kBq			57087.8 kBq		
total amount of percolate	310.3 l			284.5 l		
	kBq	%[2]	µg/l[3]	kBq	%[2]	µg/l[3]
^{14}C-carbonate	14.8	0.03	0.02	98.6	0.17	0.17
loss: not extractable not identified	13.3	0.03	0.02	11.5	0.02	0.02
water phase: not extracted not identified	241.9	0.46	0.39	255.0	0.45	0.45
CGA 108906	441.9	0.84	0.71	635.5	1.11	1.11
CGA 62826	2567.9	4.88	4.12	1416.2	2.48	2.48
active ingredient	31.6	0.06	0.05	3.0	0.01	0.01
total activity[1]	3311.4	6.29	5.31	2419.8	4.24	4.23

1) ^{14}C-balance
2) radioactivity applied in the 1st test year = 100%
3) calculated as A.I. equivalents on the basis of the spec. radioactivity (2.01 kBq/µg) and the total amount of the percolate

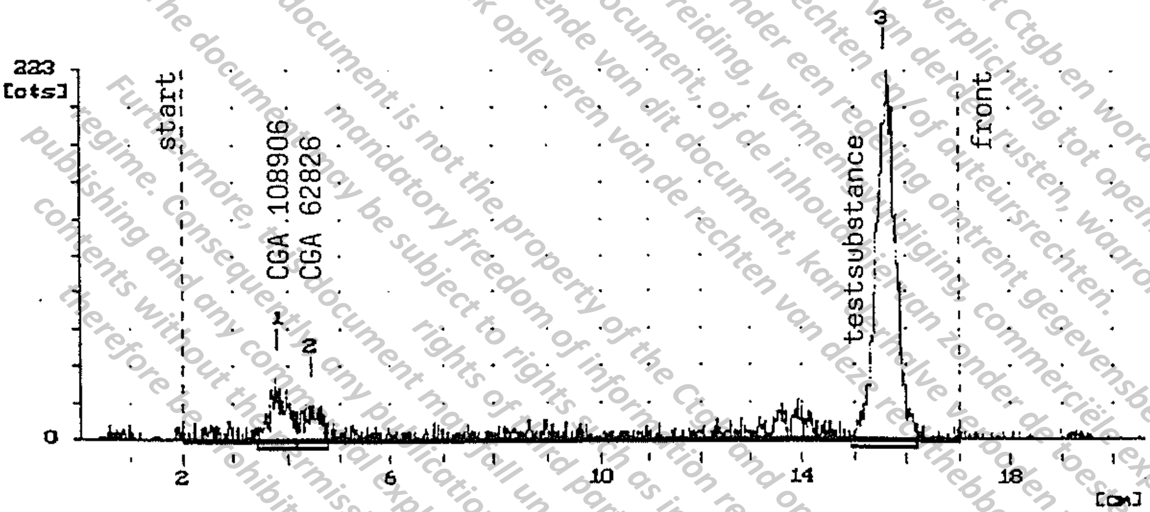
app. 12a

Percolate investigation, 1st year

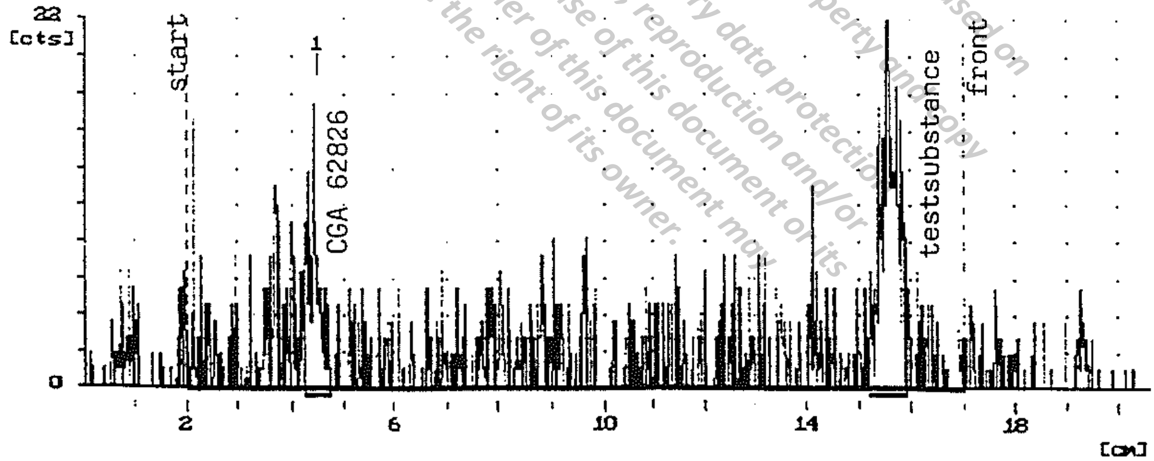
Examples of TLC analyses

Solvent : chloroform : carbon tetrachloride : ethanol = 3:1:1
Plate : silicagel $60F_{254}$

a) Chromatogram of lysimeter 19, percolate of October 05, 1992



b) Chromatogram of lysimeter 20, percolate of October 05, 1992

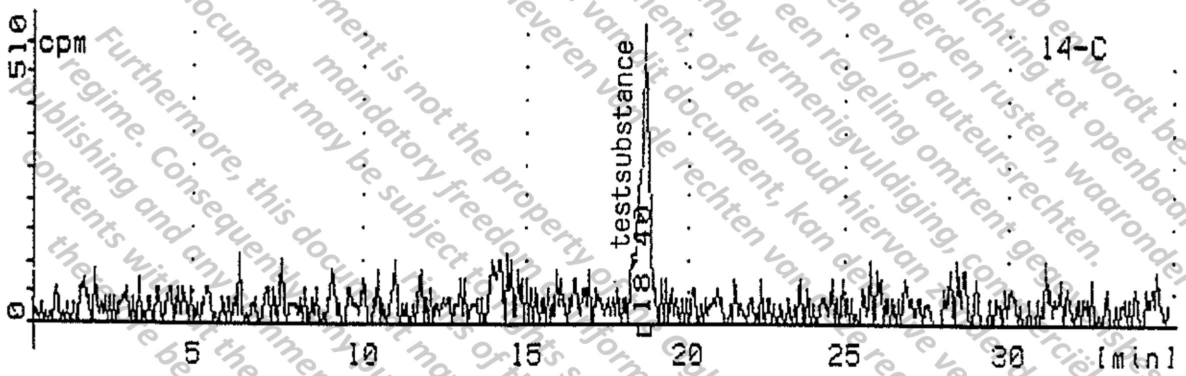


app. 12b

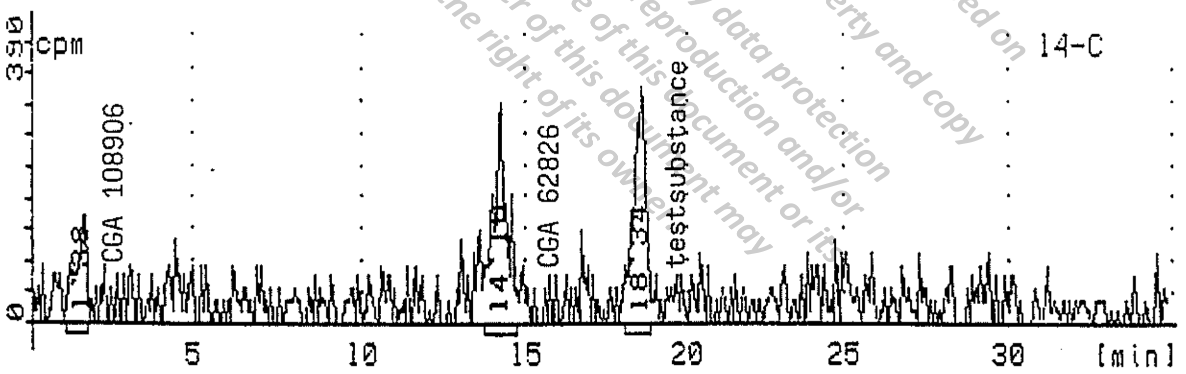
Percolate investigation, 1st year

Examples of HPLC analyses

a) Chromatogram of lysimeter 19, percolate of October 05, 1992



b) Chromatogram of lysimeter 20, percolate of October 05, 1992



app. 13

Soil investigation, 1st year

^{14}C-balance

1) 1st sampling date
(Sep. 28, 1992; 91 days after the first application)

lysimeter 19

soil layer	weight	radioactivity		
cm	kg	kBq	mg/kg[1] A.I.equivalents	%[2]
0-10	126.62	9908.02	0.04	18.82

lysimeter 20

soil layer	weight	radioactivity		
cm	kg	kBq	mg/kg[1] A.I.equivalents	%[3]
0-10	122.47	11115.38	0.05	19.47

1) calculated on the base of the spec. radioactivity of the A.I. (2.01 MBq/mg)
2) amount applied (52645.4 kBq) = 100 %
3) amount applied (57087.8 kBq) = 100 %

app. 14a

Soil extraction method, 1st year

Add 100 ml acetonitril/water (80/20; v/v) to a 50 g portion of soil

*

Shake for 90 min

*

Centrifugate with 5000 rpm

*

Repeat the soil extraction twice with 50 ml acetonitril/water for 45 min and combine all solutions

*

Dry the extracted soil at 30 °C, and homogenize it for determination of the radioactivity not extracted

*

Evaporate the extraction solution to 10 - 20 ml at 30 °C

*

Eluate the concentrated extraction solution using a prepared C-18 column (Bakerbond C-18)

*

Dry for 30 min using nitrogen

*

Extract the column using 4 x 500 µl of methanol

*

Investigate using TLC and HPLC

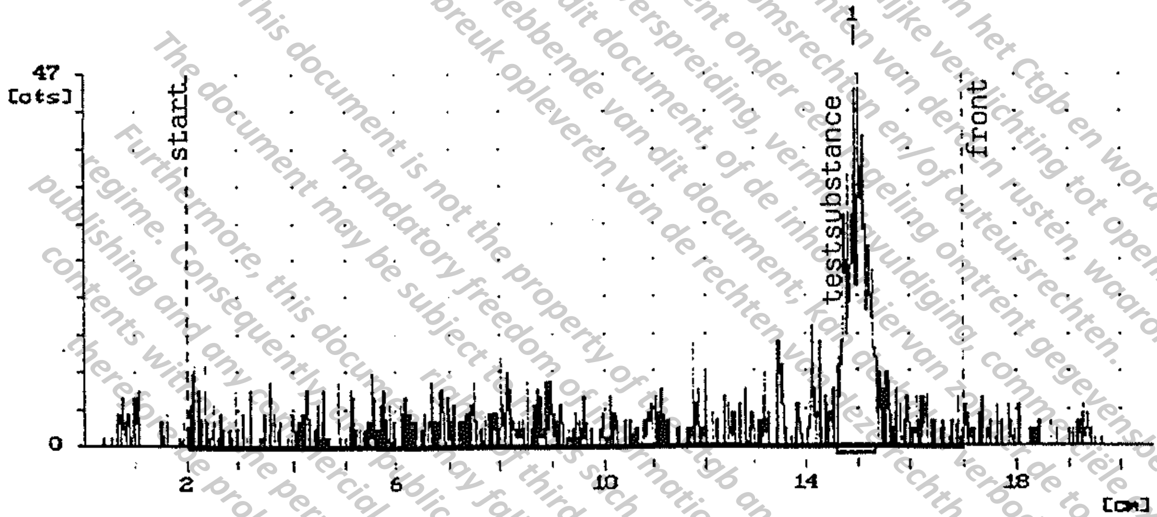
concentration µg/kg	radioactivity kBq	recovery rate kBq %
1.0	A 0.10 B 0.10	0.11 110.0 0.11 110.0

app. 14b

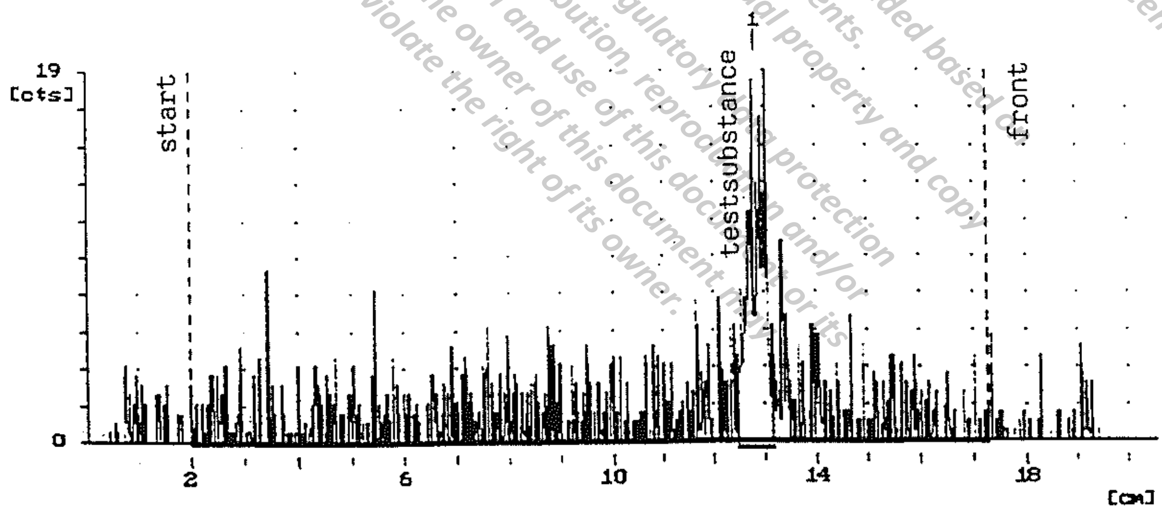
Determination and detection limits for the A.I. in soil by TLC

Solvent: chloroform : carbon tetrachloride : ethanol = 3:1:1
Plate : silicagel $60F_{254}$

a) Chromatogram of the determination limit (1.0 µg/kg)



b) Chromatogram of the detection limit (0.5 µg/kg)

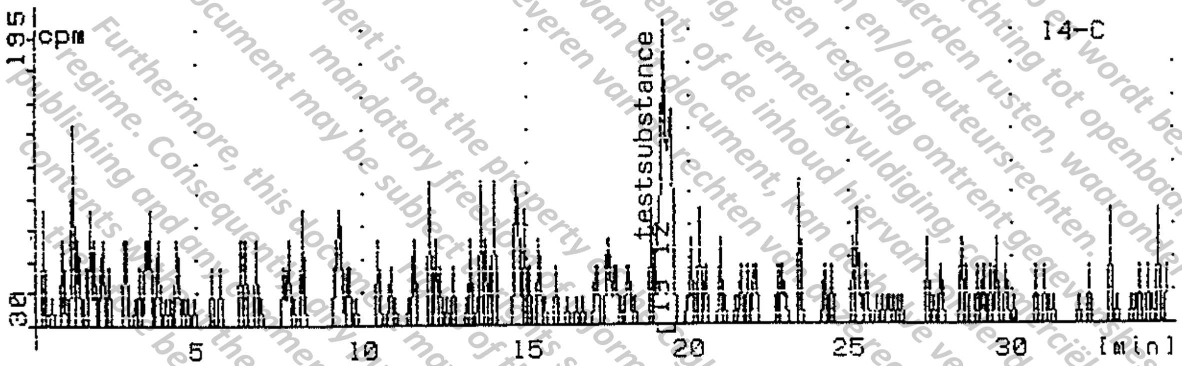


app. 14c

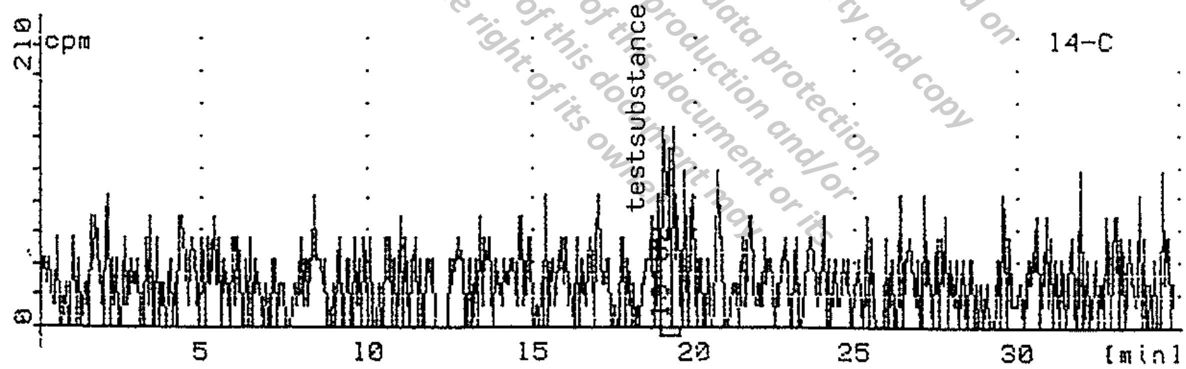
Determination and detection limits for the A.I.

in soil by HPLC

a) Chromatogram of the determination limit (1.0 µg/kg)



b) Chromatogram of the detection limit (0.5 µg/kg)



app. 15a

Soil investigation, 1st year

extraction balance of the 0-10 cm soil layer

1st sampling date (Sep. 28, 1992; 91 days after the 1st application)

lysimeter 19

	a				b			
^{14}C applied	52645.4 kBq				52645.4 kBq			
methanol extract	kBq	µg/kg[1)]	%[2)]	%[3)]	kBq	µg/kg[1)]	%[2)]	%[3)]
A.I.	21.40	10.65	27.58	5.15	18.40	9.15	25.92	4.43
CGA 108906	3.40	1.69	4.38	0.82	3.20	1.59	4.51	0.77
CGA 62826	27.60	13.73	35.57	6.64	27.40	13.63	38.59	6.59
total ^{14}C extracted	52.40	26.07	67.53	12.60	49.00	24.38	69.01	11.79
not extracted solid phase	25.20	12.54	32.47	6.06	22.00	10.95	30.99	5.29
total ^{14}C in a 1 kg soil sample	77.60	38.61	100.00	18.66	71.00	35.32	100.00	17.08

1) calculated on the base of the spec. radioactivity of the A.I. (2.01 kBq/µg)
2) radioactivity in the soil sample = 100%
3) extraction results extrapolated to the total soil sample weight (126.62 kg) and related to the radioactivity applied (100%)

app. 15b

Soil investigation, 1st year
extraction balance of the 0-10 cm soil layer
1st sampling date (Sep. 28 1992; 91 days after the 1st application)
lysimeter 20

	a				b			
^{14}C applied	57087.8 kBq				57087.8 kBq			
methanol extract	kBq	µg/kg[1)]	%[2)]	%[3)]	kBq	µg/kg[1)]	%[2)]	%[3)]
A.I.	11.40	5.67	13.38	2.45	15.20	7.56	18.27	3.26
CGA 108906	2.20	1.09	2.58	0.47	–	–	–	–
CGA 62826	21.00	10.45	24.65	4.51	17.80	8.86	21.39	3.82
not identified metabolite	4.60	2.29	5.40	0.99	4.00	1.99	4.81	0.86
total ^{14}C extracted	39.20	19.50	46.01	8.41	37.00	18.41	44.47	7.94
not extracted solid phase	46.00	22.89	53.99	9.87	46.20	22.99	55.53	9.91
total ^{14}C in a 1 kg soil sample	85.20	42.39	100.00	18.28	83.20	41.39	100.00	17.85

1) calculated on the base of the spec. radioactivity of the A.I. (2.01 kBq/µg)
2) radioactivity in the soil sample = 100 %
3) extraction results extrapolated to the total soil sample weight (122.47 kg) and related to the radioactivity applied (100 %)

app. 16a

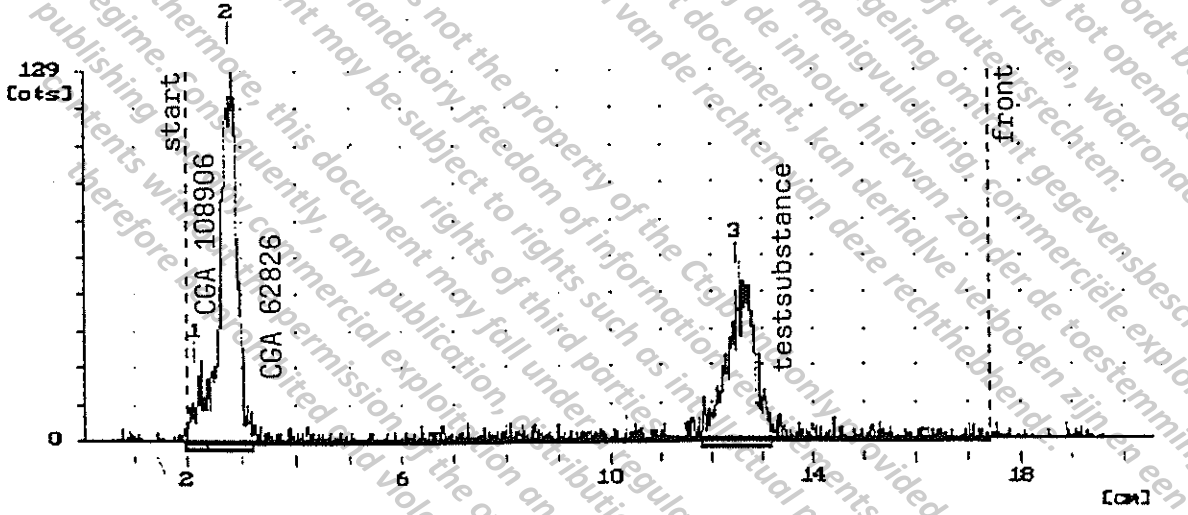
Soil investigation, 1st year

1st sampling date

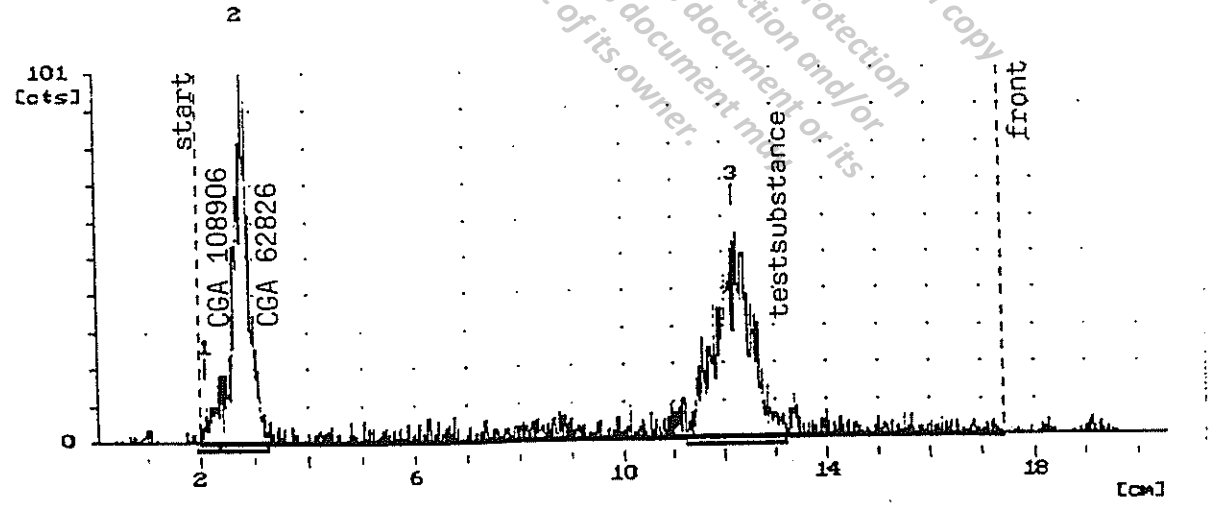
Examples of TLC analyses

Solvent : chloroform : carbon tetrachloride : Ethanol = 3:1:1
Plate : silicagel $60F_{254}$

a) Chromatogram of lysimeter 19, soil of September 28, 1992



b) Chromatogram of lysimeter 20, soil of September 28, 1992



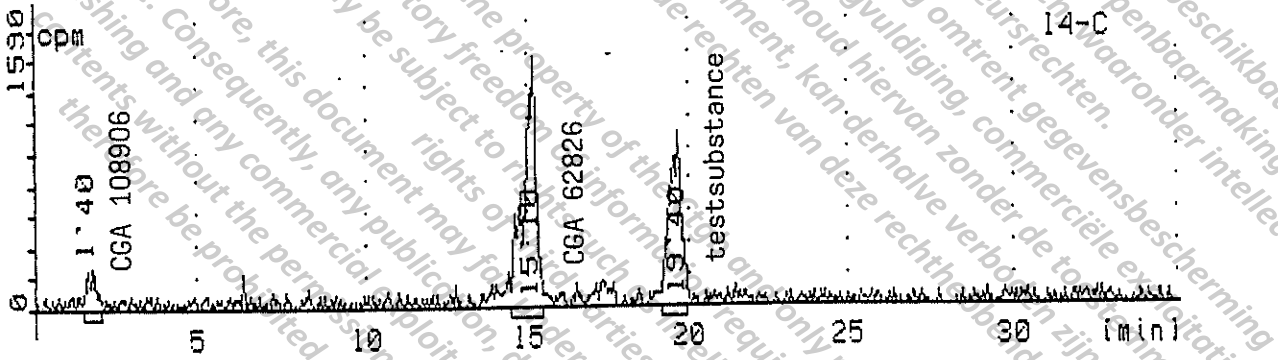
app. 16b

Soil investigation, 1st year

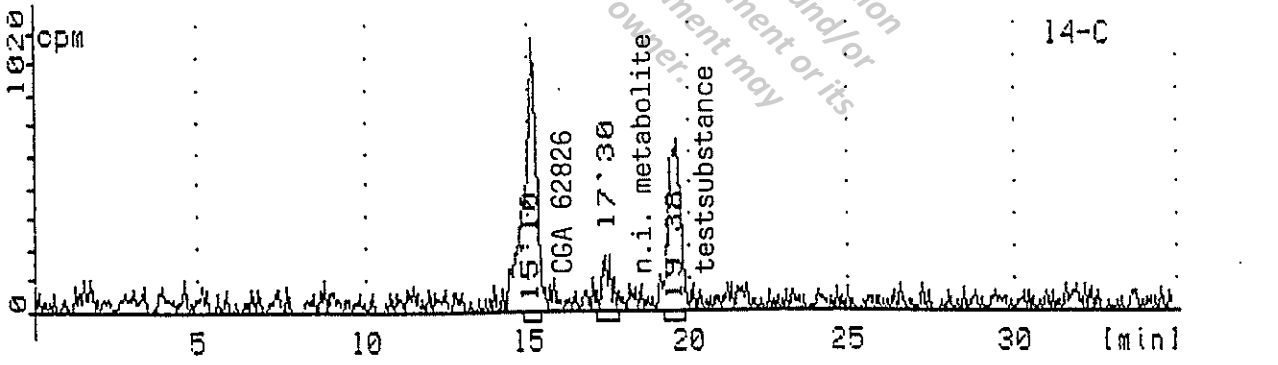
1st sampling date

Examples of HPLC analyses

a) Chromatogram of lysimeter 19, soil of September 28, 1992



b) Chromatogram of lysimeter 20, soil of September 28, 1992



app. 17

Plant investigation, 1st year

^{14}C-balance

(38 days after the 1st application)

lysimeter 19

plant fraction	dry weight g	kBq	%[1)]	mg/kg[3)]
potatoes	2808.61	259.68	0.49	0.05
leaves	216.10	10005.90	19.01	23.04
total			19.50	

lysimeter 20

plant fraction	dry weight g	kBq	%[2)]	mg/kg[3)]
potatoes	4554.02	314.86	0.55	0.03
leaves	252.00	11861.63	20.78	23.42
total			21.33	

1) radioactivity applied (52645.4 kBq) = 100 %
2) radioactivity applied (57087.8 kBq) = 100 %
3) calculated on the base of the spec. radioactivity of the A.I. (2.01 MBq/mg)

app. 18

Lysimeter investigation, 1st year

^{14}C-balance

lysimeter	19		20	
	kBq	%[1)]	kBq	%[2)]
percolate	3311.4	6.3	2419.8	4.2
soil	9908.0	18.8	11115.4	19.5
plants:				
potatoes	259.7	0.5	314.9	0.6
leaves	10005.9	19.0	11861.6	20.8
total	23485.0	44.6	25711.7	45.0

1) radioactivity applied (52645.4 kBq)= 100 %
2) radioactivity applied (57087.8 kBq)= 100 %

app. 19a

Cultivation of the lysimeter, 2nd year

1) Plants

plant		winter wheat		rape		winter barley	
lysimeter		19	20	19	20	19	20
seed time		Oct. 19, 1992		Aug. 12, 1993		Oct. 08, 1993	
harvest time		July 20, 1993		Oct. 08, 1993		June 29, 1994	
dry weight							
grain	g/lys	74.5	214.7	not investigated and left in the soil		no fractions	
	dt/ha	9.3	26.8				
chaff	g/lys	31.1	111.7				
	dt/ha	3.9	14.0				
straw	g/lys	80.0	240.0				
	dt/lys	10.0	30.0				
total	g/lys	185.6	566.4			175.0	160.0
	dt/ha	23.2	70.8			21.9	20.0

app. 19b

Cultivation of the lysimeter, 2nd year

2) Fertilization

date of fertilizing	fertilizer	kg nutrient/ ha	g nutrient/ lysimeter
Mai 16,94	ammoniumsulfate-saltpeter (25% N)	40.0 N	3.2 N

app. 20a

Experimental conditions, 2nd year

1) Temperatures

month	air [°C] 1)	soil 10 cm [°C] 1)	soil 30 cm [°C] 1)
July 93	18.9	16.6	16.4
August 93	19.2	16.4	16.5
September 93	13.7	11.9	12.1
October 93	9.0	7.7	8.0
November 93	1.7	1.5	2.2
December 93	5.3	1.5	1.5
January 94	4.4	1.3	1.6
February 94	2.3	-0.1	0.1
March 94	9.1	5.4	5.0
April 94	9.7	7.7	7.3
May 94	14.4	12.8	12.4
June 94	18.8	16.0	15.4

1) monthly mean value

app. 20b

Experimental conditions, 2nd year

2a) Precipitation and irrigation
July 1993 - November 1993

month	precipitation [mm]	irrigation		
		total [mm]	single [mm]	date
July 93	28.0	74.0	5.0	July 06, 93
			10.0	July 07, 93
			10.0	July 08, 93
			2.5	July 16, 93
			5.0	July 19, 93
			7.5	July 21, 93
			7.5	July 22, 93
			7.5	July 27, 93
			10.0	July 28, 93
			5.0	July 29, 93
			4.0	July 30, 93
August 93	9.0	48.0	3.0	Aug. 12, 93
			5.0	Aug. 16, 93
			5.0	Aug. 17, 93
			7.5	Aug. 19, 93
			5.0	Aug. 20, 93
			5.0	Aug. 23, 93
			7.5	Aug. 25, 93
			10.0	Aug. 26, 93
September 93	88.0	49.0	5.0	Sep. 02, 93
			10.0	Sep. 21, 93
			7.5	Sep. 22, 93
			5.0	Sep. 23, 93
			10.0	Sep. 27, 93
			5.0	Sep. 28, 93
			2.5	Sep. 29, 93
			4.0	Sep. 30, 93
October 93	59.0	4.0	4.0	Oct. 08, 93
November 93	14.0	-		
sum: July to November	198.0	175.0		total: 373.0 mm

app. 20c

Experimental conditions, 2nd year

2b) Precipitation and irrigation
December 1993 - June 1994

month	precipitation [mm]	irrigation		
		total [mm]	single [mm]	date
December 93	148.0	22.5	2.5	Dec. 10, 93
			10.0	Dec. 13, 93
			5.0	Dec. 14, 93
			5.0	Dec. 20, 93
January 94	69.0	-	-	-
February 94	41.0	-	-	-
March 94	32.0	-	-	-
April 94	53.0	-	-	-
May 94	34.0	13.0	3.7	May 13, 94
			3.8	May 16, 94
			2.5	May 25, 94
			3.0	May 26, 94
June 94	51.0	42.0	10.0	June 13, 94
			5.0	June 14, 94
			5.0	June 15, 94
			10.0	June 21, 94
			7.5	June 24, 94
			4.5	June 27, 94
sum July to November	198.0	175.0		**total:** 373.0 mm
sum December to June	428.0	77.5		505.5 mm
total 2nd year	626.0	252.5		878.5 mm
total 1st year	422.0	339.0		761.0 mm
sum 1st and 2nd year	1048.0	591.5		1639.5 mm

app. 21

Sampling of percolate, 2nd year

date of sampling	19 amount [l]	20 amount [l]
July 02, 1993	-[1)]	-[1)]
Aug. 02, 1993	-[1)]	-[1)]
Sep. 01, 1993	-[1)]	-[1)]
Oct. 01, 1993	27.7	8.4
Oct. 18, 1993	20.3	35.6
Nov. 02, 1993	20.4	18.0
Nov. 15, 1993	9.5	12.2
Dec. 01, 1993	5.9	8.7
Dec. 14, 1993	12.8	7.1
Jan. 05, 1994	90.7	92.7
Jan. 17, 1994	50.6	46.0
Feb. 01, 1994	7.3	15.4
Feb. 17, 1994	25.1	19.7
March 01, 1994	5.1	8.6
March 15, 1994	5.6	7.6
April 05, 1994	7.9	10.2
April 15, 1994	9.6	5.5
May 02, 1994	14.1	13.4
June 01, 1994	6.0	7.9
June 30, 1994	3.9	3.0
total percolate 2nd year	322.5	320.0
total percolate 1st year	310.3	284.5
total percolate 1st and 2nd year	632.8	604.5

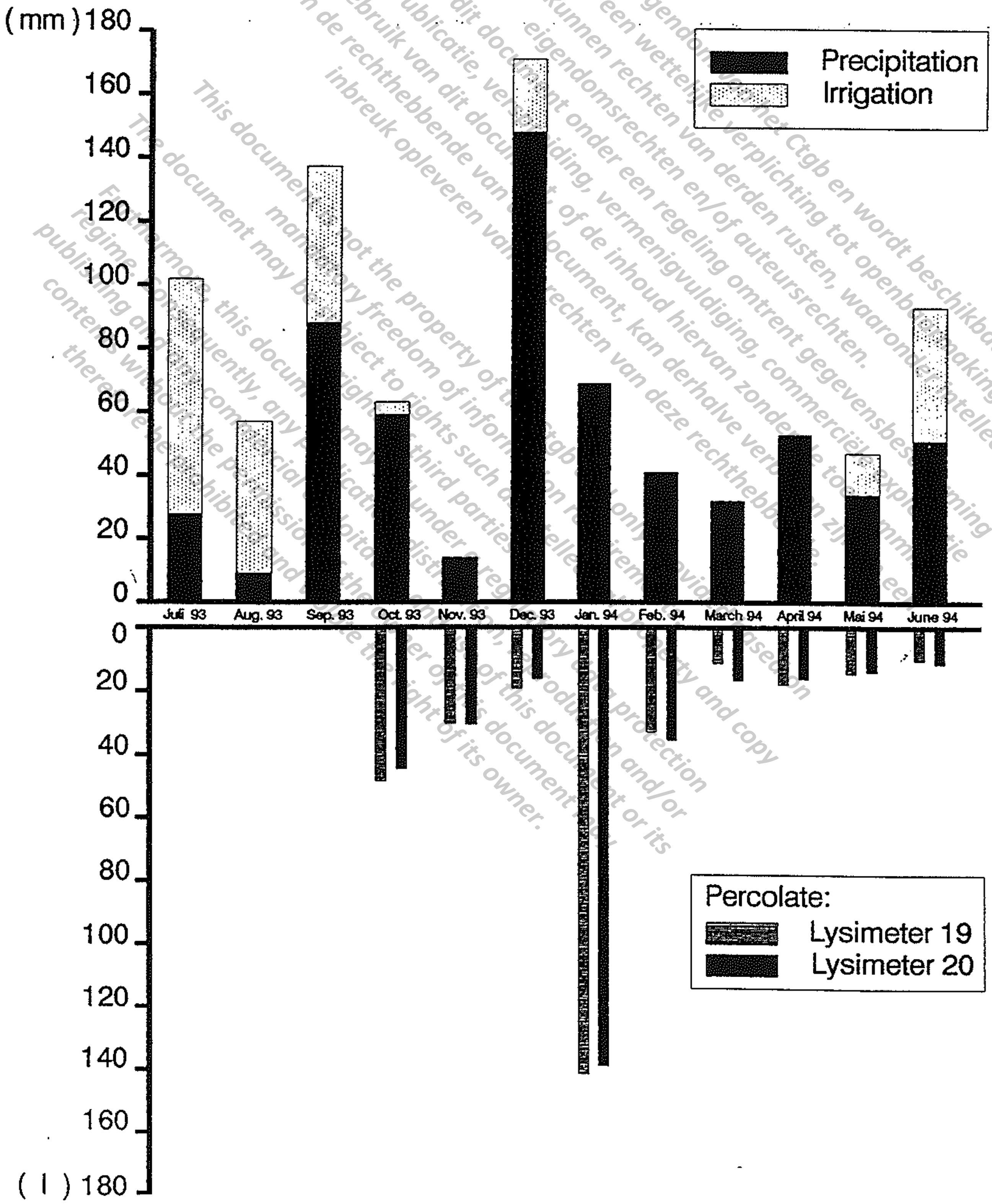
1) percolate volume < 3 l

app. 22

Precipitation, irrigation and percolate

Lysimeter 19 and 20

(second year)



app. 23a

Percolate investigation, 2nd year

Results of lysimeter 19

date of sampling	radioactivity in the percolate [l]	[kBq/l]	[µg/l][1)]	$^{14}CO_2$ [µg/l]	rep.	loss n.i.[2)]	water phase[3)] [µg/l]	extracted metabolites CGA 108906 [µg/l]	CGA 62826 [µg/l]	n.i.[2)]	active ingredient [µg/l]
Oct. 01,93	27.7	8.49	4.22	0.06	a	–	0.33	1.13	2.60	–	0.27
					b	–	0.29	1.22	2.77	–	0.22
Oct. 18,93	20.3	6.18	3.07	0.04	a	–	0.30	0.84	1.83	–	0.19
					b	–	0.31	0.82	1.86	0.07	0.22
Nov. 02,93	20.4	5.02	2.50	0.04	a	–	0.18	0.83	1.56	–	0.08
					b	–	0.22	0.78	1.60	–	0.07
Nov. 15,93	9.5	4.17	2.07	0.02	a	–	0.28	1.00	1.05	<0.05	0.10
					b	–	0.27	0.94	1.18	<0.05	0.10
Dec. 01,93	5.9	4.52	2.25	0.03	a	–	0.22	0.91	1.10	–	<0.05
					b	–	0.24	0.91	1.19	–	<0.05
Dec. 14,93	12.8	3.44	1.71	0.04	a	–	0.18	0.63	0.93	–	0.05
					b	–	0.20	0.58	0.91	<0.05	0.05
Jan. 05,94	90.7	2.32	1.15	0.05	a	–	0.17	0.41	0.58	–	–
					b	–	0.17	0.39	0.57	0.05	–
Jan. 17,94	50.6	1.60	0.80	0.03	a	–	0.11	0.35	0.37	–	–
					b	–	0.11	0.37	0.36	–	–
Feb. 01,94	7.3	1.61	0.80	0.03	a	–	0.12	0.30	0.38	–	–
					b	–	0.11	0.39	0.32	<0.05	–
Feb. 17,94	25.1	1.40	0.70	0.01	a	–	0.09	0.20	0.34	0.07	–
					b	–	0.09	0.34	0.30	–	–
Mar. 01,94	5.1	1.43	0.71	0.03	a	–	0.10	0.31	0.29	<0.05	–
					b	–	0.11	0.34	0.28	<0.05	–
Mar. 15,94	5.6	1.35	0.67	0.01	a	–	0.10	0.27	0.33	–	–
					b	–	0.09	0.31	0.27	<0.05	–
Apr. 05,94	7.9	1.37	0.68	0.03	a	–	0.10	0.28	0.29	<0.05	–
					b	–	0.11	0.35	0.22	<0.05	–
Apr. 15,94	9.6	1.35	0.67	0.06	a	–	0.10	0.36	0.08	0.13	<0.05
					b	–	0.10	0.34	0.08	0.15	<0.05
May 02,94	14.1	1.25	0.62	0.05	a	–	0.08	0.33	0.21	–	–
					b	–	0.09	0.33	0.20	–	–
June 01,94	6.0	1.21	0.60	0.05	a	–	0.10	0.32	0.17	–	–
					b	–	0.10	0.27	0.19	<0.05	–
June 30,94	3.9	1.14	0.57	0.07	a	–	0.11	0.25	0.13	0.07	–
					b	–	0.10	0.25	0.13	0.07	–

1) calculated as A.I. equivalents on the base of the spec. radioactivity (2.01 kBq/µg)
2) n.i. = not identified
3) not extracted and not identified
"–" below detection limit of 0.01 µg/l

app. 23b

Percolate investigation, 2nd year

Results of lysimeter 20

date of sampling	radioactivity in the percolate [l]	[kBq/l]	[µg/l] [1]	$^{14}CO_2$ [µg/l]	rep.	loss n.i. [2]	water phase [3] [µg/l]	extracted metabolites CGA 108906 [µg/l]	CGA 62826 [µg/l]	n.i. [2]	active ingredient [µg/l]
Oct. 01,93	8.4	3.65	1.82	0.23	a	–	0.30	0.58	0.73	<0.05	0.06
					b	–	0.30	0.51	0.71	<0.05	0.06
Oct. 18,93	35.6	2.91	1.45	0.14	a	–	0.23	0.64	0.56	–	–
					b	–	0.28	0.61	0.55	–	–
Nov. 02,93	18.0	2.63	1.31	0.12	a	–	0.21	0.73	0.32	<0.05	–
					b	–	0.21	0.77	0.33	<0.05	–
Nov. 15,93	12.2	2.39	1.19	0.12	a	–	0.22	0.73	0.30	<0.05	–
					b	–	0.22	0.80	0.21	<0.05	–
Dec. 01,93	8.7	2.66	1.32	0.10	a	–	0.23	0.74	0.34	–	–
					b	–	0.20	0.81	0.28	–	–
Dec. 14,93	7.1	2.19	1.09	0.09	a	–	0.17	0.63	0.22	<0.05	–
					b	–	0.17	0.60	0.26	<0.05	–
Jan. 05,94	92.7	1.71	0.85	0.11	a	–	0.18	0.37	0.20	<0.05	–
					b	–	0.18	0.40	0.22	–	–
Jan. 17,94	46.0	1.64	0.82	0.12	a	–	0.18	0.36	0.20	<0.05	–
					b	–	0.17	0.40	0.20	<0.05	–
Feb. 01,94	15.4	1.85	0.92	0.12	a	–	0.20	0.54	0.15	<0.05	–
					b	–	0.20	0.57	0.16	–	–
Feb. 17,94	19.7	1.89	0.94	0.14	a	–	0.14	0.54	0.16	<0.05	–
					b	–	0.11	0.60	0.15	<0.05	–
Mar. 01,94	8.6	1.88	0.94	0.12	a	–	0.17	0.60	0.13	<0.05	–
					b	–	0.16	0.57	0.15	<0.05	–
Mar. 15,94	7.6	1.95	0.97	0.10	a	–	0.20	0.54	0.17	<0.05	–
					b	–	0.18	0.54	0.14	0.06	–
Apr. 05,94	10.2	1.87	0.93	0.08	a	–	0.14	0.62	0.10	0.05	–
					b	–	0.17	0.64	0.07	0.05	–
Apr. 15,94	5.5	1.76	0.88	0.13	a	–	0.16	0.61	0.07	<0.05	–
					b	–	0.16	0.64	0.06	<0.05	–
May 02,94	13.4	1.89	0.94	0.14	a	–	0.18	0.60	0.10	<0.05	–
					b	–	0.19	0.65	0.06	<0.05	–
June 01,94	7.9	1.66	0.83	0.12	a	–	0.18	0.54	0.07	<0.05	–
					b	–	0.17	0.53	0.07	<0.05	–
June 30,94	3.0	1.20	0.60	0.10	a	–	0.14	0.44	–	–	–
					b	–	0.15	0.44	–	–	–

1) calculated as A.I. equivalents on the base of the spec. radioactivity (2.01 kBq/µg)
2) n.i. = not identified
3) not extracted and not identified
"–" below detection limit of 0.01 µg/l

app. 23c

Percolate analysis 2nd year/total

	lysimeter 19			lysimeter 20		
radioactivity applied	52645.4 kBq			57087.8 kBq		
total amount of percolate	322.5 l			320.0 l		
	kBq	%[2)]	µg/l[3)]	kBq	%[2)]	µg/l[3)]
^{14}C-carbonate	26.9	0.05	0.04	79.6	0.14	0.12
loss: not extracted not identified	-	-	-	-	-	-
water phase: not extracted not identified	110.6	0.21	0.17	124.7	0.22	0.19
CGA 108906	339.9	0.65	0.52	336.3	0.59	0.52
CGA 62826	532.1	1.01	0.82	159.6	0.28	0.25
not identified metabolite	13.6	0.03	0.02	15.0	0.03	0.02
active ingredient	29.7	0.06	0.05	1.1	<0.01	<0.01
total activity[1)]	1052.8	2.00	1.62	716.3	1.25	1.11

1) ^{14}C-balance
2) radioactivity applied in the 1st test year = 100 %
3) calculated as A.I. equivalents on the basis of the spec. radioactivity (2.01 kBq/µg) and the total amount of the percolate

app. 23d

Percolate analysis 1st and 2nd year/total

	lysimeter 19			lysimeter 20		
radioaktivity applied	52645.4 kBq			57087.8 kBq		
total amount of percolate	632.8 l			604.5 l		
	kBq	%2)	µg/l3)	kBq	%2)	µg/l3)
^{14}C-carbonate	41.7	0.08	0.03	178.2	0.31	0.15
loss: not extracted not identified	13.3	0.03	0.01	11.5	0.02	0.01
water phase: not extracted not identified	352.5	0.67	0.28	379.7	0.67	0.31
CGA 108906	781.8	1.49	0.61	971.8	1.70	0.80
CGA 62826	3100.0	5.89	2.44	1575.8	2.76	1.30
not identified metabolite	13.6	0.03	0.01	15.0	0.03	0.01
active ingredient	61.3	0.12	0.05	4.1	0.01	<0.01
total activity1)	4364.2	8.29	3.43	3136.1	5.49	2.58

1) ^{14}C-balance
2) radioactivity applied in the 1st test year = 100 %
3) calculated as A.I. equivalents on the basis of the spec. radioactivity (2.01 kBq/µg) and the total amount of the percolate

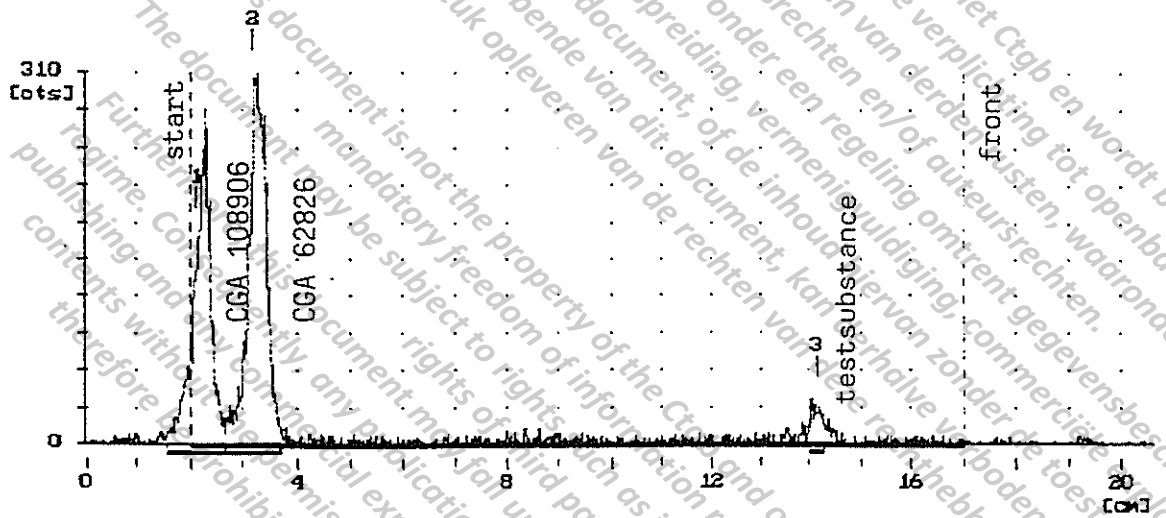
app. 24a

Percolate investigation, 2nd year

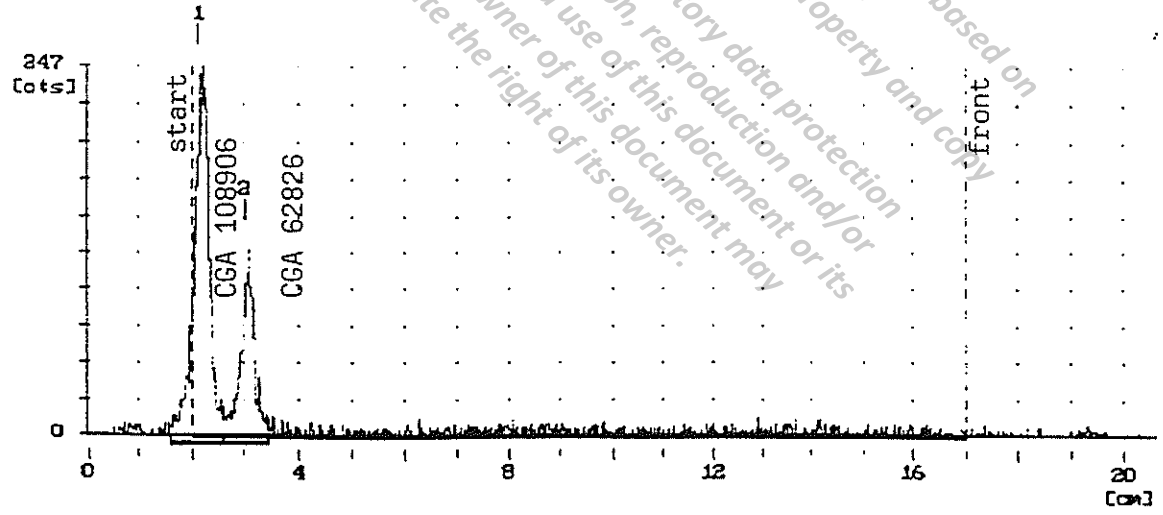
Examples of TLC analyses

Solvent : chloroform : carbon tetrachloride : ethanol = 3:1:1
Plate : silicagel $60F_{254}$

a) Chromatogram of lysimeter 19, percolate of December 14, 1993



b) Chromatogram of lysimeter 20, percolate of December 14, 1993

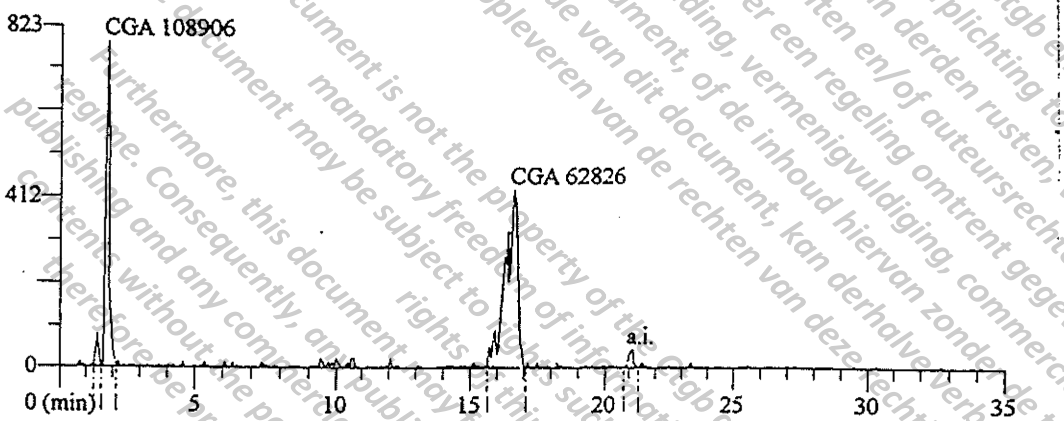


app. 24b

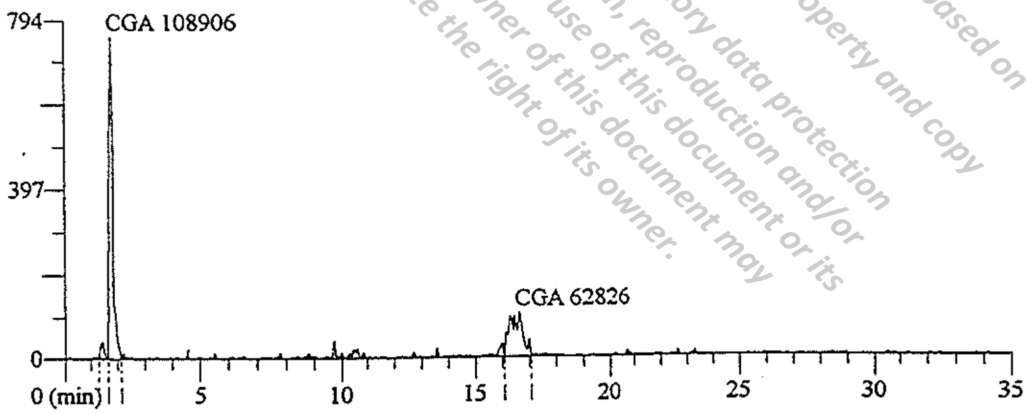
Percolate investigation, 2nd year

Examples of HPLC analyses

a) Chromatogram of lysimeter 19, percolate of December 14, 1993



b) Chromatogram of lysimeter 20, percolate of December 14, 1993



app. 25a

Soil investigation, 2nd year

^{14}C-balance

1) 2nd sampling date
(Oct. 08, 1993; 373 days after the first application)

lysimeter 19

soil layer	weight	radioactivity		
cm	kg	kBq	mg/kg[1] A.I.equivalents	%[2]
0-10	110.43	1800.01	0.01	3.42

lysimeter 20

soil layer	weight	radioactivity		
cm	kg	kBq	mg/kg[1] A.I.equivalents	%[3]
0-10	117.37	3748.80	0.02	6.57

1) calculated on the base of the spec. radioactivity of the A.I. (2.01 MBq/mg)
2) amount applied (52645.4 kBq) = 100%
3) amount applied (57087.8 kBq) = 100%

app. 25b

Soil investigation, 2nd year

^{14}C-balance

2) 3rd sampling date
(June 30, 1994; 731 days after the first application)

lysimeter 19

soil layer cm	weight kg	radioactivity kBq	mg/kg[1] A.I.equivalents	%[2]
0- 10	107.4	1435.94	0.01	2.73
10- 20	101.1	1424.50	0.01	2.71
20- 30	104.8	981.98	<0.01	1.87
30- 40	112.0	682.08	<0.01	1.30
40- 50	112.0	329.28	<0.01	0.63
50- 60	112.0	367.36	<0.01	0.70
60- 70	112.0	285.60	<0.01	0.54
70- 80	112.0	284.48	<0.01	0.54
80- 90	112.0	273.28	<0.01	0.52
90-100	112.0	201.60	<0.01	0.38
100-110	112.0	166.88	<0.01	0.32
110-120	112.0	192.64	<0.01	0.37
120-130	112.0	483.84	<0.01	0.92
0-130		7109.46		13.50

lysimeter 20

soil layer cm	weight kg	radioactivity kBq	mg/kg[1] A.I.equivalents	%[3]
0- 10	105.2	2905.62	0.01	5.09
10- 20	114.9	3006.93	0.01	5.27
20- 30	108.0	1137.24	0.01	1.99
30- 40	112.0	481.60	<0.01	0.84
40- 50	112.0	154.56	<0.01	0.27
50- 60	112.0	124.32	<0.01	0.22
60- 70	112.0	105.28	<0.01	0.18
70- 80	112.0	156.80	<0.01	0.27
80- 90	112.0	96.32	<0.01	0.17
90-100	112.0	105.28	<0.01	0.18
100-110	112.0	133.28	<0.01	0.23
110-120	112.0	147.84	<0.01	0.26
120-130	112.0	285.60	<0.01	0.50
0-130		8840.67		15.49

1) calculated on the base of the spec. radioactivity of the A.I. (2.01 MBq/mg)
2) amount applied (52645.4 kBq) = 100%
3) amount applied (57087.8 kBq) = 100%

app. 26a

Soil investigation, 2nd year

extraction balance of the 0-10 cm soil layer

2nd sampling date (Oct. 09, 1993; 373 days after the 1st treatment)

lysimeter 19

	a				b			
^{14}C applied	52645.4 kBq				52645.4 kBq			
	kBq	µg/kg[1]	%[2]	%[3]	kBq	µg/kg[1]	%[2]	%[3]
methanol extract	not detected				not detected			
A.I.	not detected				not detected			
CGA 108906	not detected				not detected			
CGA 62826								
total ^{14}C extracted	1.80	0.90	10.71	0.38	1.60	0.80	9.64	0.34
not extracted solid phase	15.00	7.46	89.29	3.15	15.00	7.46	90.36	3.15
total ^{14}C in a 1 kg soil sample	16.80	8.36	100.00	3.52	16.60	8.26	100.00	3.48

1) calculated on the base of the spec. radioactivity of the A.I. (2.01 kBq/µg)
2) radioactivity in the soil sample = 100 %
3) extraction results extrapolated to the total soil sample weight (110.43 kg) and related to the radioactivity applied (100%)

app. 26b

Soil investigation, 2nd year

extraction balance of the 0-10 cm soil layer

2nd sampling date (Oct. 08, 1993; 373 days after the 1st application)

lysimeter 20

	a				b			
^{14}C applied	57087.8 kBq				57087.8 kBq			
methanol extract	kBq	µg/kg[1)]	%[2)]	%[3)]	kBq	µg/kg[1)]	%[2)]	%[3)]
A.I.	below detection limit				below detection limit			
CGA 108906	below detection limit				below detection limit			
CGA 62826	below detection limit				below detection limit			
total ^{14}C extracted	1.60	0.80	5.16	0.33	1.40	0.70	4.64	0.29
not extracted solid phase	29.40	14.63	94.84	6.04	28.80	14.33	95.36	5.92
total ^{14}C in a 1 kg soil sample	31.00	15.42	100.00	6.37	30.20	15.02	100.00	6.21

1) calculated on the base of the spec. radioactivity of the A.I. (2.01 kBq/µg)
2) radioactivity in the soil sample = 100%
3) extraction results extrapolated to the total soil sample weight (117.37 kg) and related to the radioactivity applied (100%)

app. 27a

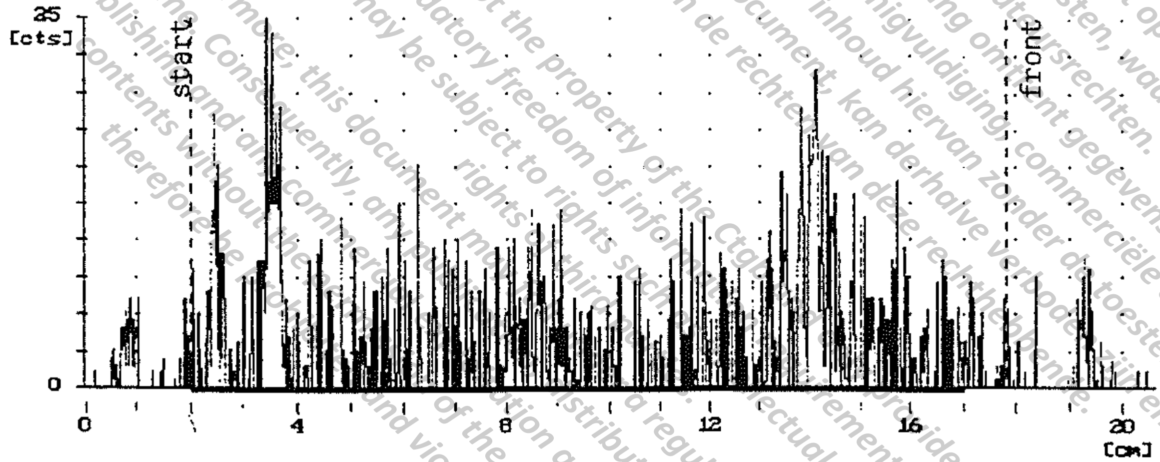
Soil investigation, 2nd year

2nd sampling date (October 08,1993)

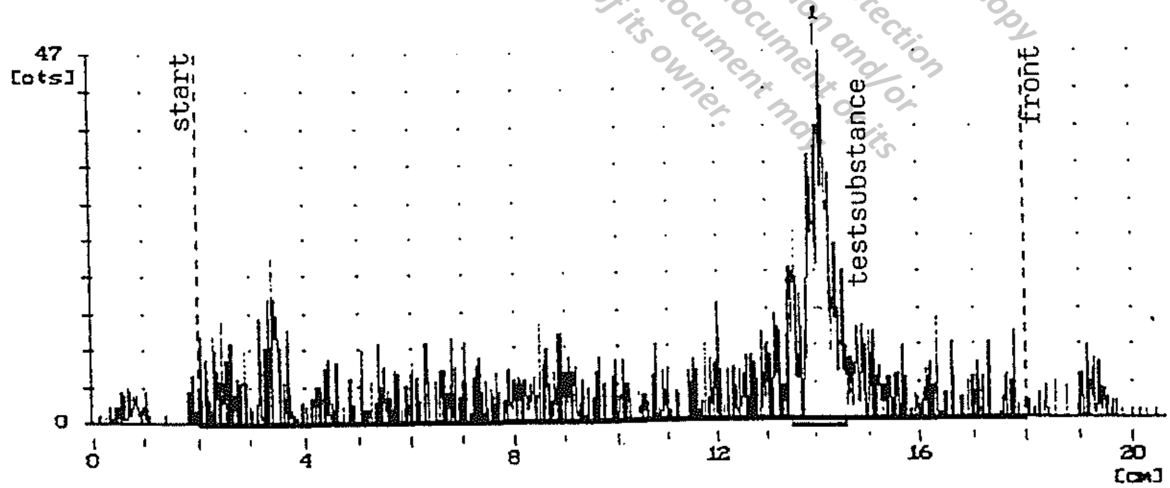
Examples of TLC analyses

Solvent : chloroform : carbon tetrachloride : Ethanol = 3:1:1
Plate : silicagel $60F_{254}$

a) Chromatogram of lysimeter 19, soil of October 08, 1993



b) Chromatogram of lysimeter 20, soil of October 08, 1993



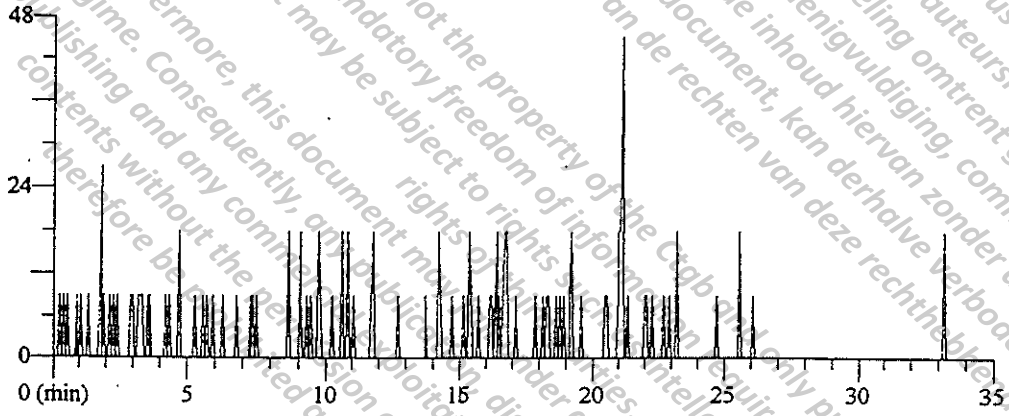
app. 27b

Soil investigation, 2nd year

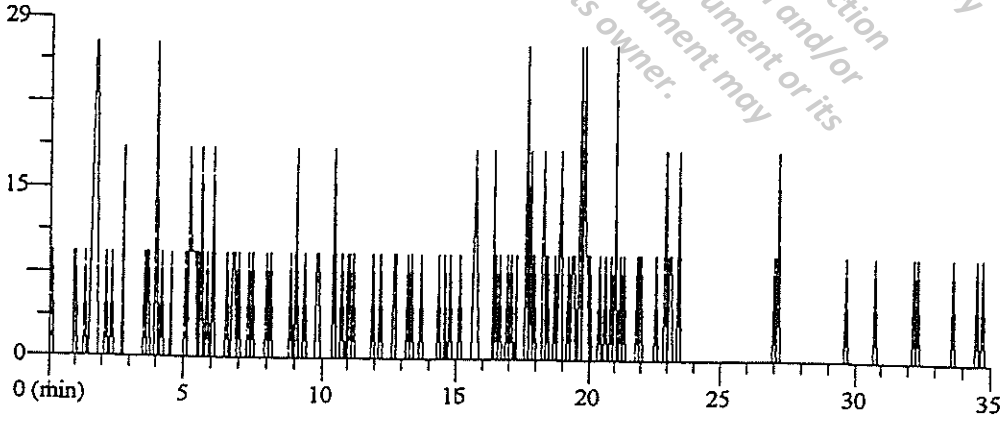
2nd sampling date (October 08, 1993)

Examples of HPLC analyses

a) Chromatogram of lysimeter 19, soil of October 08, 1993



b) Chromatogram of lysimeter 20, soil of October 08, 1993



app. 28

Soil extraction method

2nd year, 3rd sampling date

Add 100 ml acetonitril/water (80/20; v/v) to a 50 g portion of soil

*

Shake for 90 min

*

Centrifugate with 5000 rpm

*

Repeat the soil extraction twice with 50 ml acetonitril/water for 45 min and combine all solutions

*

Add 100ml 0.01 M $CaCl_2$ to the soil and shake it (horizontal shaker) for ≥ 15 hours

*

Centrifugate with 5000 rpm

*

Determinate the radioactivity of the $CaCl_2$-phase

*

Dry the extracted soil at 30 °C, and homogenize it for determination of the radioactivity not extracted

*

Evaporate the extraction solution to 10 - 20 ml

*

Eluate the concentrated extraction solution using a prepared C-18 column (Bakerbond C-18), determinate the radioactivity of the water phase

*

Dry for 30 min using nitrogen

*

Extract the column using 4 x 500 μl of methanol

*

Investigate using TLC and HPLC

app. 29a

Soil investigation, 2nd year

extraction balance

3rd sampling date (June 30, 1994; 731 days after the 1st application)

lysimeter 19

soil layer [cm]	rep.	extracted: organic phase[3] µg/kg[1]	%[2]	extracted: water phase µg/kg[1]	%[2]	extracted: $CaCl_2$ phase µg/kg[1]	%[2]	not extractable soil-bound µg/kg[1]	%[2]	total µg/kg[1]	%[2]
0- 10	a	0.40	6.81	0.10	1.70	0.20	3.41	5.17	88.07	5.87	100.00
	b	0.40	7.58	0.10	1.89	0.20	3.79	4.58	86.74	5.28	100.00
10- 20	a	0.50	8.80	0.10	1.76	0.20	3.52	4.88	85.92	5.68	100.00
	b	0.50	8.80	0.10	1.76	0.20	3.52	4.88	85.92	5.68	100.00
20- 30	a	0.30	6.02	0.10	2.01	0.20	4.02	4.38	87.95	4.98	100.00
	b	0.30	6.41	0.10	2.14	0.20	4.27	4.08	87.18	4.68	100.00
30- 40	a	0.10	4.02	0.10	4.02	0.10	4.02	2.19	87.95	3.19	100.00
	b	0.10	3.14	0.10	3.14	0.10	3.14	2.89	90.88	3.18	100.00
40- 50	a	0.10	6.71	0.10	6.71	0.10	6.71	1.19	79.87	1.49	100.00
	b	0.10	7.19	0.10	7.19	0.10	7.19	1.09	78.42	1.39	100.00
50- 60	a	0.10	6.29	0.10	6.29	0.10	6.29	1.29	81.13	1.59	100.00
	b	0.10	6.71	0.10	6.71	0.10	6.71	1.19	79.87	1.49	100.00
60- 70	a	0.10	7.69	0.10	7.69	0.10	7.69	1.00	76.92	1.30	100.00
	b	0.10	7.69	0.10	7.69	0.10	7.69	1.90	76.92	1.30	100.00
70- 80	a	0.30	20.00	0.10	6.67	0.10	6.67	1.00	66.67	1.50	100.00
	b	0.40	25.00	0.10	6.25	0.10	6.25	1.00	62.50	1.60	100.00
80- 90	a	0.10	9.09	0.10	9.09	0.10	9.09	0.80	72.73	1.10	100.00
	b	0.10	9.09	0.10	9.09	0.10	9.09	0.80	72.73	1.10	100.00
90-100	a	0.10	12.50	-	-	0.10	12.50	0.60	75.00	0.80	100.00
	b	0.10	12.50	-	-	0.10	12.50	0.60	75.00	0.80	100.00
100-110	a	0.10	14.29	-	-	0.10	14.29	0.50	71.43	0.70	100.00
	b	0.10	16.67	-	-	0.10	16.67	0.40	66.67	0.60	100.00
110-120	a	0.10	14.29	-	-	0.10	14.29	0.50	71.43	0.70	100.00
	b	0.10	12.50	-	-	0.10	12.50	0.60	75.00	0.80	100.00
120-130	a	0.20	11.17	-	-	0.10	5.59	1.49	83.24	1.79	100.00
	b	0.20	10.58	0.10	5.29	0.10	5.29	1.49	78.84	1.89	100.00

1) calculated on the base of the spec. radioactivity of the A.I. (2.01 MBq/mg)
2) radioactivity in the soil sample = 100 %
3) radioactivity in the organic phase (A.I. + metabolites) was too low for chromatographic indification
"-" = below 0.01 kBq in the extracted soil sample (50g)

app. 29b

Soil investigation, 2nd year

extraction balance

3rd sampling date (June 30, 1994; 731 days after the 1st application)

lysimeter 20

soil layer [cm]	rep.	extracted: organic phase[3] µg/kg[1]	%[2]	water phase µg/kg[1]	%[2]	$CaCl_2$ phase µg/kg[1]	%[2]	not extractable soil-bound µg/kg[1]	%[2]	total µg/kg[1]	%[2]
0- 10	a	0.50	3.64	0.10	0.73	0.40	2.91	12.74	92.79	13.74	100.00
	b	0.50	4.02	0.10	0.80	0.30	2.41	11.54	92.77	12.44	100.00
10- 20	a	0.40	4.06	0.10	1.01	0.40	4.06	8.96	90.87	9.86	100.00
	b	0.40	3.94	0.10	0.99	0.40	3.94	9.25	91.13	10.15	100.00
20- 30	a	0.20	4.46	0.10	2.23	0.20	4.46	3.98	88.84	4.48	100.00
	b	0.20	4.37	0.10	2.18	0.20	4.37	4.08	89.08	4.58	100.00
30- 40	a	0.10	5.59	0.10	5.59	0.10	5.59	1.49	83.24	1.79	100.00
	b	0.10	5.59	0.10	5.92	0.10	5.92	1.49	82.25	1.79	100.00
40- 50	a	0.10	14.29	–	–	–	–	0.60	85.71	0.70	100.00
	b	0.10	14.29	–	–	–	–	0.60	85.71	0.70	100.00
50- 60	a	0.10	16.67	0.10	16.67	–	–	0.40	66.67	0.60	100.00
	b	–	–	–	–	–	–	0.40	100.00	0.40	100.00
60- 70	a	0.10	16.67	–	–	–	–	0.50	83.33	0.60	100.00
	b	–*	–	–	–	–	–	0.40	100.00	0.40	100.00
70- 80	a	0.10	20.00	–	–	–	–	0.40	80.00	0.50	100.00
	b	0.10	16.67	–	–	–	–	0.50	83.33	0.60	100.00
80- 90	a	0.10	20.00	–	–	–	–	0.40	80.00	0.50	100.00
	b	–	–	–	–	–	–	0.30	100.00	0.30	100.00
90-100	a	0.10	12.50	0.10	12.50	0.10	12.50	0.50	62.50	0.80	100.00
	b	0.10	12.50	0.10	12.50	0.10	12.50	0.50	62.50	0.80	100.00
00-110	a	0.10	12.50	0.10	12.50	0.10	12.50	0.50	62.50	0.80	100.00
	b	0.20	22.22	0.10	11.11	0.10	11.11	0.50	55.56	0.90	100.00
10-120	a	0.10	11.11	0.10	11.11	0.10	11.11	0.60	66.67	0.90	100.00
	b	–	–	0.10	11.11	0.10	11.11	0.70	77.78	0.90	100.00
20-130	a	0.10	6.71	0.10	6.71	0.20	13.42	1.09	73.15	1.49	100.00
	b	0.20	13.33	0.10	6.67	0.20	13.33	1.00	66.67	1.50	100.00

1) calculated on the base of the spec. radioactivity of the A.I. (2.01 MBq/mg)
2) radioactivity in the soil sample = 100 %
3) radioactivity in the organic phase (A.I. + metabolites) was to low for chromatographic indification

"–" = below 0.01 kBq in the extracted soil sample (50g)
"–*" = below detection limit of ^{14}C

app. 30a

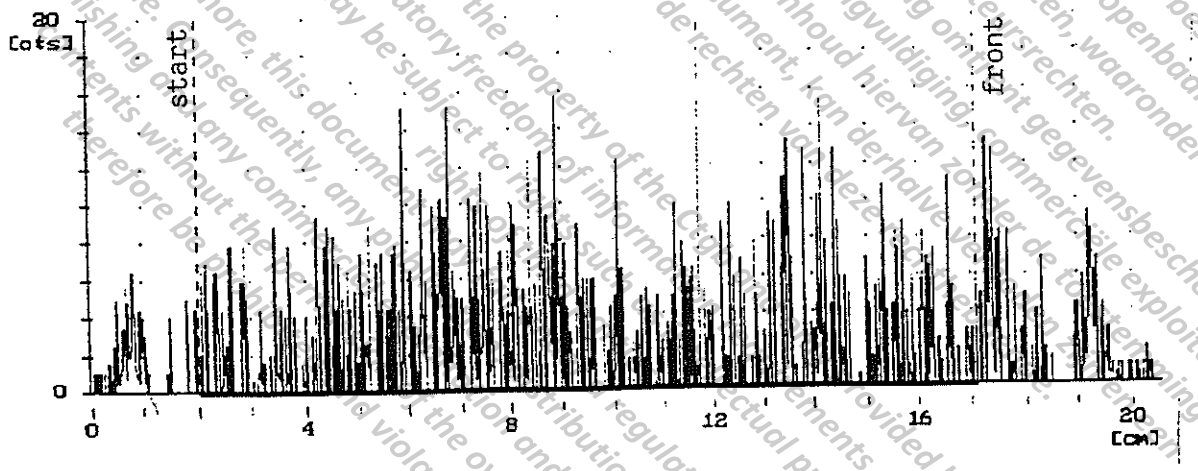
Soil investigation, 2nd year

3rd sampling date (June 30, 1994)

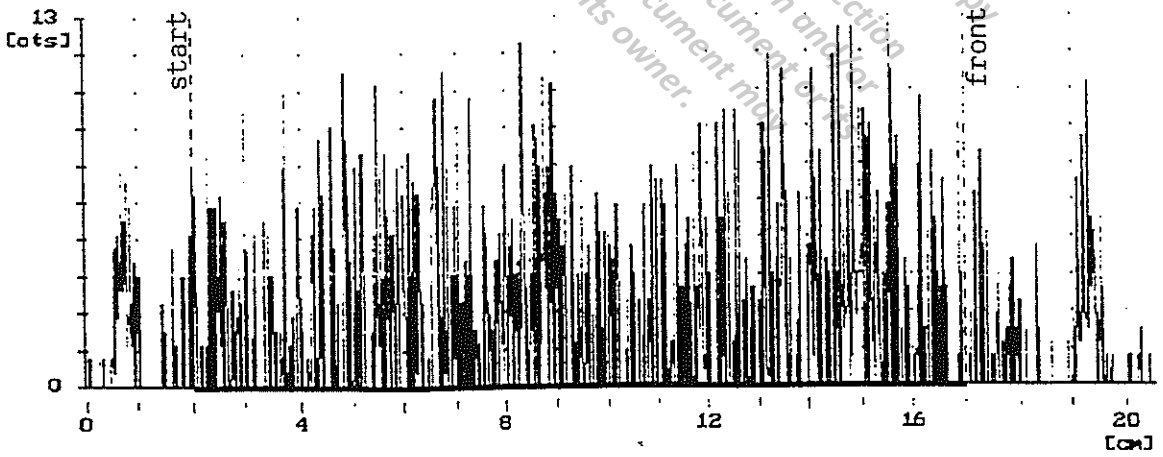
Examples of TLC analyses

Solvent : chloroform : carbon tetrachloride : Ethanol = 3:1:1
Plate : silicagel $60F_{254}$

a) Chromatogram of lysimeter 19, layer 10-20 cm, soil of June 30, 1994



b) Chromatogram of lysimeter 20, layer 0-10 cm, soil of June 30, 1994



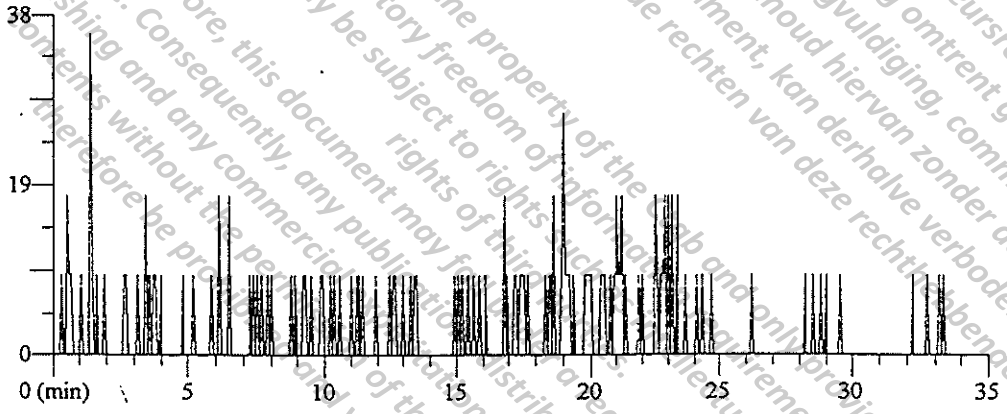
app. 30b

Soil investigation, 2nd year

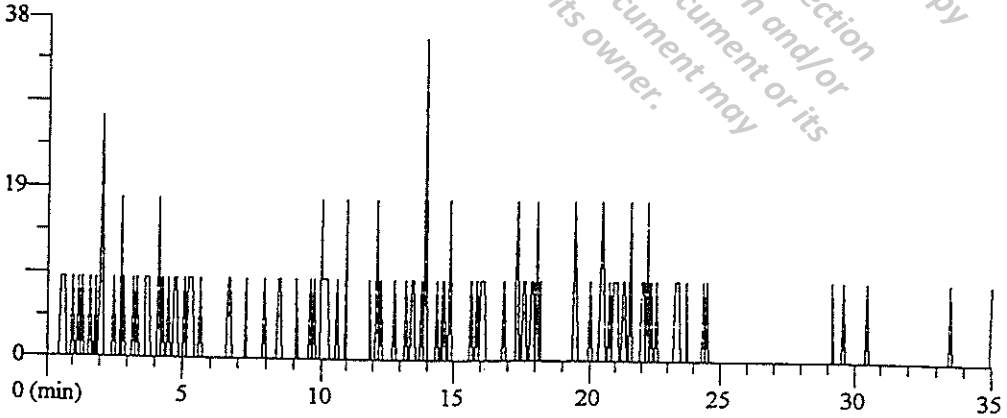
3rd sampling date (June 30, 1994)

Examples of HPLC analyses

a) Chromatogram of lysimeter 19,layer 10-20 cm, soil of June 30, 1994



b) Chromatogram of lysimeter 20, layer 0-10 cm, soil of June 30, 1994



app. 31a

Plant investigation, 2nd year

^{14}C-balance

1) Winter wheat (harvest time: July 20, 1993)

lysimeter 19

plant fraction	dry weight g	kBq	%[1]	mg/kg[3]
grain	74.5	0.67	<0.01	<0.01
chaff	31.1	0.89	<0.01	0.01
straw	80.0	7.18	0.01	0.04
total	185.6	8.74	0.02	

lysimeter 20

plant fraction	dry weight g	kBq	%[1]	mg/kg[3]
grain	214.7	1.12	<0.01	<0.01
chaff	111.7	1.15	<0.01	0.01
straw	240.0	12.09	0.02	0.03
total	566.4	14.36	0.03	

1) radioactivity applied (52645.4 kBq) = 100%
2) radioactivity applied (57087.8 kBq) = 100%
3) calculated on the base of the spec. radioactivity of the A.I. (2.01 MBq/mg)

app. 31b

Plant investigation, 2nd year

^{14}C-balance

2) Winter barley (harvest time: June 29, 1994)

lysimeter 19

plant fraction	dry weight g	kBq	%[1)]	mg/kg[3)]
no fractions	175.0	1.44	<0.01	<0.01

lysimeter 20

plant fraction	dry weight g	kBq	%[1)]	mg/kg[3)]
no fractions	160.0	1.15	<0.01	<0.01

1) radioactivity applied (52645.4 kBq) = 100 %
2) radioactivity applied (57087.8 kBq) = 100 %
3) calculated on the base of the spec. radioactivity of the A.I. (2.01 MBq/mg)

app. 32

Lysimeter investigation, 1st and 2nd year

total ^{14}C-balance

lysimeter		19		20	
		kBq	%[1]	kBq	%[2]
percolate	1st year	3311.4	6.3	2419.8	4.2
	2nd year	1052.8	2.0	716.3	1.3
soil [3]	2nd year	7109.5	13.5	8840.7	15.5
plants:					
potatoes	1st year	259.7	0.5	314.9	0.6
leaves		10005.9	19.0	11861.6	20.8
winter wheat	2nd year	8.7	<0.1	14.4	<0.1
winter barley	2nd year	1.4	<0.1	1.2	<0.1
total		21749.4	43.5	24168.9	42.3

1) radioactivity applied (52645.4 kBq)= 100 %
2) radioactivity applied (57087.8 kBq)= 100 %
3) ^{14}C-balance of the last sampling date (June 30, 1994)
soil layer 0-130 cm

Copy of GLP certificate

RheinlandPfalz

Ministerium für Arbeit, Soziales,
Familie und Gesundheit

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Telefax: 06131/16-2452
Bearbeiterin/Bearbeiter: Herr

12.06.1992

G L P - B e s c h e i n i g u n g

Bescheinigung	Certificate
Hiermit wird bestätigt, daß die Prüfungseinrichtung	It is hereby certified that the test facility
Abteilung Phytomedizin der Landes-Lehr- und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau Breitenweg 71 6730 Neustadt-Mußbach	department Phytomedizin of the Landes-Lehr- und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau Breitenweg 71 6730 Neustadt-Mußbach
Bundesrepublik Deutschland	Federal Republic of Germany
vom 24. - 26. Februar 1992 von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist	from 24. - 26. february 1992 was inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

Copy of GLP certificate

-2-

Es wird hiermit bestätigt, daß die Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Im Auftrag

Dr.

Die Ablichtung stimmt mit dem Original überein.
Neustadt, den 23.6.92

Copy of GLP certificate

-3-

Anlage zur GLP -Bescheinigung der Abteilung Phytomedizin in der Landes-Lehr- und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau Breitenweg 71, 6730 Neustadt-Mußbach
vom 12.06.1992

Von der Prüfeinrichtung können folgende Studientypen unter Einhaltung der Grundsätze der Guten Laborpraxis durchgeführt werden:

1. Verhalten von Pflanzenschutzmitteln (PSM) in Boden, Wasser und Luft

2. Einfluß von Pflanzenschutzmitteln auf die Bodenmikroflora

3. Nützlingsprüfungen im Labor (Raubmilben)

4. Verarbeitung von Erntegut zu Rückstandsproben (Keltertrauben)

Die Ablichtung stimmt mit dem Original überein.
Neustadt, den 23.6.92

Spectroscopy Report
Identification of Metabolites of Metalaxyl in percolated water of lysimeter.

METALAXYL, STRUCTURE ELUCIDATION OF METABOLITES

Prepared by:
MS Spectroscopy: Dr. 5.1.2.e Woo
CIBA-GEIGY Ltd.
Division Plant Protection
Divisional Unit R&D
Product Safety
Metabolism (PP 2.52)
CH 4002 Basel

Basel, Switzerland, June 11, 1993

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INTRODUCTION

This appendix describes the structure elucitation of metalaxyl metabolites isolated from water of lysimeter by mass spectrometry.

SPECTROSCOPIC METHODS

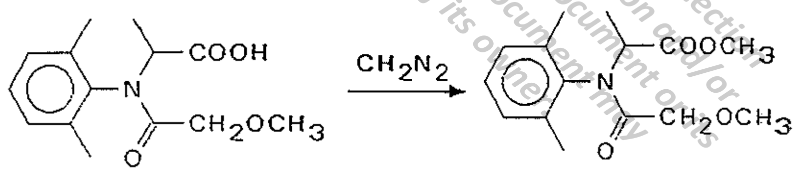
The instruments used for the spectroscopic investigations, the instrument settings and the chromatographic conditions are tabulated together with the appropriate metabolite structures.
The original spectra are filed at CIBA-GEIGY, PP 2.52.

INTERPRETATION

Direct GC injection of the samples showed now signals in the radio gas chromatograms. After derivatization of the samples with diazomethane, two radio active peaks could be detected by the gas chromatography. All samples showed peaks with the same chromatographic retention times of 11 min and 12,75 min.
The mass spectrometric data show ammonia clusters of the molecular ions in the positive chemical ionization mode of m/z 297 (m/z 279 + NH_4) and m/z 341 (m/z 223 + NH_4) and in the negative chemical ionization mode, the deprotonated molecular ion of m/z 278 (m/z 279 - H) and the molecular ion of m/z 323.
The corresponding electron impact spectrum of the metabolite with the molecular ion of m/z 279 is consistent with the fragmentation pattern of the active ingredient metalaxyl (CGA 48988), whereas the spectrum with the molecular ion of m/z 323 after methylation with diazomethane is consistent with the reference compound CGA 109 097.
The structures of the original metabolites of the percolated water samples are therefore CGA 62 826 and CGA 108 906 or CGA 108 905, which will form after methylation CGA 48 988 and CGA 109 097, respectively.

Metabolite I

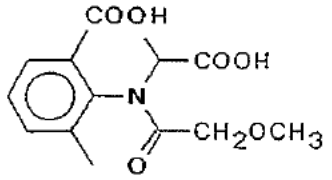
CGA 62 826:

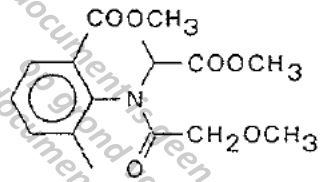


:CGA 48988

Metabolite II

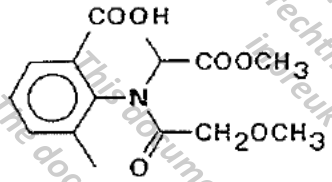
CGA 108 906:





:CGA 109 097

CGA 108 905:



CERTIFICATION OF AUTHENTICITY

We, the undersigned, hereby declare that the experimental work described in this report was performed under our supervision and that the report provides a true and accurate record of the data obtained. The testing facility worked under the survillance of GLP and the interpretation represents original scientific work and was carried out in accordance with the standards of the profession.

5.1.2.e Woo

5.1.2.e Woo Dr. phil.

Date

Chromatographic Parameters

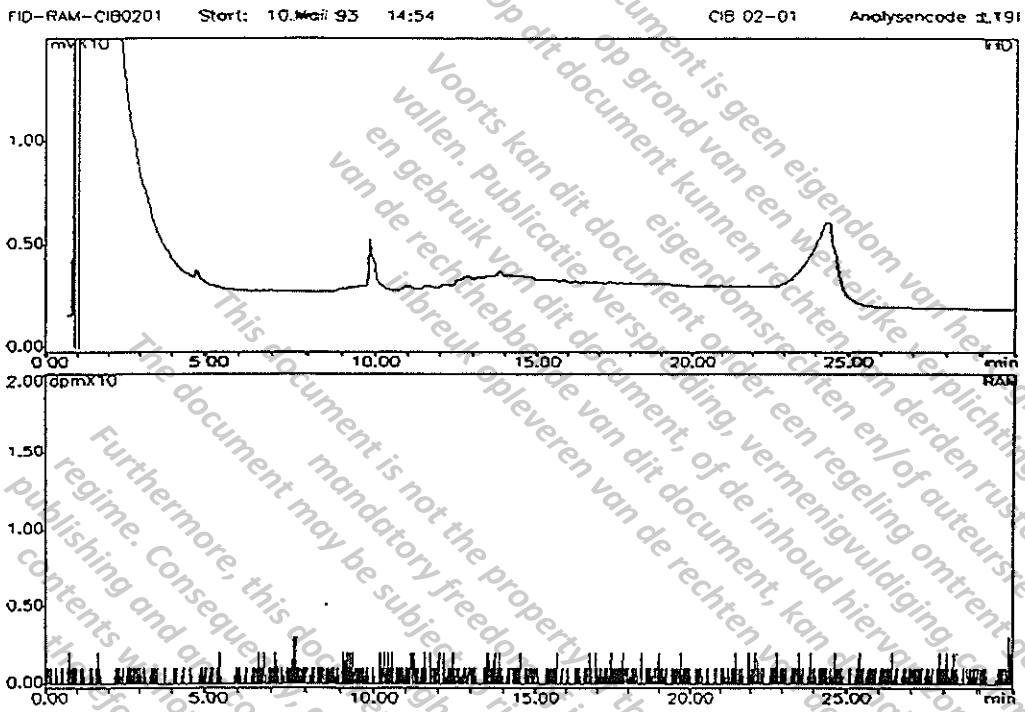
Mass Spectrometer	
Instrument:	Finnigan 4500 GC-MS
GC-Interface:	Direct coupling
Temp. Manifold:	50 - 80 °C
Temp. Ion source:	120 °C
Temp. GC-Transfer line:	220 °C
Temp. Interface oven:	230 °C
Ionization voltage:	40 eVolt
Multiplier voltage:	1 300 Volt
Emission current:	0,15 mA

GC-MS Conditions	
Ionization technique:	EI PCI NCI
Reactant gas:	ammonia
Column type:	DB-17
Length:	15 m
Diameter:	0,530 mm
Injection port temperature:	250 °C
Oven temperature program:	From 100 °C to 250 °C; Rate : 5 resp. 15 °C per min
Computer files:	CIB0201, CIB0202, CIB0203, CIB21CIP, CIB21CIN, CIB22CIP, CIB22CIN, CIB23CIP, CIB23CIN

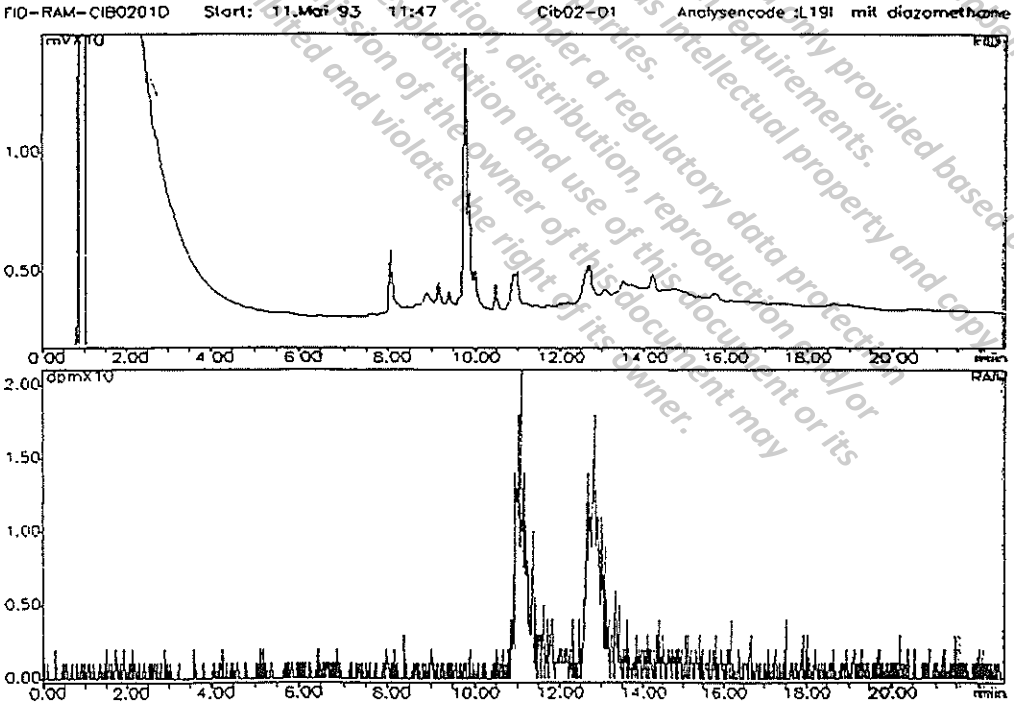
GC-RAM Conditions	
Split RAM-FID	1:7
Column type:	DB-17
Length:	15 m
Diameter:	0,530 mm
Injection port temperature:	250 °C
Oven temperature program:	From 100 °C to 250 °C; Rate : 5 resp. 15 °C per min
Computer files:	CIB0201, CIB0202, CIB0203

Study: CIB02
Sample identification: L 19 I
Lysimeter: 19
Samplingdate: march 1,1993

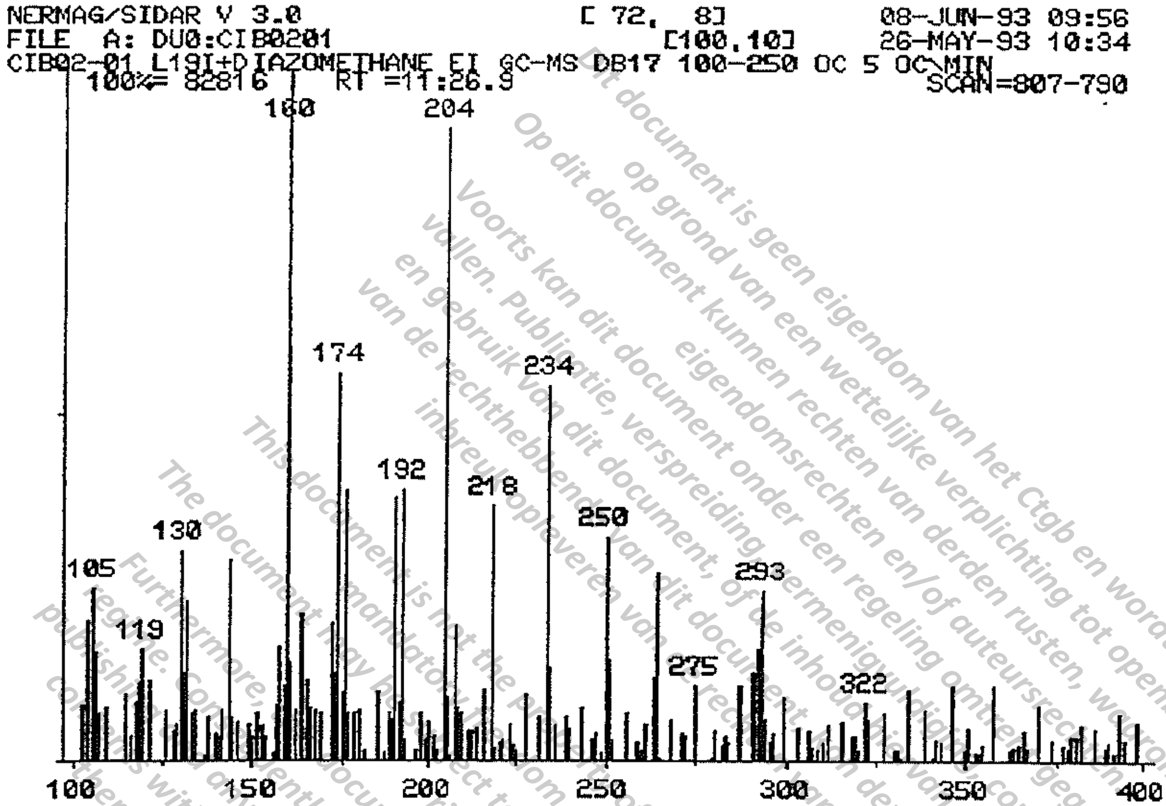
Radio gas chromatogram, upper trace: FID, lower trace: RAM



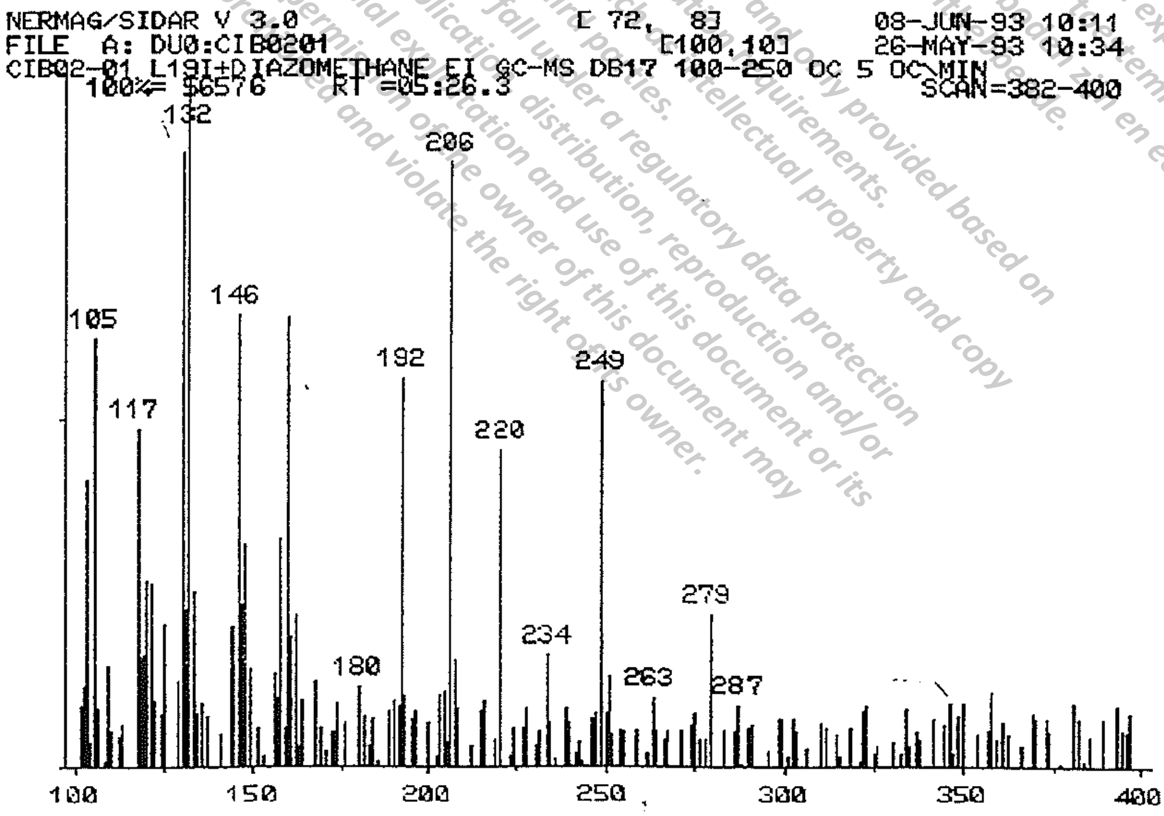
Radio gas chromatogram after methylation, upper trace: FID, lower trace: RAM



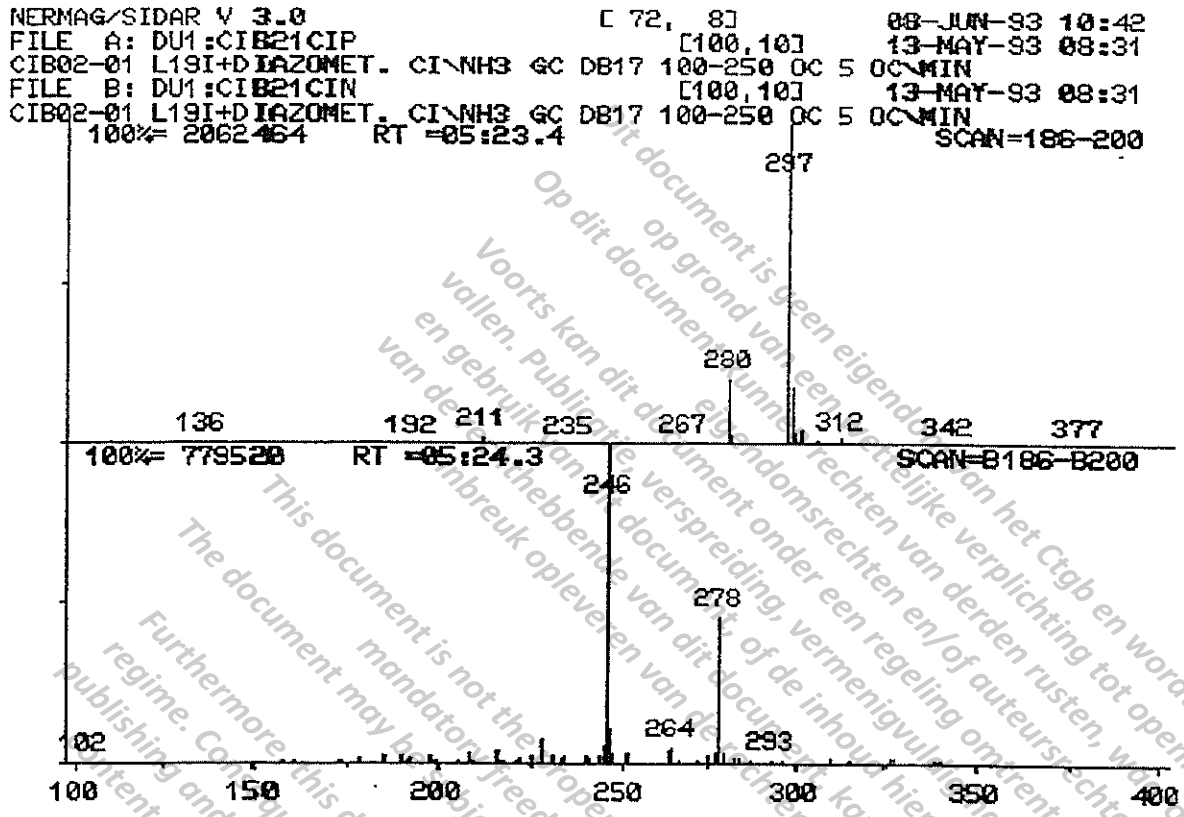
Mass spectra EI mode; 1st peak: CGA 62 826 methylated.



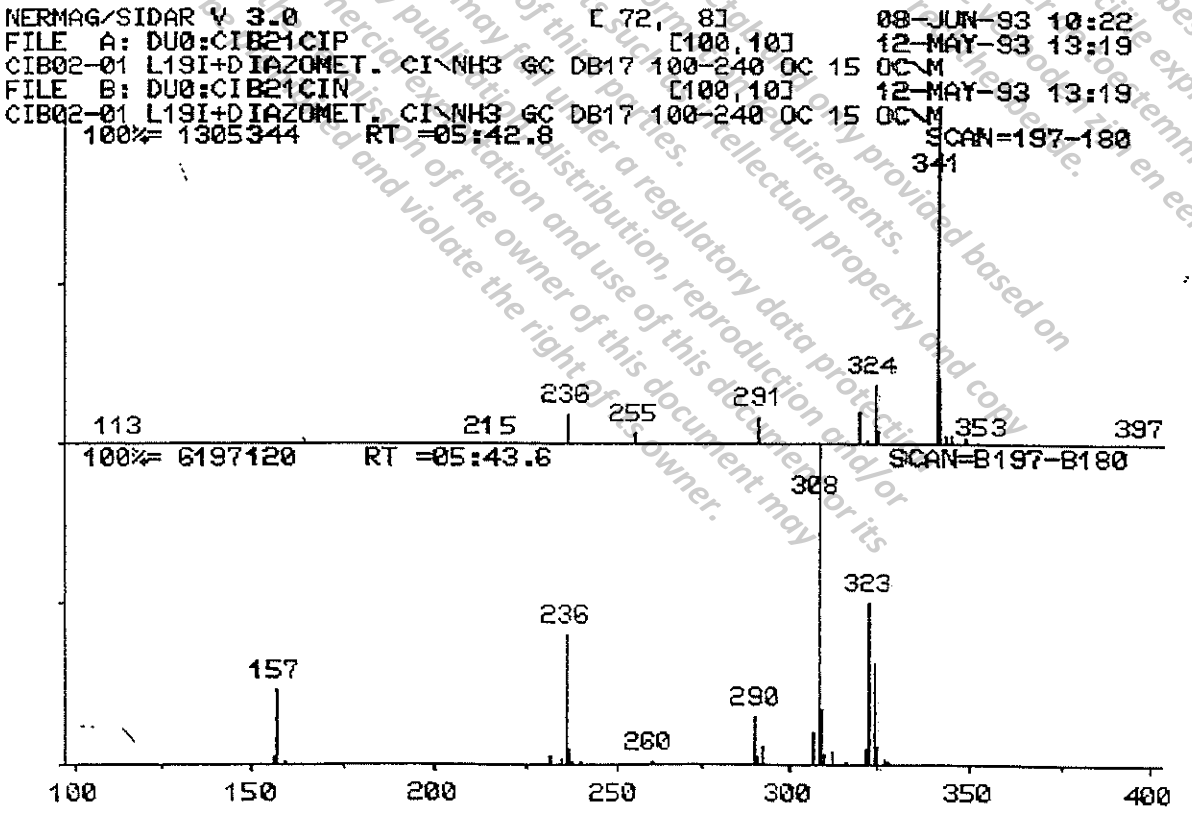
Mass spectra EI mode; 2nd peak: CGA 109 097 methylated.



Mass spectra CI mode NH_3; 1st peak: CGA 62 826 methylated.

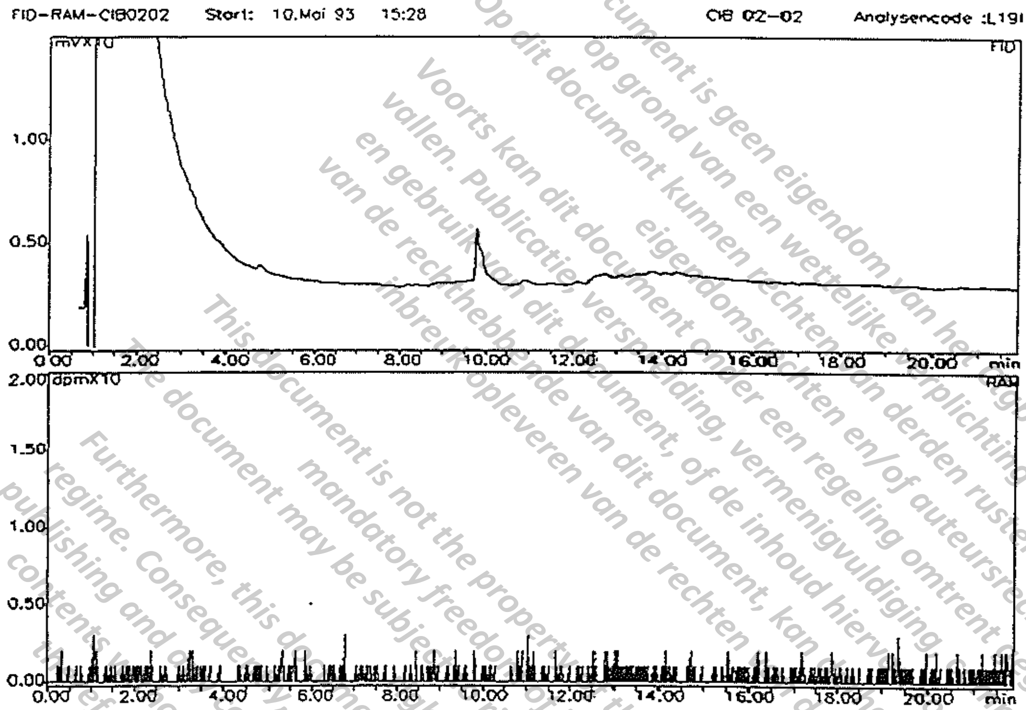


Mass spectra CI mode NH_3; 2nd peak: CGA 109 097 methylated.

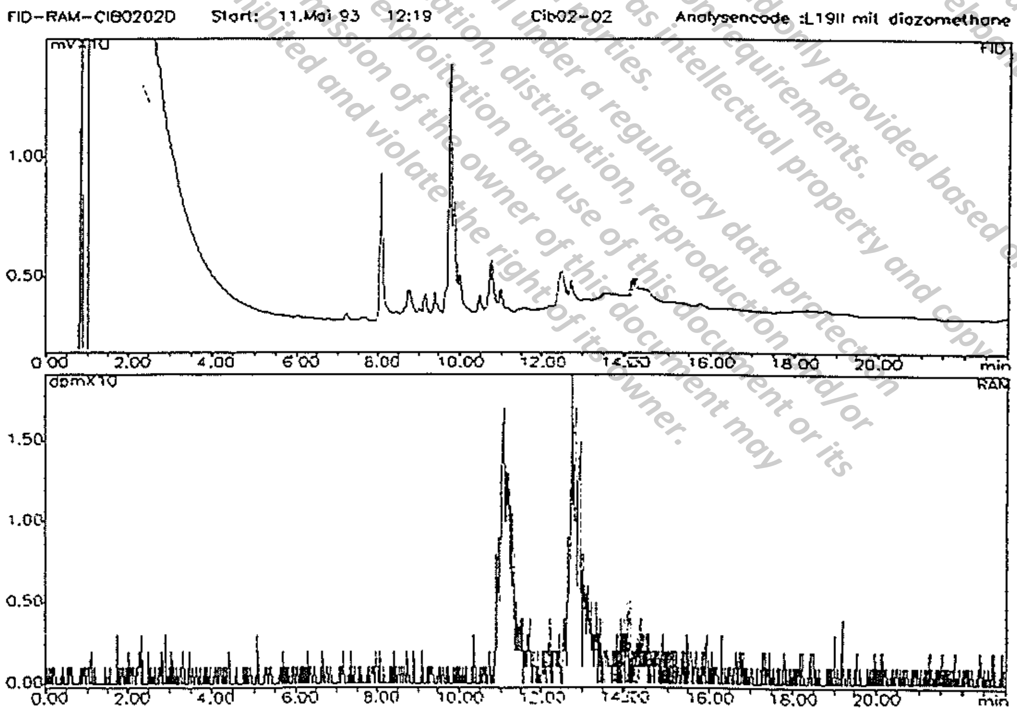


Study: CIB02
Sample identification: L 19 II
Lysimeter: 19
Samplingdate: march 1,1993

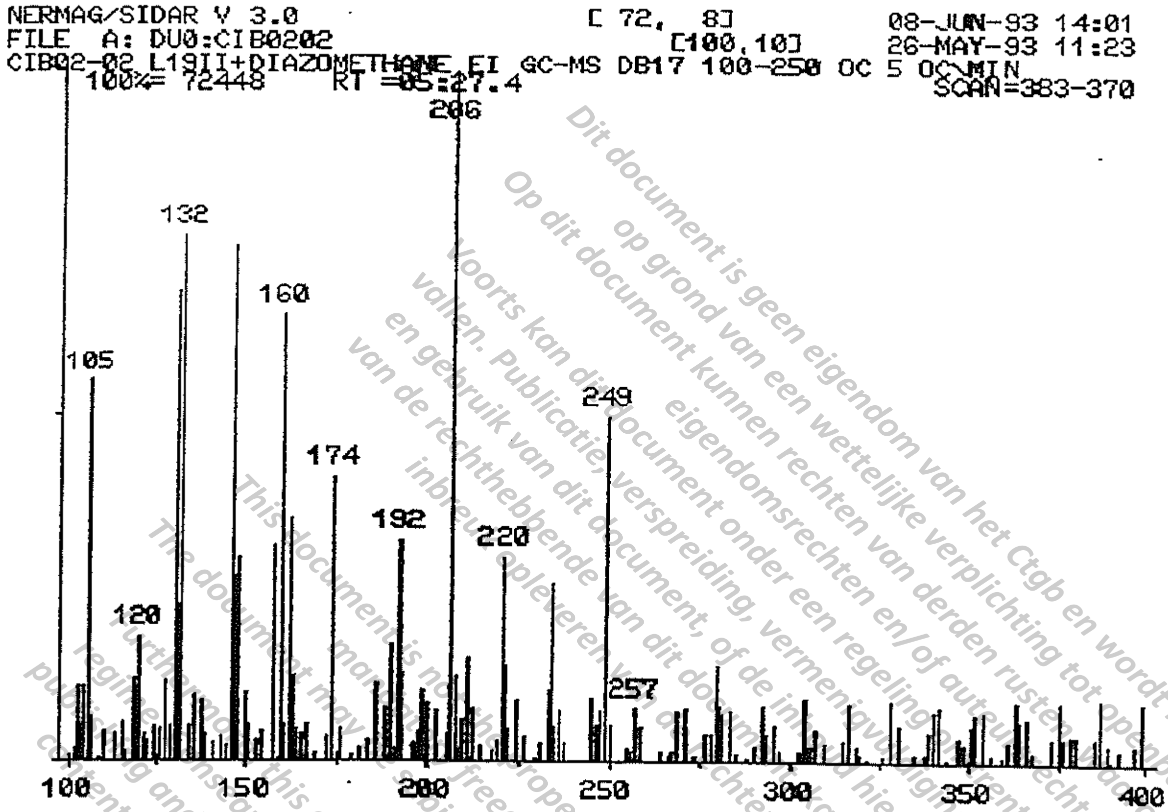
Radio gas chromatogram, upper trace: FID, lower trace: RAM



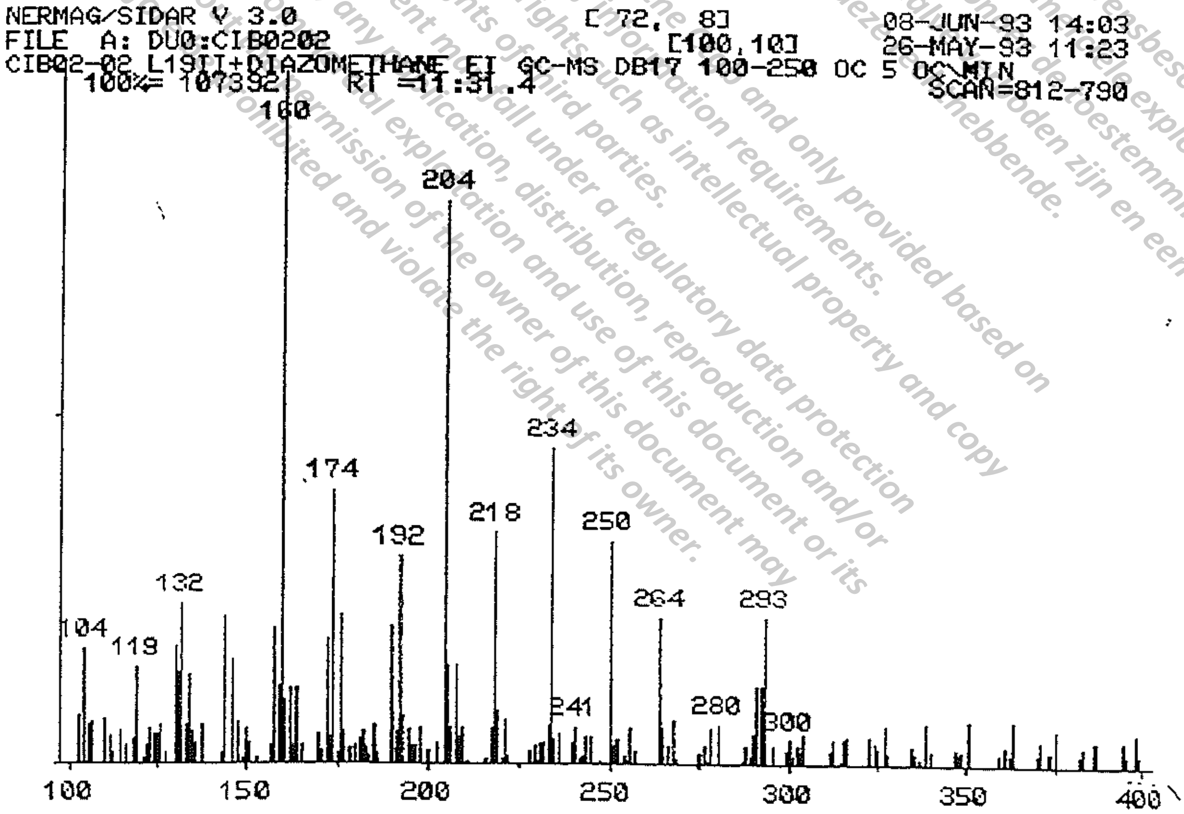
Radio gas chromatogram after methylation, upper trace: FID, lower trace: RAM



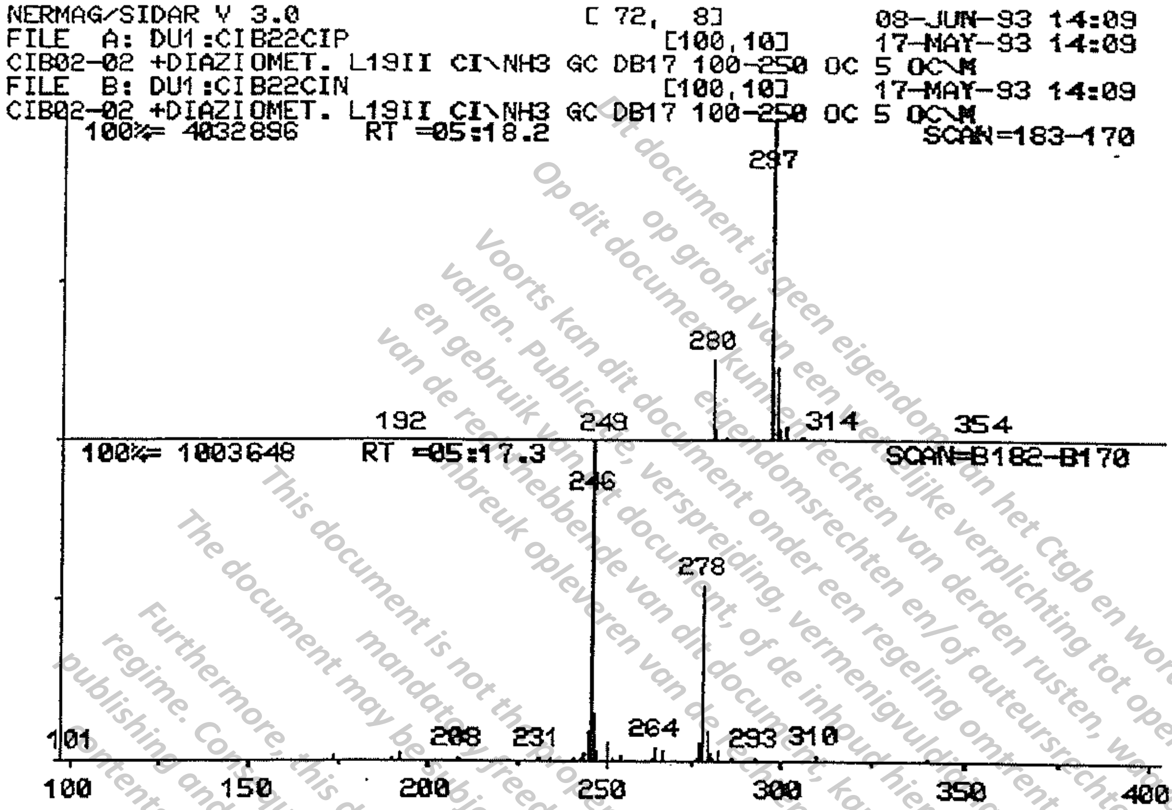
Mass spectra EI mode; 1st peak: **CGA62826** methylated.



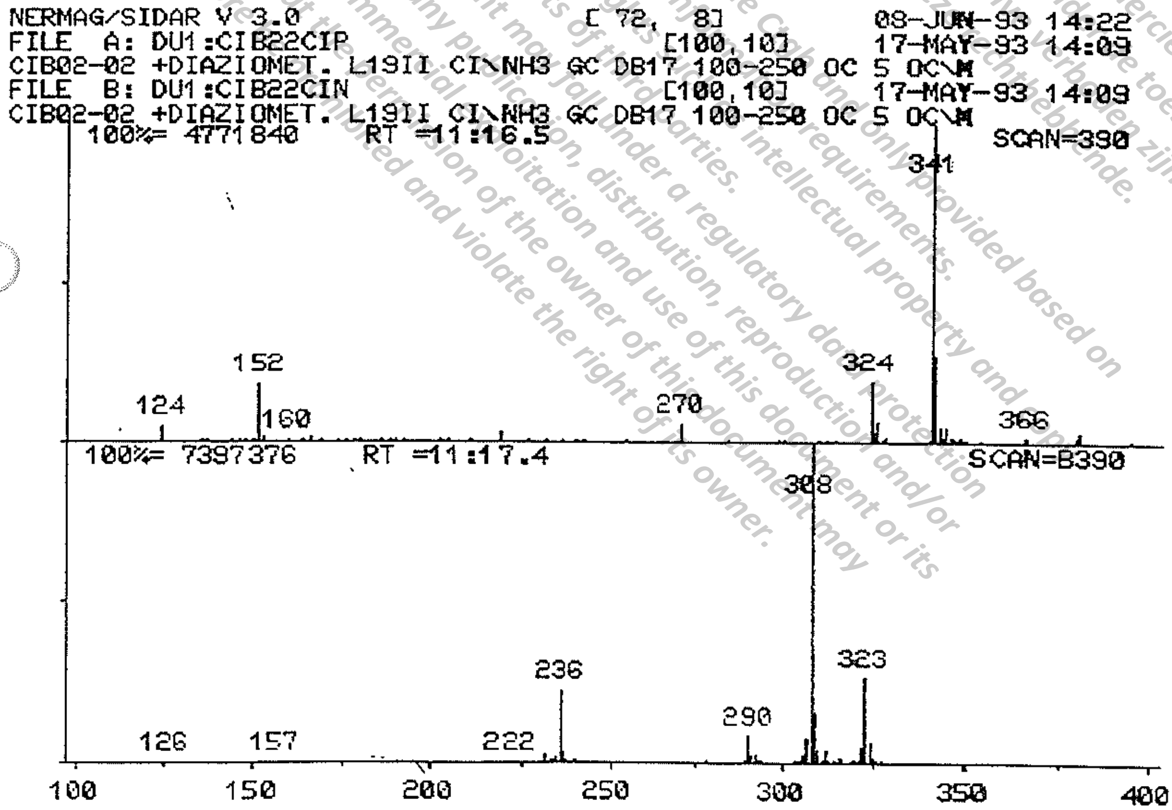
Mass spectra EI mode; 2nd peak: CGA 109 097 methylated.



Mass spectra CI mode NH_3; 1st peak: CGA 62 826 methylated.

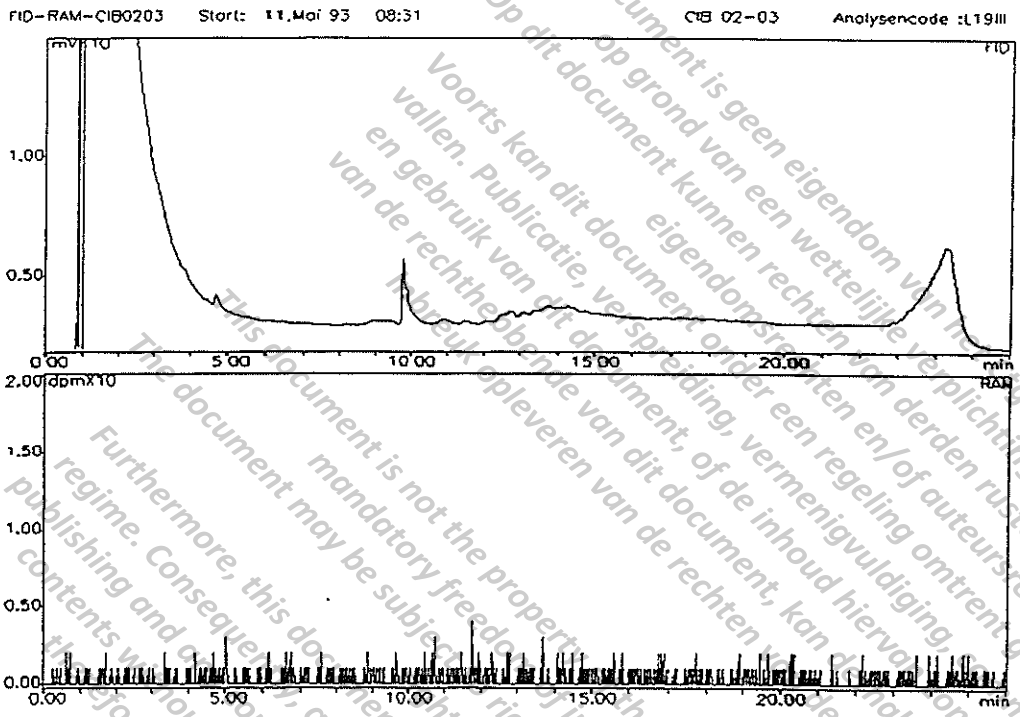


Mass spectra CI mode NH_3; 2nd peak: CGA 109 097 methylated.

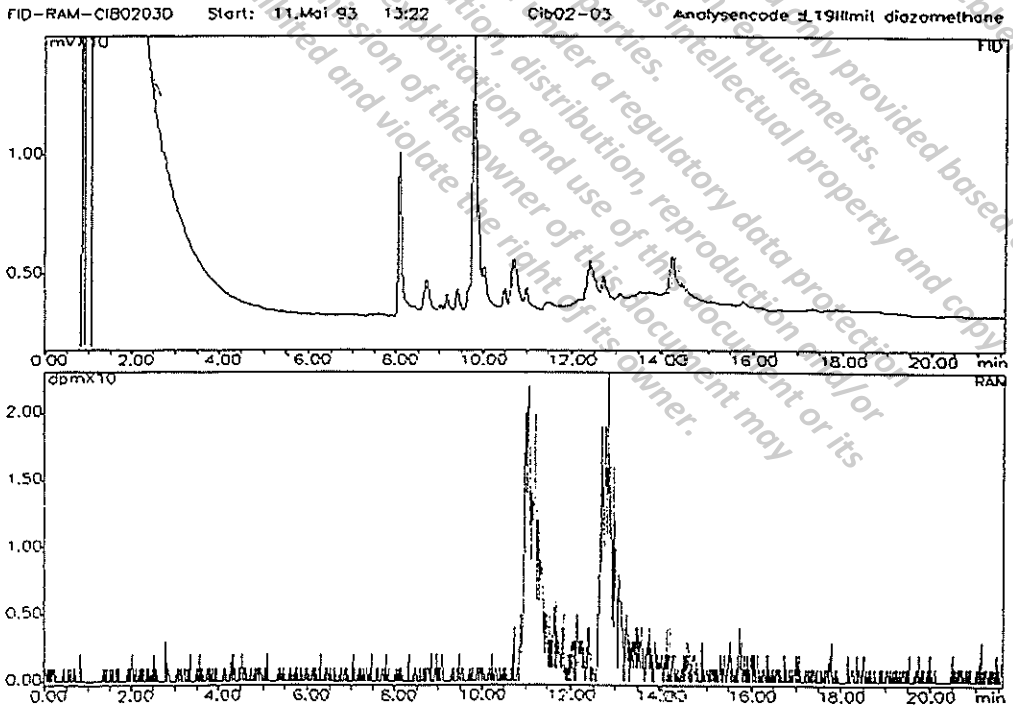


Study: CIB03
Sample identification: L 19 III
Lysimeter: 19
Sampling date: march 1,1993

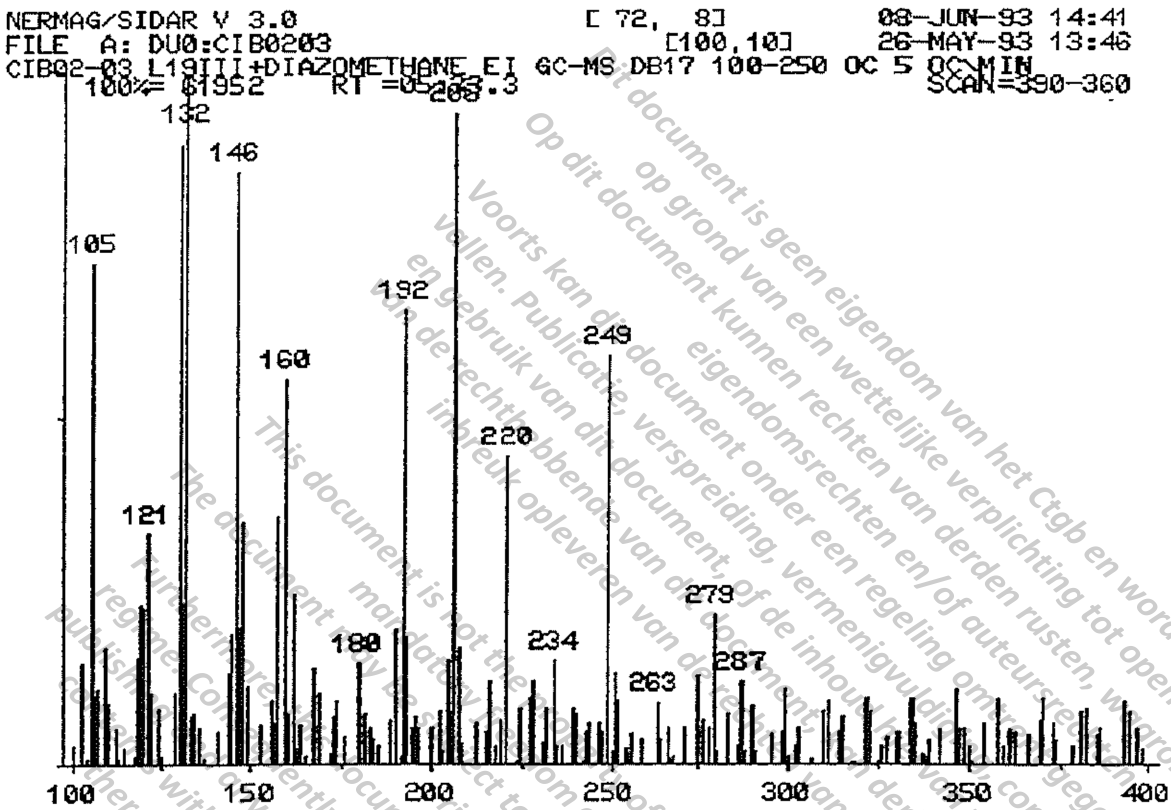
Radio gas chromatogram, upper trace: FID, lower trace: RAM



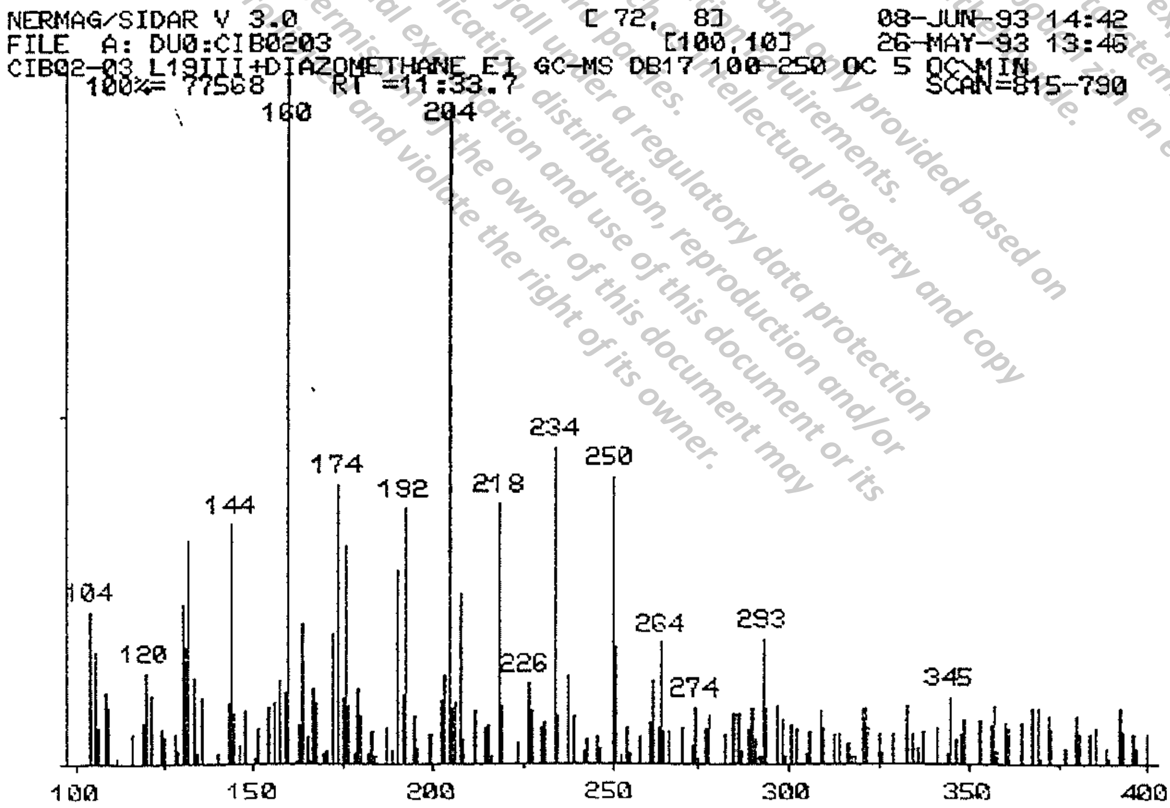
Radio gas chromatogram after methylation, upper trace: FID, lower trace: RAM



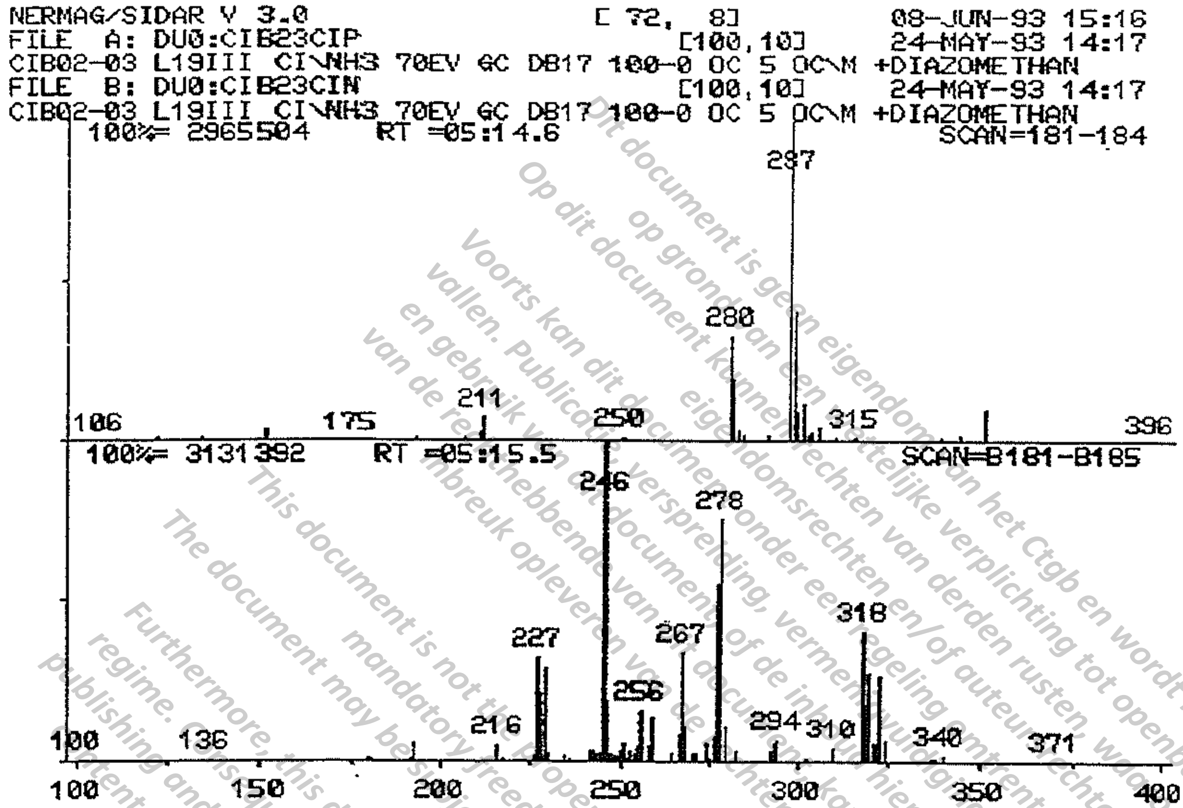
Mass spectra EI mode; 1st peak: CGA 62 826 methylated.



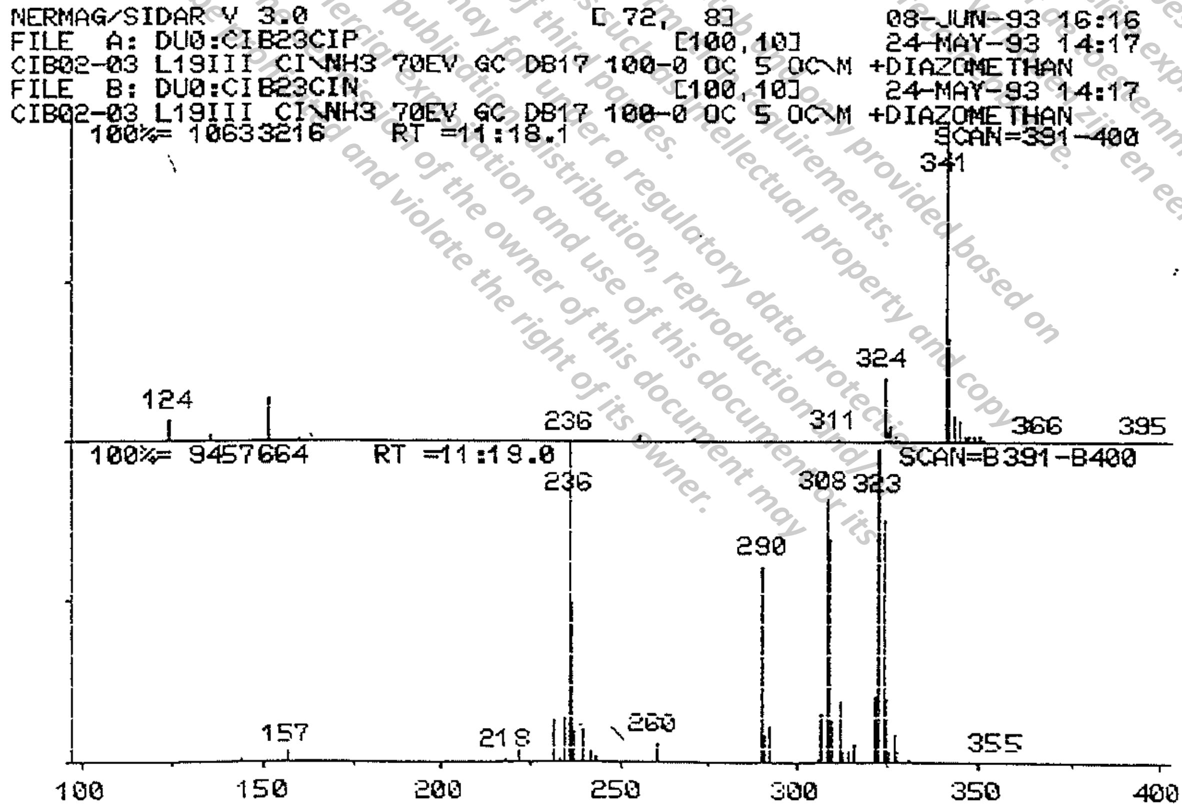
Mass spectra EI mode; 2nd peak: CGA 109 097 methylated.



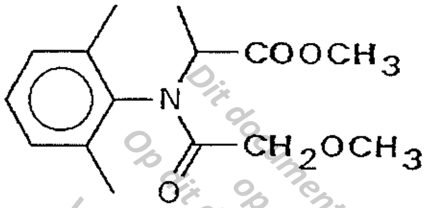
Mass spectra CI mode NH_3; 1st peak: CGA 62 826 methylated.



Mass spectra CI mode NH_3; 2nd peak: CGA 109 097 methylated.

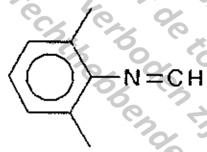


Reference: CGA 48 988



$C_{15}H_{21}NO_4$

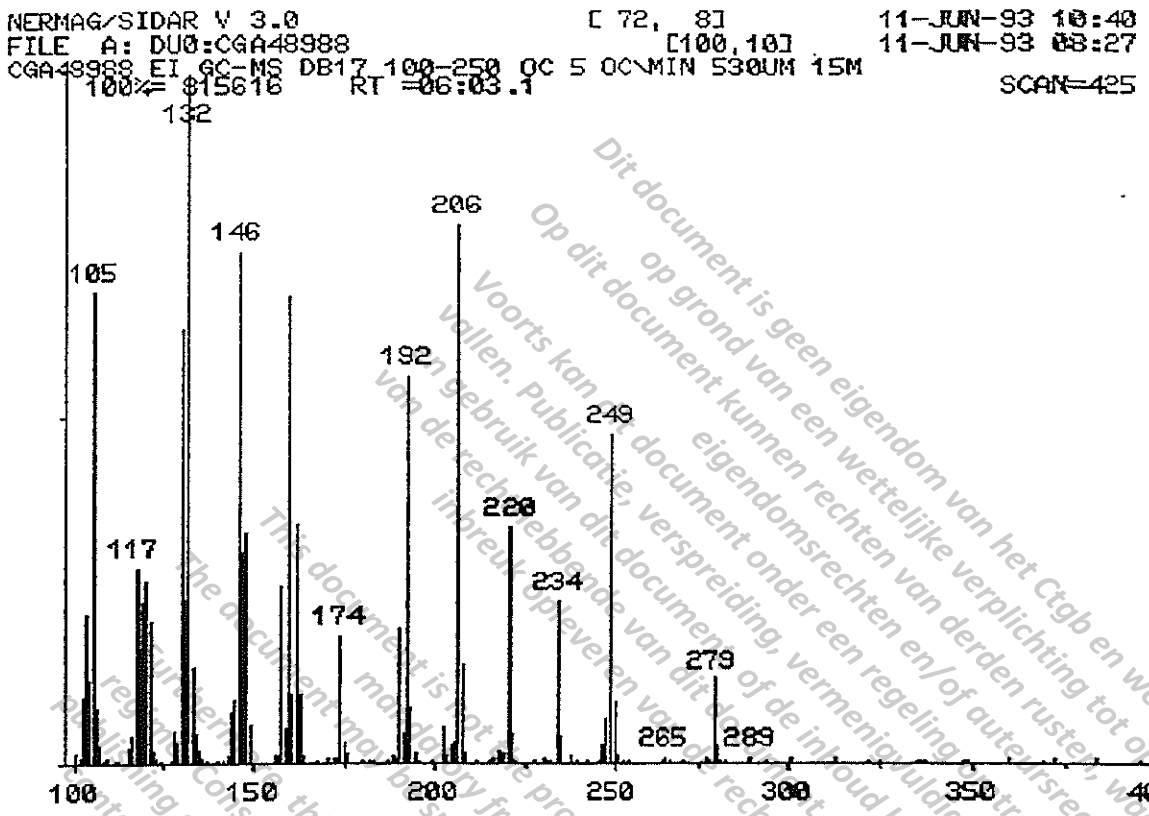
EI-MS

amu	rel.Intensity	Ion Registration	Assignments	Fragments
279	13	positive	M	
249	48	positive	M- 30	⇒ CH_2O
234	25	positive	M- 45	⇒ CH_2OCH_3
220	35	positive	M- 59	⇒ $COOCH_3$
206	78	positive	M- 73	⇒ $COCH_2OCH_3$
192	57	positive	M- 87	⇒ $CH_3CHCOOCH_3$
174	37	positive	206 - 32	⇒ CH_3OH
162	34	positive	249 - 87	⇒ $CH_3CHCOOCH_3$
160	69	positive	192 - 32	⇒ CH_3OH
148	34	positive	220 - 72	⇒ $O=C=CHOCH_3$
146	75	positive	206 - 60	⇒ $HCOOCH_3$
132	100	positive	M - 147	= 

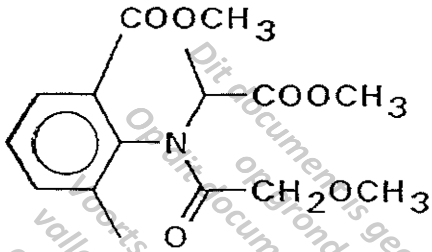
DCI-MS

amu	rel.Intensity	Ion Registration	Assignments	Fragments
297	100	positive	$M+NH_4$	
280	47	positive	M + H	

amu	rel.Intensity	Ion Registration	Assignments	Fragments
278	38	negative	M- H	
246	100	negative	278 - 32	⇒ CH_3OH

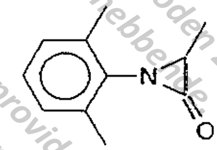


Reference: CGA 109 097 Target: CGA 48 988



$C_{16}H_{21}NO_6$

EI-MS

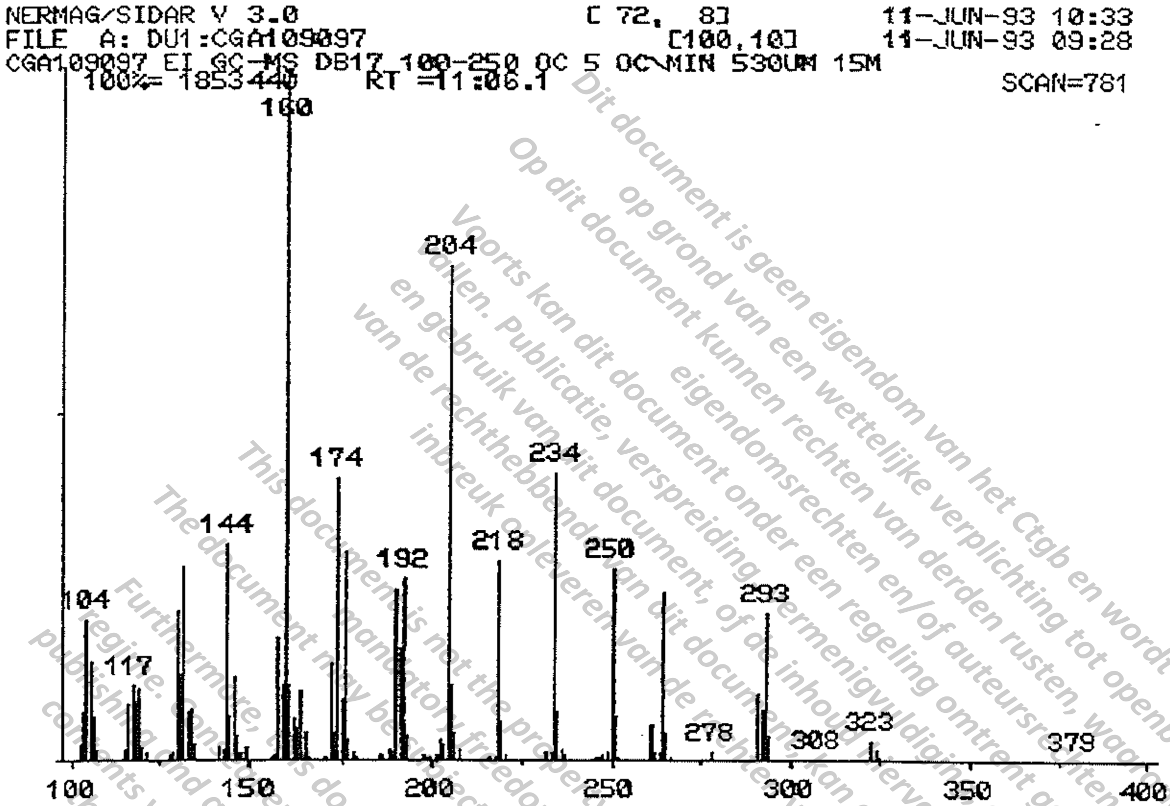
amu	rel.Intensity	Ion Registration	Assignments	Fragments
323	3	positive	M	
293	22	positive	M- 30	⇒ $CH_2=O$
278	2	positive	M- 45	⇒ CH_2OCH_3
264	25	positive	M- 59	⇒ $COOCH_3$
250	28	positive	M- 73	⇒ $COCH_2OCH_3$
234	42	positive	239 - 59	⇒ $COOCH_3$
218	31	positive	278 - 60	⇒ $HCOOCH_3$
204	72	positive	234 - 32	⇒ CH_3OH
192	27	positive	264 - 72	⇒ $O=C=CHOCH_3$
174	40	positive	234 - 60	⇒ $HCOOCH_3$
160	100	positive	M- 163	= 

DCI-MS

amu	rel.Intensity	Ion Registration	Assignments	Fragments
341	100	positive	$M+NH_4$	

amu	rel.Intensity	Ion Registration	Assignments	Fragments
323	28	negative	M	
308	100	negative	M- 15	⇒ CH_3

Mass spectrum EI mode: CGA 109 097



ad 48988/3732

Ciba-Geigy Ltd.
Crop Protection Division
Product Safety

CHARACTERIZATION OF BOUND ^{14}C-RESIDUES OF SOIL SAMPLES CIBO2/L19

(Lysimeter study with Metalaxyl; CIBO2, [redacted], Neustadt, Germany)

Exper. Start 11/9/1994

Exper. Termination 01/21/1995

Reporting Date 11/24/1995

Author

A. Summary

1. Origin of the samples

Soil samples were collected 731 days after application of study CIBO2 (DEGRADATION AND LEACHING OF ^{14}C-METALAXYL IN TWO SAND LYSIMETERS UNDER OUTDOR CONDITIONS AFTER APPLICATION TO POTATOES) by [illegible] SLFA Neustadt/Weinstrasse, Germany. Samples from different layers (0-10 cm, 70-80 cm, and 120-130 cm) were subjected to extraction under different conditions involving microwaves. More details about the experimental conditions are present in Part B.

2. Results

The following percentages were extracted:
(expressed in % of total activity present per sample).

Extraction method	Soil depth		
	0 - 10 cm	70 - 80 cm	120 - 130 cm
Soxhlet Reflux Methanol	7.1	4.7	2.9
Microwaves aq. n-Propanol	64.2	20.9	44.8

Metabolites CGA 62826 and CGA 67868 and/or parent metalaxyl could be identified from the extracts of the 0-10 cm layer.

3. Conclusion

A considerable part of the bond residues, i.e. not extractable with methanol was released under the influence of microwaves and, partially, be identified by co-chromatography with known standards as metabolites CGA 62826 and CGA 67868 and/or parent metalaxyl. From this, it can be concluded that an essential part of "non-extractable" radioactivity consist of physically bound residues.

B. Experimental part

The total radioactivity in the individual soil samples was determined after combustion of three aliquots in a Harvey Biological Material Oxidizer (Zinsser Analytik, Frankfurt, Germany). The ^{14}C-labelled material in the samples is thus converted to $^{14}CO_2$, absorbed in an appropriate scintillation cocktail and counted in a Packard scintillation counter.
Extraction of the soil samples was performed, on one hand, in a soxhlet apparatus with boiling methanol for about 16 hours, and on the other hand, by a microwave extraction of 10 g aliquots in a MLS 1200 Mega microwave digestion unit under the following conditions:

Microwave extraction:

Solvent n-Propanol/Water 8:2

Step 1: 20 min at 100 °C (200 W)
Step 2: 10 min at 100 °C (200 W), followed by 20 min at 150 °C (250 W).
Step 3: 10 min at 100 °C (200 W), 20 min at 150 °C (250 W) followed by 20 min at 180 °C (300 W).
Step 4: same as step 3

Solvent n-Propanol/aq. Tetramethylammoniumhydroxide (12.5%) 8:2

Step 5 20 min at 120 °C (220 W)
Step 6: 10 min at 120 °C (220 W), followed by 20 min at 150 °C (250 W).

Thin layer chromatography:

Detection of metabolites of the soil extracts was performed by using precoated silica gel 60 F_{254} plates with a thickness of 0.25 mm. The plates were developed without chamber saturation. Reference compounds were visualized under UV-ligth (254 nm).
The following solvent systems were used to separate the soluble radioactivity:

Analytical System I:
Ethylacetate-Acetic acid 9:1

Analytical System II:
1. direction Hexane-Ethylacetate-Ethanol-Formic acid 50:25:25:0,4
2. direction Ethylacetate-Acetic acid 9:1

Radioactive zones on thin layer plates were localized with a Berta Camera (Raytest, Straubenhardt, Germany). Quantitation of the radioactivity was performed by scraping off the individual spots from the plate and counting in a Packard scintillation counter.

Figure 1: Procedure for the analysis of soil layer 0-10cm

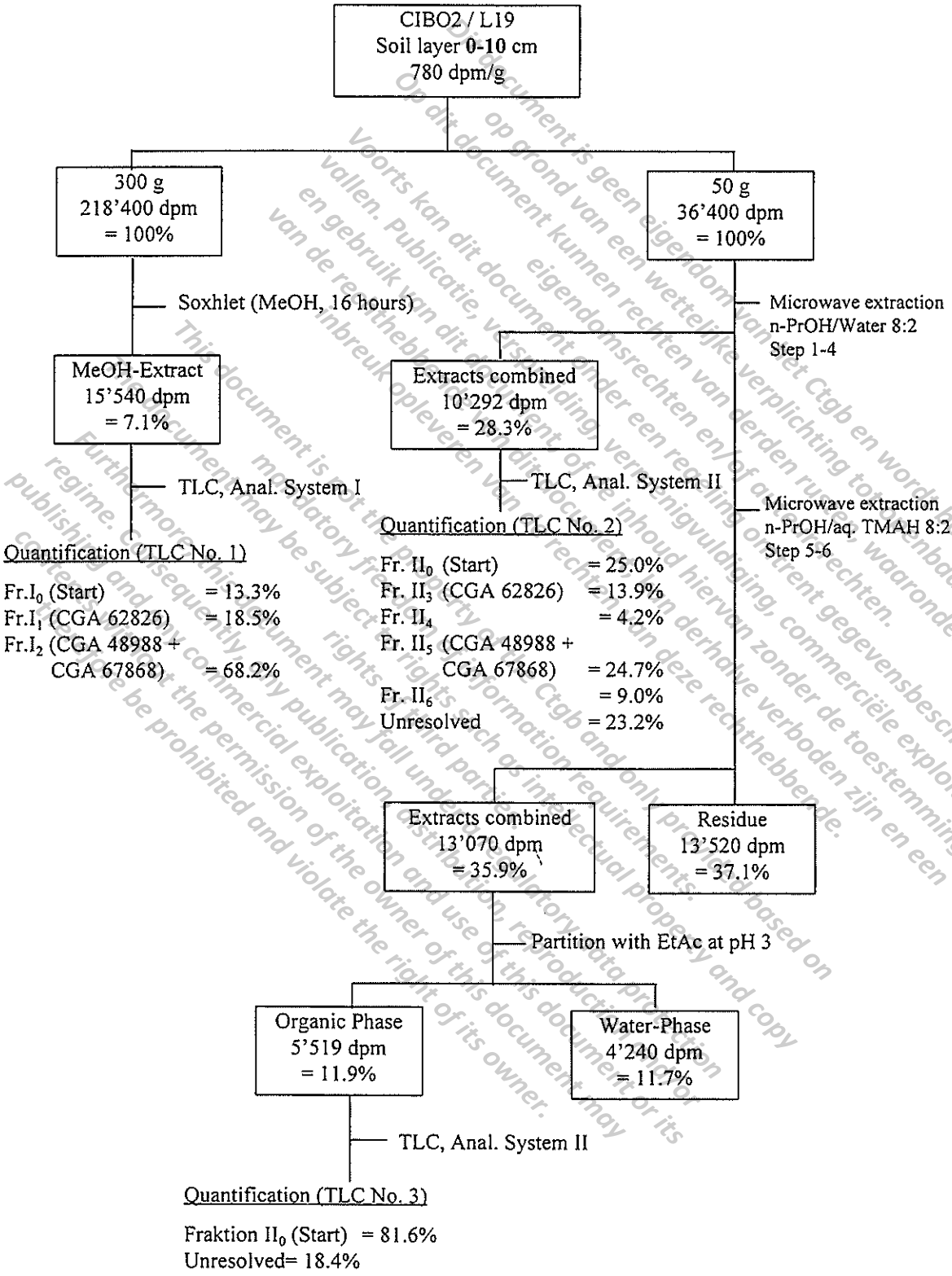


Figure 2: Procedure for the analysis of soil layer 70-80cm

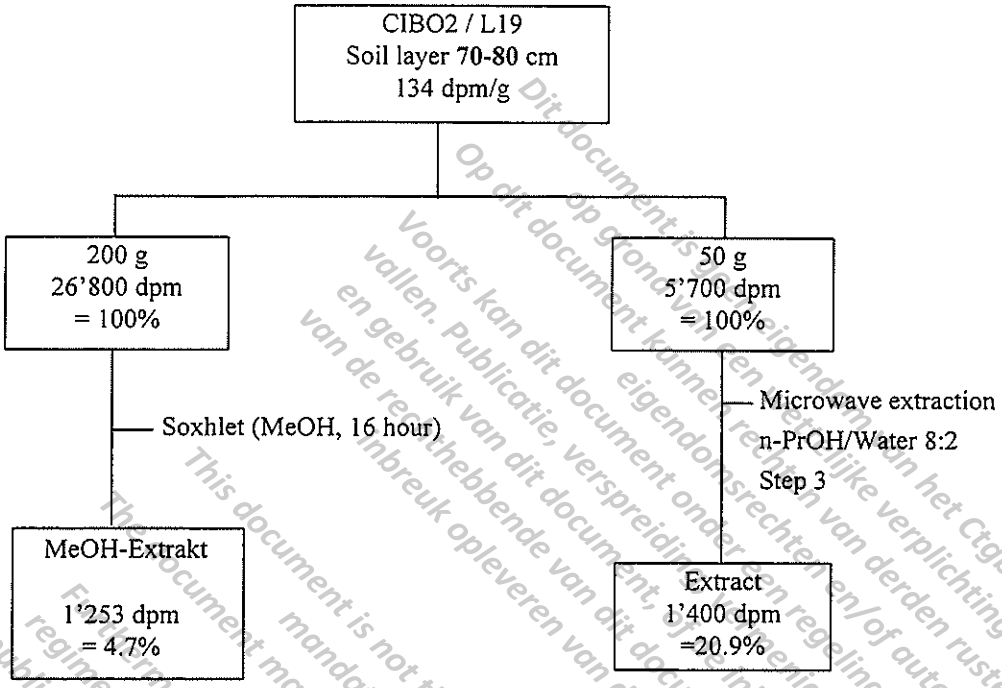


Figure 3: Procedure for the analysis of soil layer 120-130cm

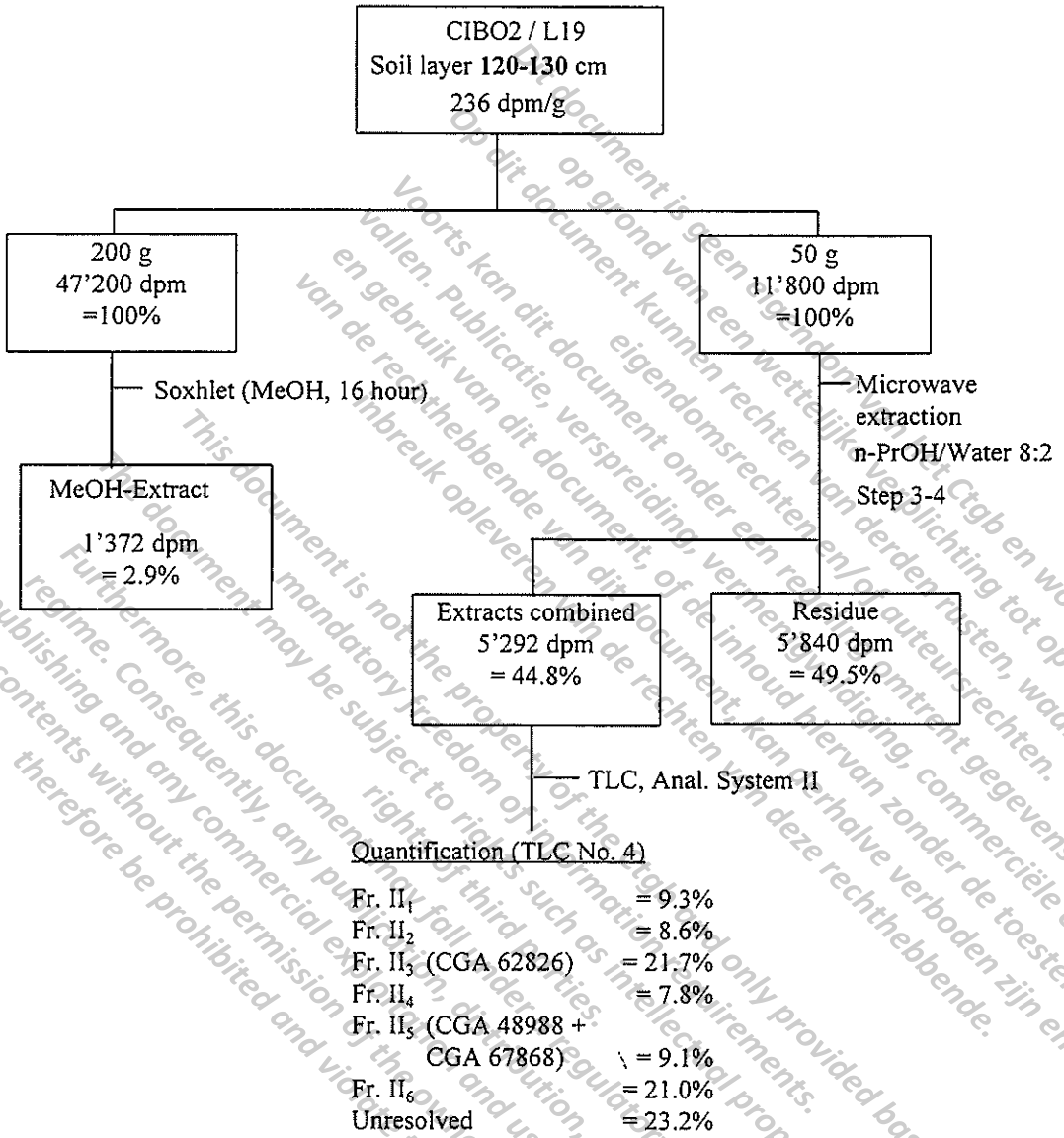
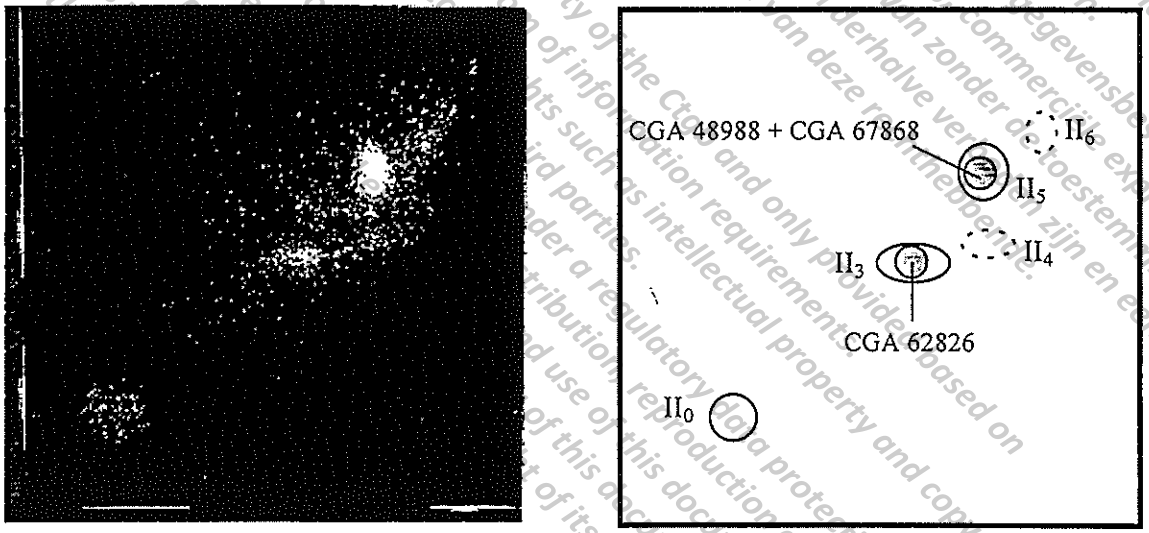


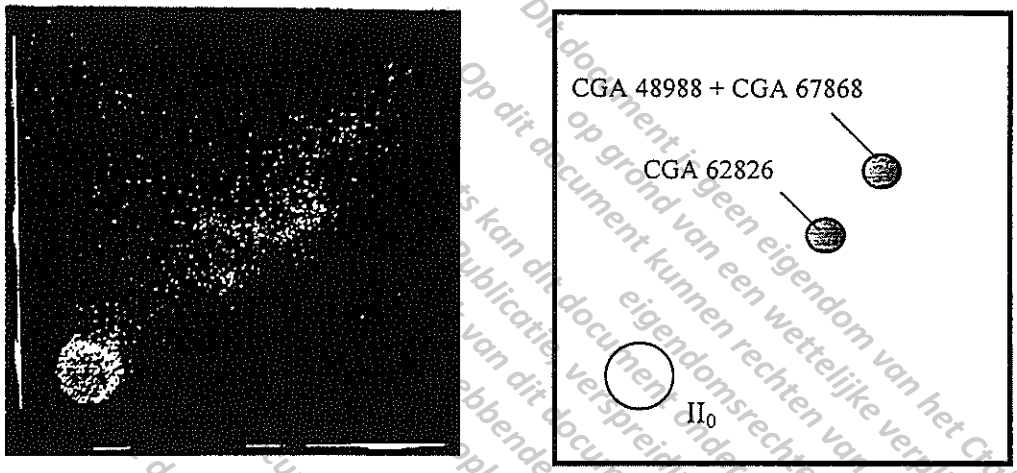
Figure 4: Metabolite Fractions found in soil extracts

TLC No.1: Soxhlet extract of soil layer 0-10 cm (Anal. System I)

TLC No.2: Microwave extract (step 1-4) of soil layer 0-10 cm (Anal. System II)



TLC No.3: Microwave extract (step 5-6) of soil layer 0-10 cm (Anal. System II)



TLC No.4: Microwave extract (step 3-4) of soil layer 120-130 cm (Anal. System II)