

1. Interim Report
CIB03

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Study title

: DEGRADATION AND LEACHING OF ¹⁴C-METALAXYL IN
TWO SAND LYSIMETERS UNDER OUTDOOR CONDITIONS
AFTER APPLICATION TO VINE

Results from the 1st experimental year

Study

Requirement

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

* BBA = Federal Biological Institute
(Braunschweig; FRG)

Code of
the study

: CIB03

Author

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STATEMENT OF COMPLIANCE

The study described in this report was conducted in compliance with the Good Laboratory Practice regulations (as modified in the German Chemicals Act, §19 Chemg. March 14, 1990) and this report provides an accurate record of the results obtained.

Study director:

October 22, 93
(Date)

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I. ZUSAMMENFASSUNG

Das Verlagerungsverhalten von [Phenyl-¹⁴C]Metalaxyl (methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alaninat) wurde nach Applikation zu Reben in zwei Lysimetern (15 und 16), die einen ungestörten Sandboden enthielten, unter Freilandbedingungen untersucht. In Übereinstimmung mit dem empfohlenen Einsatz wurden zwei Behandlungen mit ca. 160 g und 240 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 ersten Versuchsjahr der laufenden Studie wurden folgende Ergebnisse erzielt:

In den Sickerwässern beider Lysimeter wurden 3.7 % (Lys. 15) und 3.2 % (Lys. 16) der insgesamt applizierten Radioaktivität innerhalb des ersten Versuchsjahres gefunden. Unter Berücksichtigung des gesamten Sickerwasservolumens (235.6 l/Lys. 15 und 242.3 l/Lys. 16) wurde eine mittlere Konzentration von 4.65 und 3.44 µg Wirkstoffäquivalente/l Sickerwasser berechnet.

Nur eine geringe mittlere Metalaxyl-Konzentration war im Sickerwasser des Lysimeters 15 im ersten Versuchsjahr vorhanden die sich auf 0.01 µg/l belief während der Wirkstoff im Sickerwasser von Lysimeter 16 zu keinem Zeitpunkt auftauchte.

Neben dem Wirkstoff wurden auch die Metaboliten CGA 62826 (2-[(2,6-Dimethyl-phenyl)-(2-methoxyacetyl)-amino]-propionsäure) und CGA 108906 (2-[(1-carboxy-ethyl)-(2-methoxyacetyl)-amino]-3-methyl benzoessäure) durch HPLC, DC und Massenspektroskopie charakterisiert. Die mittleren Konzentrationen von CGA 62826 betragen 3.86 bis 2.42 µg/l, die mittleren Konzentrationen von CGA 108906 betragen 0.48 bis 0.76 µg/l. Weiterhin wurde ¹⁴CO₂ in mittleren Konzentrationen von 0.11 µg/l in beiden Lysimeter gefunden.

Zum Zeitpunkt der Traubenernte wurden in den untersuchten Rebenfraktionen (Trauben, Blätter, Holzschnitt) 6.1 (Lys. 15) und 3.9 % (Lys. 16) der applizierten Radioaktivität gefunden, die sich hauptsächlich in den Trauben und Blättern befand.

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

I. SUMMARY

The leaching behaviour of [Phenyl-¹⁴C]Metalaxyl, i.e. methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate, applied to vine was studied under outdoor conditions, using two lysimeters (15 and 16) containing an undisturbed sandy soil. According to the recommended use, two treatments were carried out, at rates corresponding to 160 and 240 g A.I./ha. The 1st treatment was carried out in a divided spray application at June 29, 1992 and July 14, 1992. At July 27, 1992 the 2nd treatment was performed.

In the 1st experimental year of the ongoing study the following results were obtained:

In leachates of both lysimeters 3.7 % (lys. 15) and 3.2 % (lys. 16) of the total radioactivity applied, appeared within the first year. When related to the total volumes of leachate collected (235.6 l/lys. 15 and 242.3 l/lys. 16) an average concentration of 4.65 and 3.44 µg parent equivalents per l of leachate was calculated.

Only a minor average concentration of metalaxyl was present in the percolate of lysimeter 15 in the 1st year and accounted for 0.01 µg/l whereas no A.I. was detected in any percolate of lysimeter 16.

Beside the parent compound, CGA 62826, i.e. 2-[(2,6-Dimethyl-phenyl)-(2-methoxyacetyl)-amino]-3-methyl benzoic acid could be characterized by HPLC, TLC and mass spectroscopy. Average concentrations of CGA 62826 ranged from 3.86 to 2.42 µg/l and CGA 108906 from 0.48 to 0.76 µg/l, respectively. Furthermore ¹⁴CO₂ was detected in average concentrations of 0.11 µg/l in both lysimeters.

At harvest time of grapes 6.1 % (lys. 15) and 3.9 % (lys. 16) of the radioactivity applied were found in the vines, mainly concentrated in the grapes and leaves.

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

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Lysimeter investigation, 1st year
percolate

lysimeter	L 15	L 16
date of application	1st a) June 29, 92 b) July 14, 92	June 29, 92 July 14, 92
	2nd July 27, 92	July 27, 92
radioactivity applied [kBq]	58807.9	53184.3
spec. radioactivity [kBq/mg]	2010.0	2010.0
radioactivity percolated [kBq]	2198.6	1675.6
¹⁴ C-carbonate in the percolate [kBq]	63.1	57.7
radioactivity percolated without ¹⁴ C-carbonate [kBq]	2135.5	1617.9
amount of percolate [l]	235.6	242.3
radioactivity of A.I. in the percolate [kBq]	2.8	-
radioactivity in the percolate without A.I. and ¹⁴ C-carbonate [kBq]	2132.7	1617.9
radioactivity percolated (% of the amount applied)	3.73	3.15
radioactivity percolated without ¹⁴ C-carbonate (% of the amount applied)	3.62	3.04
concentration of A.I. in the percolate [µg/l]	0.01	-
concentration of the metabolites [µg/l]		
CGA 108906	0.48	0.76
CGA 62826	3.86	2.42

II. 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 three year study was started using two lysimeters, filled with a sandy soil. The lysimeters were planted with one vine each which was three years old at the beginning of the experiment.

III INVESTIGATION

1. Test substance

The test substance (active ingredient) used was:

[Phenyl- ^{14}C]Metalaxyl

The details concerning the test substance are listed in appendix 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:

$\geq 97\%$

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 analytical standards of reference substances were used for metabolite identification.

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 investigated standards 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 04. The sampling area was Birkenheide, Rhineland Palatinate, Germany. Date of sampling was December 20, 1990.

2.2 Size of lysimeters

The lysimeters (appendix 02) 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 m². 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.02).

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 03. The distance between lysimeter L15 and L16 was 110 cm. The surroundings of the two lysimeters were used as a control area (app. 03) 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

For both lysimeters dates of applications in the 1st experimental year were:

1st treatment (divided): a) June 29, 92

b) July 14, 92

2nd treatment: July 27, 92

3.2 Amount (mg/m² and g/ha)

The planned application rate of the test substance was:

1st treatment: 13 mg A.I./lysimeter = 160 g A.I./ha

2nd treatment: 19 mg A.I./lysimeter = 240 g A.I./ha

The quantity of test substance required for application was calculated for the surface area of the lysimeters (0.8 m²). 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.).

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 1.5 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. 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 acetone 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.

4. Cultivation of the lysimeters

4.1 Plants

During the whole study

Vine : One vine (*Vitis vinifera*, var: Müller Thurgau) was planted in each lysimeter. Grapes, leaves and wood were harvested for investigation in autumn.

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

4.1.1 Planting time

When the experiment started the age of the vine was 3 years.

4.2 Fertilization

The details concerning the fertilization are given in appendix 06b.

4.3 Plant protection treatment

The fungicide Topas was sprayed on June 24, 1992, June 14, 1993 and July 8, 1993.

4.4 Further cultivation treatment

The weeds were eliminated manually and remained on the lysimeters.

4.5 Harvest time

1st year

Grapes: Sept. 22, 1992 (app. 06a)

Leaves: Oct. 26, 1992

4.6 Crop yield

1st year

Grapes and leaves (app. 06a)

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.

5.2 Soil temperatures

Continuous measurements of the soil temperatures (10, 30 and 80 cm) were conducted in a control lysimeter. The monthly mean values are given in appendix 07a.

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.

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.

6. Percolate sampling

At intervals of 4 (June- Oct.) or 2 (Nov. - May) weeks, the percolate from each of the two lysimeters was collected using a suction pump and the exact volume was determined.

6.1 Amount of percolate and sampling date

Cf. appendix 08 and appendix 09.

6.2 Total amount of percolate after the 1st year

1st year (see app. 08)

Lysimeter 15: 235.6 l
Lysimeter 16: 242.3 l

6.3 Percolate storage

After determination of the total radioactivity (^{14}C -balance) and the $^{14}\text{CO}_2$ 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. The results were confirmed by mass spectroscopic analysis of representative samples of the lysimeter study CIB02 (Degradation and leaching of ^{14}C -Metalaxyl in two sand lysimeters under outdoor conditions after application to potatoes). For details see the CIBA-Geigy Spectroscopy Report of [redacted] (Identification of Metabolites of Metalaxyl in percolated water of Lysimeter) of June 1993. 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.

Lysimeter 15: 2198.6 kBq = 3.73 % of the radioactivity applied
(app. 11c)

Lysimeter 16: 1675.6 kBq = 3.15 % of the radioactivity applied
(app. 11c).

Examples of chromatographic analysis are given in app. 12a and 12b.

7.1.1 Active ingredient (^{14}C -metalaxyl) in individual leachates of the 1st year

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

Lysimeter L15:

2nd, and 3rd percolate sample after application (app. 11a).

Lysimeter L16:

In no percolate sample (app. 11b).

Examples of the chromatographic analysis are given in appendices 12a, b.

7.1.2 Metabolites in individual leachates of the 1st year

¹⁴CO₂:

0.02 to 0.51 µg/l A.I. equivalents were determined as ¹⁴CO₂ in most of the percolate samples of lysimeter 15 (app. 11a).

0.02 to 0.25 µg/l A.I. equivalents were determined as ¹⁴CO₂ in all percolate samples of lysimeter 16 (app. 11b).

Metabolite CGA 108906:

This metabolite appeared in all percolate samples of both lysimeters in concentrations from 0.01 to 1.89 µg A.I. equivalents/l (app. 11a, b).

Metabolite CGA 62826:

This metabolite appeared in all percolate samples of both lysimeters in concentrations from 0.40 to 6.64 µg A.I. equivalents/l (app. 11a, b).

7.1.3 Unidentified radioactivity in individual leachates of the 1st year

Lysimeter 15: The amount of radioactivity which could not be identified because of loss or unextractability ranged between no unidentified radioactivity and 0.78 µg A.I. equivalents/l (app. 11a).

Lysimeter 16: The amount of radioactivity which could not be identified because of loss or unextractability ranged between no unidentified radioactivity and 0.55 µg A.I. equivalents/l (app. 11b).

7.1.4 Average concentration of active ingredient (¹⁴C-metaxyl) in the total leachate of the 1st year

Average concentration of the A.I. was 0.01 µg/l (lys. 15) and no A.I. was detected in lysimeter 16 (app. 11c).

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

¹⁴CO₂:

0.11 (lys. 15) and 0.11 (lys. 16) µg A.I. equivalents/l (app. 11c).

CGA 108906:

0.48 (lys. 15) and 0.76 (lys. 16) µg A.I. equivalents/l (app. 11c).

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CGA 62826:

3.86 (lys. 15) and 2.42 (lys. 16) μg A.I. equivalents/l (app. 11c).

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

The amount of non-extracted (not determined polar) radioactivity or lost during extraction procedure was 0.17 $\mu\text{g}/\text{l}$ (lysimeter 15) and 0.14 $\mu\text{g}/\text{l}$ (lysimeter 16) (app. 11c).

7.2 Soil

No intermediate soil sampling was carried out in this experiment.

7.3. Plants

For details concerning the cultivation of vine in the lysimeters see appendix 6a, b.

7.3.1 Total radioactivity in plants

Leaves and wood

3.05 % (lys. 15) and 2.82 % (lys. 16) of the radioactivity applied to the vine were detected in the total amount of leaves at the time of grape harvest (app. 13a).

The wood collected in the course of cutting procedure of the vines was investigated also and contained 0.07 (lys. 15) and 0.04 % of the radioactivity applied (app. 13a).

Grapes

The total amount of grapes of each lysimeter was mixed and 100 g samples were extracted as described in app. 13b.

The total grape material contained 2.94 (lys. 15) and 1.06 % of the radioactivity applied. 57 days after the last application (time of grape harvest). 61.25 % (lys. 15) and 58.51 % of the radioactivity in the grapes represented the unchanged test substance. The metabolites CGA 108906 and CGA 62826 also were detected in small concentrations (app. 13c, 14a, 14b)

8. Total radioactivity

Because intermediate soil sampling was carried out, no balance could be calculated up to now.

9. Analysis conditions

Determination of ^{14}C :

Four to six aliquots of 0.5 g air-dried and homogenized plant material were mixed with cellulose and combusted (Sample Oxidizer 406, Packard Tec.). The $^{14}\text{CO}_2$ 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 scanned with a Linear Analyser (LB 283 Berthold) and evaluated with the help of a data processing system (1-Dimensional Chroma version 7.25, Berthold on Compaq 386). For TLC-conditions see appendix 10b.

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Extraction of percolate samples:

1000 ml of percolate were extracted as described in app. 10a. The determination limit was 0.05 $\mu\text{g/l}$. The detection limit was 0.01 $\mu\text{g/l}$. For A.I. concentrations between determination and detection limit the base for further calculations was the half of the determination limit (0.025 $\mu\text{g/l}$). The recovery rate of this method was 110 and 100% for the A.I.

Extraction of grape samples:

The extraction scheme for grapes is given in app. 13b. Because extraction of grapes was mainly used to obtain the ^{14}C balance in the vine no recovery rate was carried out and no determination and detection limits were fixed.

IV DISCUSSION

1. Percolate

No major differences between the two lysimeters (app. 11c) were observed with respect to the total amount of percolate. Only in the first leachates after applicaiton small amounts of the A.I. were found in lysimeter 15 indicating that preferential flow may be possible under these experimental conditions whereas no test substance appeared in the leachates of lysimeter 16. The metabolites CGA 108906 and CGA 62826 were found in each percolate sample of both lysimeters in mean concentrations significantly above 0.1 $\mu\text{g/l}$. This metabolites must be classified as leachable compounds. Part of the total radioactivity in the percolate was very polar and could not be identified. Also $^{14}\text{CO}_2$ was determined in the percolate indicating the mineralization of the labelling position of the A.I. in the soil.

2. Plants

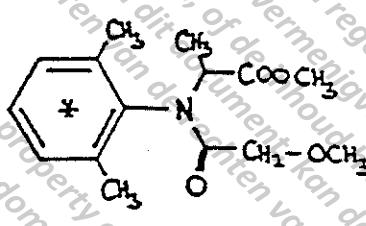
The grapes harvested 57 days after the last application and the leaves collected up to 91 days after the last application contained together 6.0 (lys. 15) and 3.9 % (lys. 16) of the ^{14}C applied. In the wood investigated only negligible amounts of radioactivity were detected. Although the radioactivity was applied to the plants the amounts detected at the time of grape harvest were low. No intermediate soil sampling was carried out but the results obtained from the percolate investigation showed that wash off by rain or dew was of importance.

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.

app. 01a

Test substance, 1st year
lysimeter 15 and 16

test substance (active ingredient)	^{14}C -CGA 48'988
common name	Metalaxyl
chemical name (IUPAC)	D,L-N-(2,6 dimethyl- ^{14}C (U)phenyl)-N-(2,-methoxyacetyl)-alanin-methylester
empirical formula	$\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}$
structural formula	 <p>* = [U-^{14}C]</p>
molar mass	279.34 g/mol
melting point	71.8-72.3 °C
formulation	EC- 240 in A-7192 B
carrier substance	A-7192 B containing Antarox CO 720 ricinusoil-polyglykolether 36-37 dodecyl-benzol-sulfoaere linear Ca-salt r-Butyrolacton Rest aromatic organic solvent 230
radiochem.purity	≤ 97%
spec. radioactivity	2.01 kBq/μg

app. 01b

List of reference standards

product	CGA 42447
batch no.	LU 440
amount	30 mg
purity	no declaration
certification date	no declaration
producer	no declaration

product	CGA 48988
batch no.	ANS 175/104
amount	100 mg
purity	no declaration
certification date	no declaration
producer	no declaration

product	CGA 37734
batch no.	LU 646
amount	30 mg
purity	no declaration
certification date	no declaration
producer	no declaration

app. 01c

List of reference standards

product 1st delivery	CGA 119857
batch no.	LU 1208
amount	30 mg
purity	no declaration
certification date	no declaration
producer	no declaration

product	CGA 67869
batch no.	RV-/9/2/1
amount	30 mg
purity	no declaration
certification date	no declaration
producer	no declaration

product	CGA 67868
batch no.	RV-2228/2
amount	30 mg
purity	no declaration
certification date	no declaration
producer	no declaration

app. Old

List of reference standards

product	CGA 67867
batch no.	LU 598
amount	30 mg
purity	no declaration
certification date	no declaration
producer	no declaration

product	CGA 62826
batch no.	RV-1595/3
amount	31 mg
purity	no declaration
certification date	no declaration
producer	no declaration

product	CGA 68125
batch no.	LU 603
amount	33 mg
purity	no declaration
certification date	no declaration
producer	no declaration

app. 01e

List of reference standards

Product 1st delivery	CGA 68124
batch no.	LU 602
amount	30 mg
purity	no declaration
certification date	no declaration
producer	no declaration

product	CGA 67866
batch no.	99844,8
amount	31 mg
purity	no declaration
certification date	no declaration
producer	no declaration

product 2nd delivery	CGA 68124
batch no.	LU 602
amount	30 mg
purity	99 %
certification date	April 5, 93
producer	CIBA-GEIGY Ltd Analytical Development Münchwilen, CH

app. 01f

List of reference standards

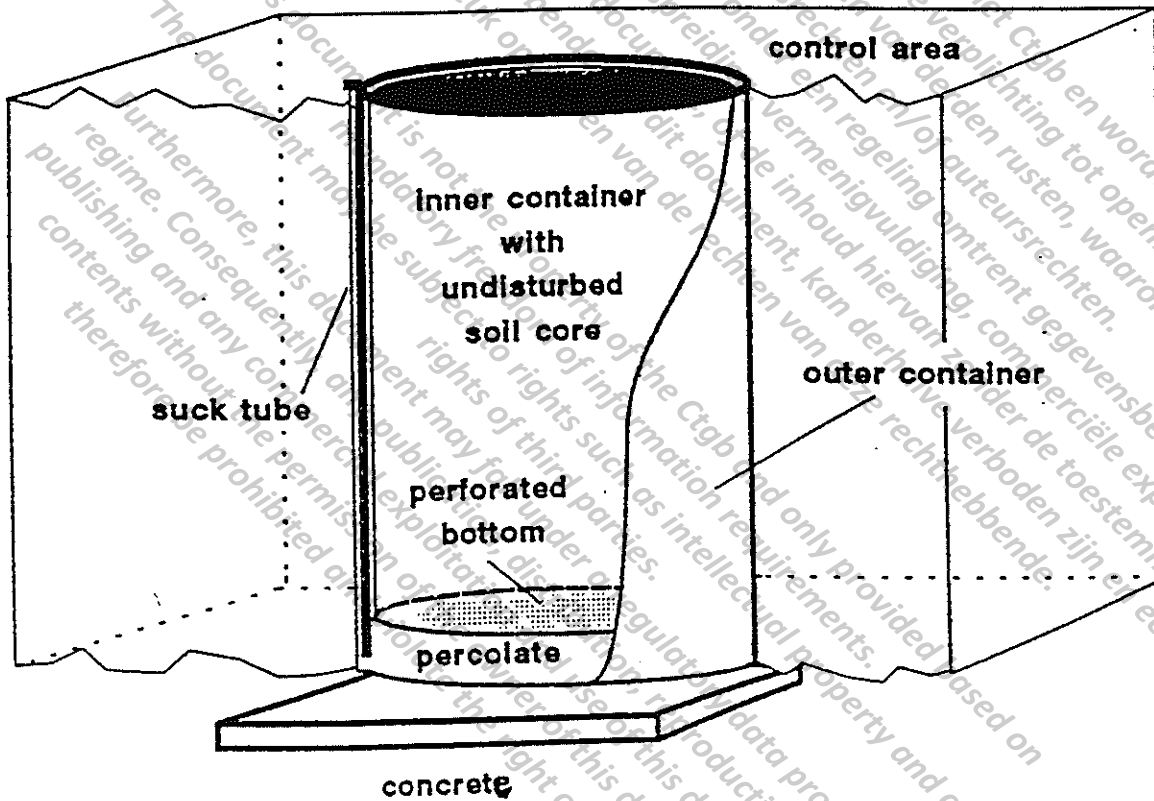
product 2nd delivery	CGA 119857
batch no.	LU 1208
amount	30 mg
purity	99 %
certification date	April 1, 93
producer	CIBA-GEIGY Ltd Analytical Develop- ment Münchwilen, CH

product	CGA 108905
batch no.	GB-XX-40D
amount	5 mg
purity	no declaration
certification date	no declaration
producer	no declaration

product	CGA 108906
batch no.	GB-XIX-127B
amount	5 mg
purity	no declaration
certification date	no declaration
producer	no declaration

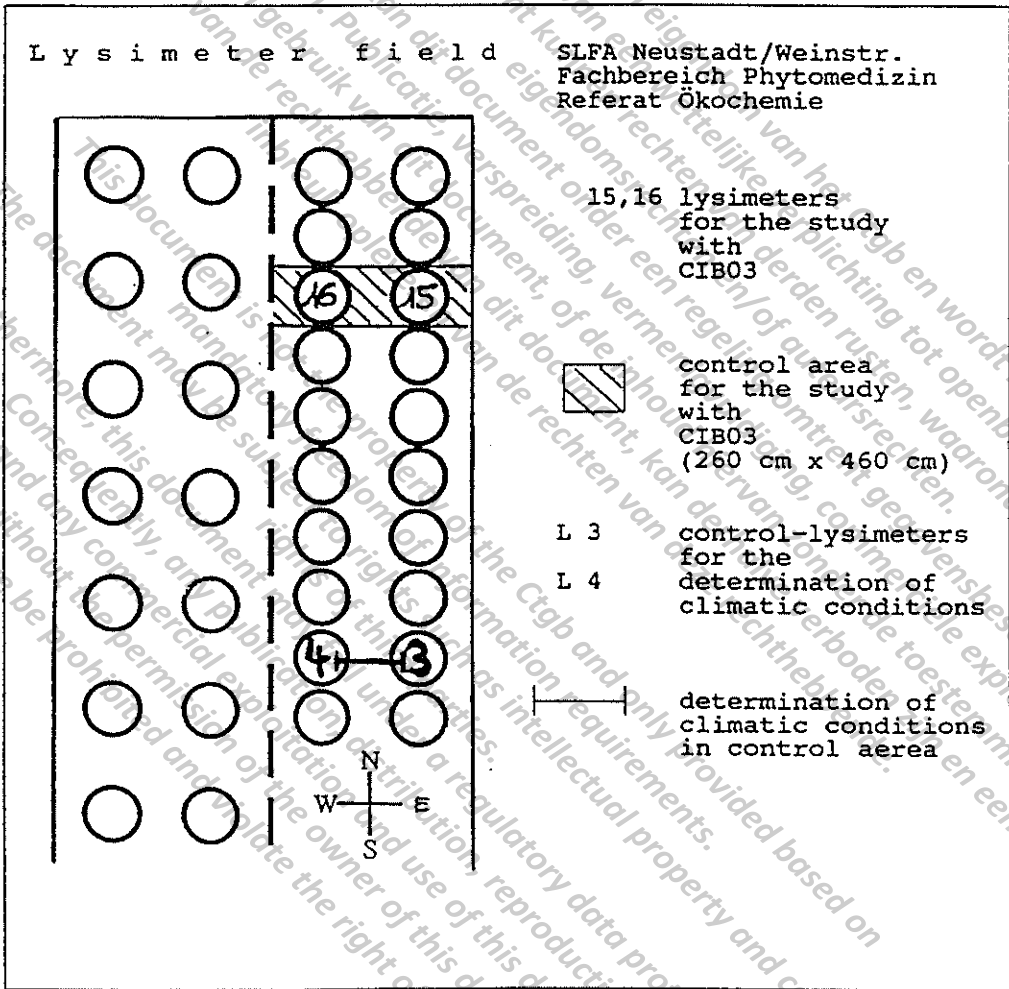
app. 02

Lysimeter with undisturbed soil profile



app. 03

Arrangement of lysimeters



app. 04

Data of test soil

soil parameter ¹⁾	0 - 30 cm	30 - 60 cm	60 - 120 cm
clay [%] (0.0-0.002 mm)	3.6	4.6	5.6
silt [%] (0.002-0.0635 mm)	15.6	15.6	12.5
sand [%] (0.063-2.0 mm)	80.8	79.7	81.6
C _{org} [%]	1.0	0.2	0.1

1) investigated by LUFA, Speyer, FRG

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app. 05a

Application details, 1st year

1st application

lysimeter number	15	16
date of application	a) June 29, 1992 b) July 14, 1992	
amount a.i./ha	a) 87.5 g* b) 92.5 g	a) 88.8 g* b) 67.5 g
amount used (a.i./lys.) and radioactivity used	a) 7.0 mg* b) 7.4 mg a) 14130.9 kBq b) 14777.9 kBq	a) 7.1 mg* b) 5.4 mg a) 14280.0 kBq b) 10803.6 kBq
kind of formulation	EC 240 (A-7192 B)	
kind of mixture 1) a.i. 2) amount of solution	1) a) 7.0 mg* b) 7.4 mg 2) a)+b) 34 ml water/lys.= 425.0 l/ha	1) a) 7.1 mg* b) 5.4 mg 2) a)+b) 34 ml water/lys.= 425.0 l/ha
application technique	sprayer AMTP 208 spray pressure 1.5 bar	
application losses	a) 13.5%	a) 16.7%
radioactivity applied	12227.0 kBq	11899.8 kBq
amount of active ingredient after deduction of appli- cation losses	6.1 mg a.i.*	5.9 mg a.i.*
	b) 9.8%	b) 16.3%
	13330.6 kBq	9045.8 kBq
	6.6 mg a.i.*	4.5 mg a.i.*
spec. radioactivity	2.01 MBq/mg	

* calculated as a.i. equivalents on the base of the spec. radioactivity (2.01 kBq/μg)

app. 05b

Application details, 1st year

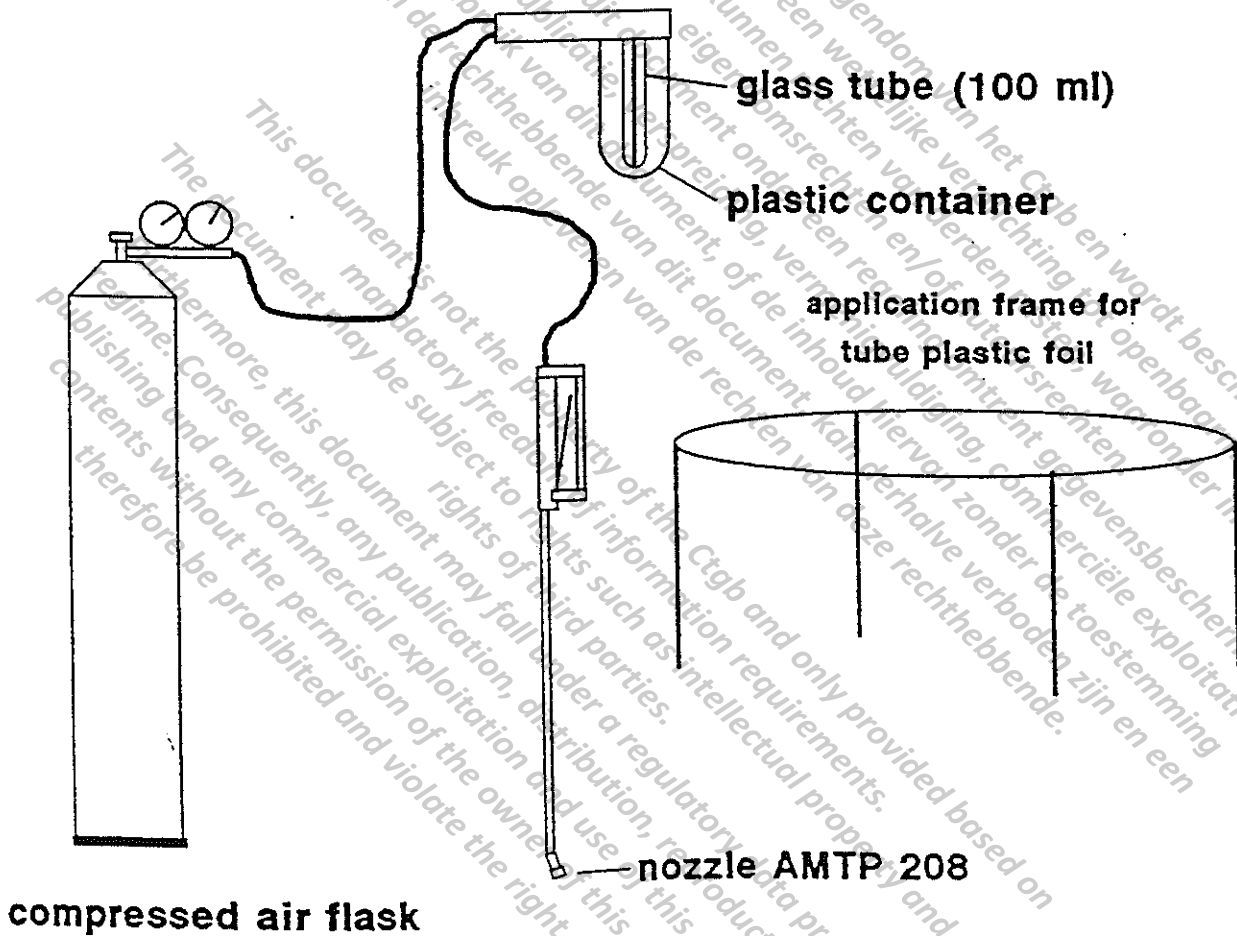
2nd application

lysimeter number	15	16
date of application	July 27, 1992	
amount a.i./ha	231.3 g*	222.5 g*
amount used (a.i./lys.) and radioactivity used	18.5 mg* 37201.6 kBq	17.8 mg* 35786.3 kBq
kind of formulation	EC 240 A-7192 B	
kind of mixture 1) a.i. 2) amount of solution	1) 18.5 mg* 2) 34 ml water/lys.= 425.0 l/ha	1) 17.8 mg* 2) 34 ml water/lys.= 425.0 l/ha
application technique	sprayer AMTP 208 spray pressure 1.5 bar	
application losses radioactivity applied	10.6% 33250.3 kBq	9.9% 32238.7 kBq
amount of active ingredient after deduction of appli- cation losses	16.5 mg a.i.*	16.0 mg a.i.*
spec. radioactivity	2,01 MBq/mg	

* calculated as a.i. equivalents on the base of the spec.
radioactivity (2.01 kBq/ μ g)

app. 05c

Figure of sprayer equipment



app. 06b

Cultivation of the lysimeter, 1st year

2) Fertilization

date of fertilizing	fertilizer	kg nutrient/ha	g nutrient/lysimeter
April 08,92	KAS (27% N)	40.0 N	3.2 N
March 08,93	Ammoniumsulfat-salpeter (25% N)	53.8 N	4.3 N
	Superphosphat (18% P ₂ O ₅)	90.0 P ₂ O ₅	7.2 P ₂ O ₅
	Kaliummagnesia (30:10)	450.0 K ₂ O 150.0 MgO	36.0 K ₂ O 12.0 MgO

3) Plant protection

date	kind of pesticide	trade name	concentration
June 24,92	fungicide	Topas	0.015%
June 14,93	fungicide	Topas	0.015%
July 08,93	fungicide	Topas	0.045%

app. 07b

Experimental conditions, 1st year

2a) Precipitation and irrigation

July 1992 - February 1993

month	precipitation [mm]	irrigation		
		total [mm]	single [mm]	date
July 92	42.0	-	-	
August 92	37.0	19.0	10.0	Aug. 3, 92
			9.0	Aug. 4, 92
September 92	25.0	49.0	10.0	Sep. 21, 92
			10.0	Sep. 22, 92
			10.0	Sep. 23, 92
			10.0	Sep. 24, 92
			9.0	Sep. 25, 92
October 92	61.0	39.0	10.0	Oct. 21, 92
			10.0	Oct. 23, 92
			10.0	Oct. 26, 92
			10.0	Oct. 31, 92
			9.0	
November 92	71.0	-	-	
December 92	38.0	-	-	
January 93	27.0	23.0	5.0	Jan. 13, 93
			10.0	Jan. 15, 93
			6,3	Jan. 26, 93
			1,7	Jan. 29, 93
February 93	9.0	46.0	3.8	Feb. 03, 93
			10.0	Feb. 19, 93
			10.0	Feb. 22, 93
			10.0	Feb. 24, 93
			6.0	Feb. 25, 93
			6.2	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
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			
Mai 93	60.0	41.0	7.5	Mai 12, 93
			5.0	Mai 24, 93
			10.0	Mai 25, 93
			10.0	Mai 27, 93
			8.5	Mai 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 year	422.0	339.0		761.0 mm

app. 08

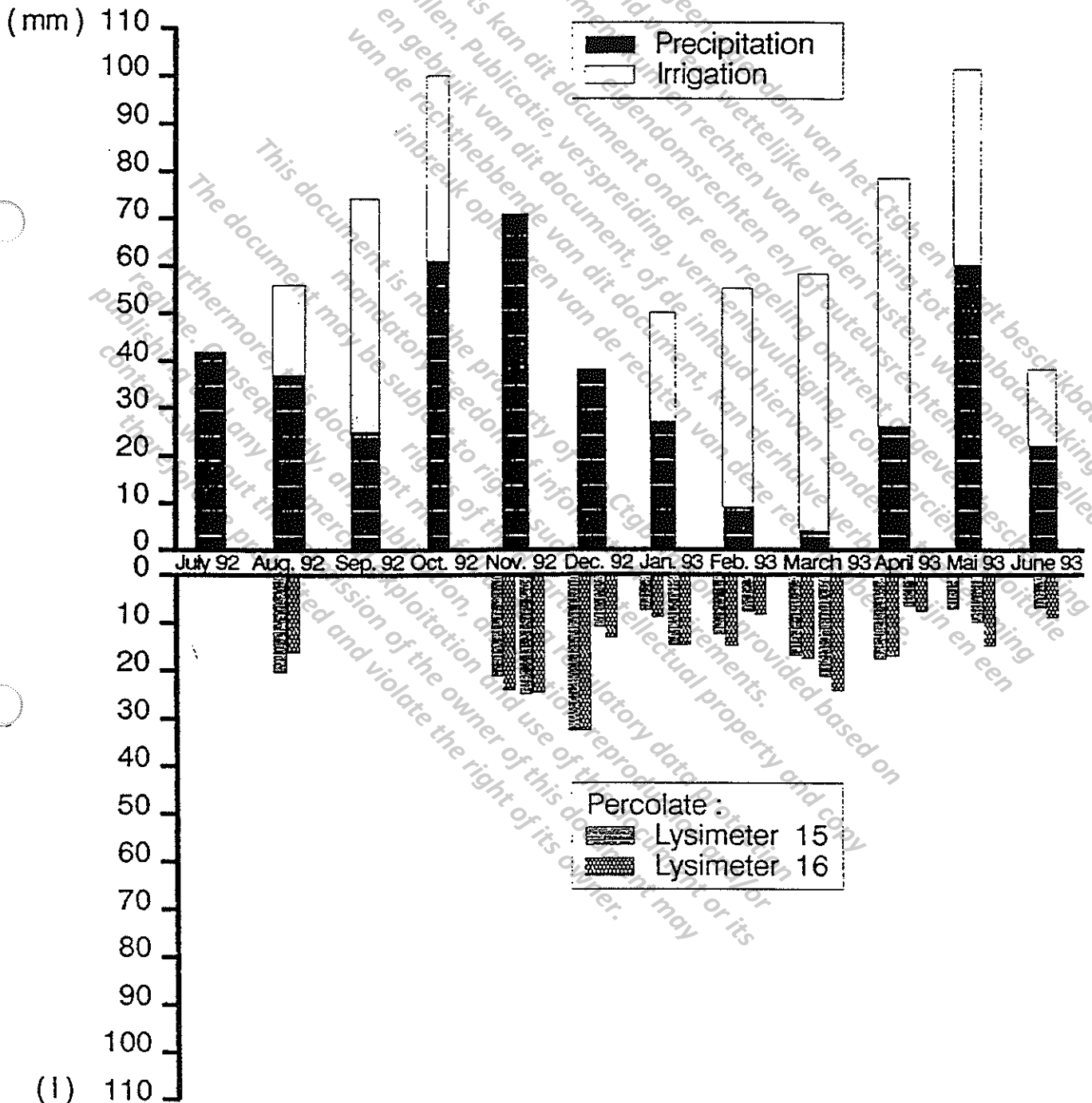
Sampling of percolate, 1st year

date of sampling	15	16
July 02, 1992	-1)	-1)
Aug. 03, 1992	22.1	15.7
Sep. 03, 1992	-1)	-1)
Oct. 05, 1992	-1)	-1)
Nov. 02, 1992	20.8	23.7
Nov. 19, 1992	24.5	24.2
Dec. 08, 1992	32.4	32.5
Dec. 12, 1992	10.6	12.9
Jan. 04, 1993	7.2	8.6
Jan. 18, 1993	14.6	14.4
Feb. 01, 1993	12.1	14.5
Feb. 15, 1993	7.4	7.9
March 01, 1993	16.6	17.2
March 16, 1993	21.0	23.9
April 06, 1993	17.5	16.7
April 19, 1993	6.0	7.1
May 03, 1993	6.6	-1)
May 19, 1993	9.6	14.5
June 02, 1993	6.6	8.5
total percolate	235.6	242.3

1) percolate volume < 3 l

app. 09

Precipitation, irrigation and percolate
(first year)
lysimeter 15 and 16



(1)

app. 10a

Extraction scheme, percolate

Acidified 1000 ml percolate with 6 N H₂SO₄ to pH 2

*

Activate a C₁₈-cartouche with methanol and water

*

Clean up the percolate over the cartridge

*

¹⁴C-measurement from the eluated water

*

Desiccate the cartouche for 30 min with nitrogen

*

Eluate the cartridge with 4 * 500 µl methanol

*

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	103.9
1.00	A 1.0	1.0	100.0
	B 1.0	1.0	100.0
0.05	A 0.10	0.11	110.0
	B 0.10	0.10	100.0

app. 10b

TLC conditions

solvent	chloroform : carbon tetrachloride : 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 : acetonitrile B : ammoniumbicarbonate 0.1%																					
gradient profile : <table border="1"> <thead> <tr> <th>time</th> <th>flow (ml/min)</th> <th>%B</th> </tr> </thead> <tbody> <tr><td>0</td><td>1.5</td><td>0</td></tr> <tr><td>5</td><td>1.5</td><td>10</td></tr> <tr><td>10</td><td>1.5</td><td>5</td></tr> <tr><td>21</td><td>1.5</td><td>90</td></tr> <tr><td>30</td><td>1.5</td><td>0</td></tr> <tr><td>35</td><td>1.5</td><td>0</td></tr> </tbody> </table>	time	flow (ml/min)	%B	0	1.5	0	5	1.5	10	10	1.5	5	21	1.5	90	30	1.5	0	35	1.5	0
time	flow (ml/min)	%B																			
0	1.5	0																			
5	1.5	10																			
10	1.5	5																			
21	1.5	90																			
30	1.5	0																			
35	1.5	0																			
detector : LB 506 C (Berthold)																					
R _T -a.i. : 18m38s-18m48s																					

R_T-Metabolites

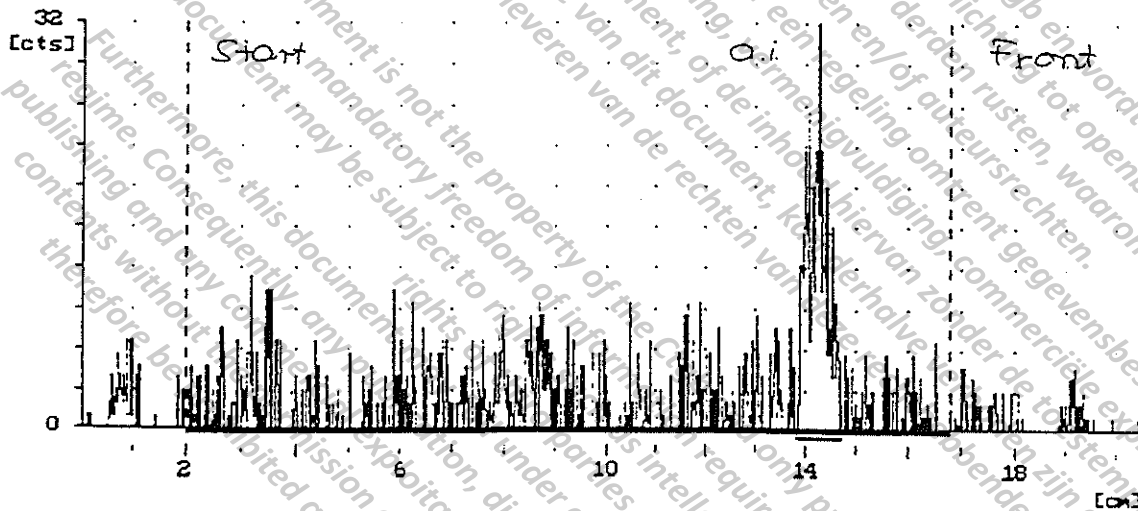
metabolite number	R _T -value
CGA 108906	1m36s-1m54s
CGA 62826	14m10s-14m28s

app. 10c

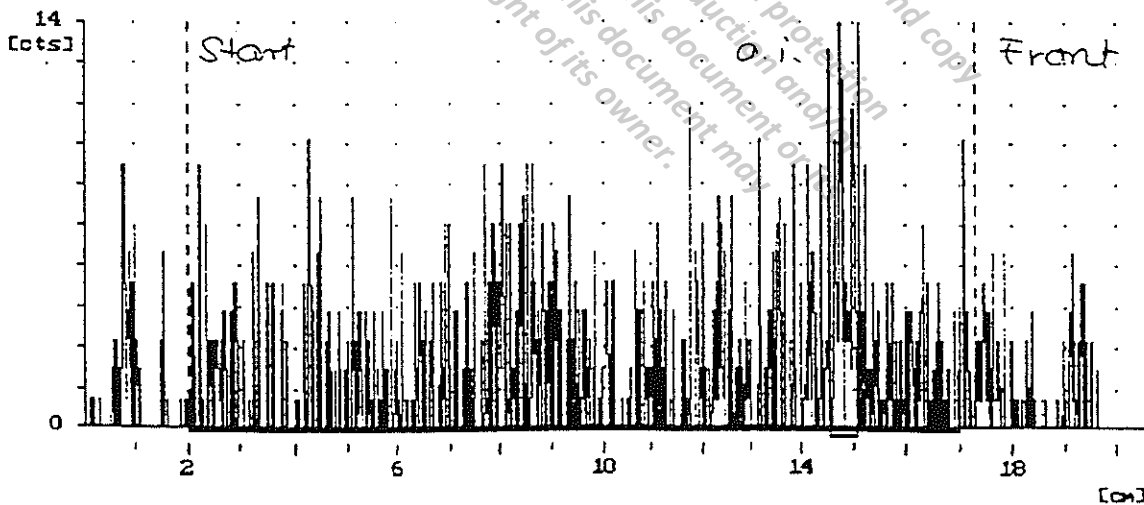
Determination and detection limits for the a.i.
in percolate sampling by TLC

Solvent : chloroform : carbon tetrachloride : ethanol = 3:1:1
Plate : silicagel 60F₂₅₄

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



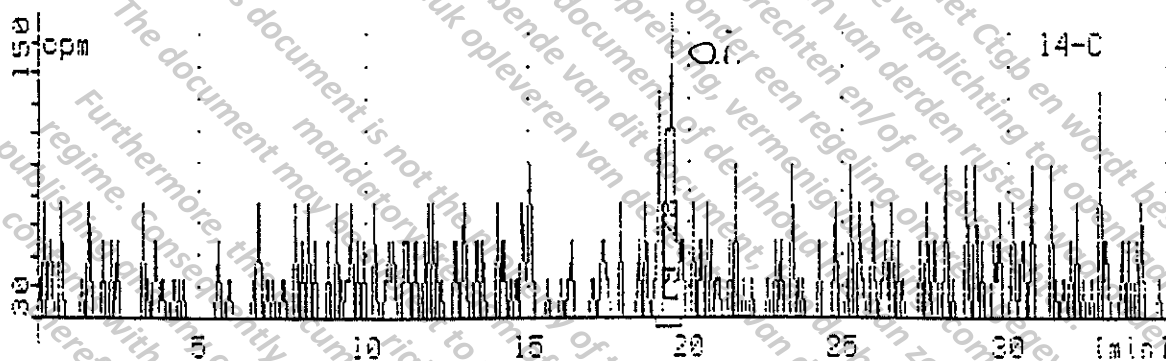
b) Chromatogram of the detection limit (0.01 µg/l)



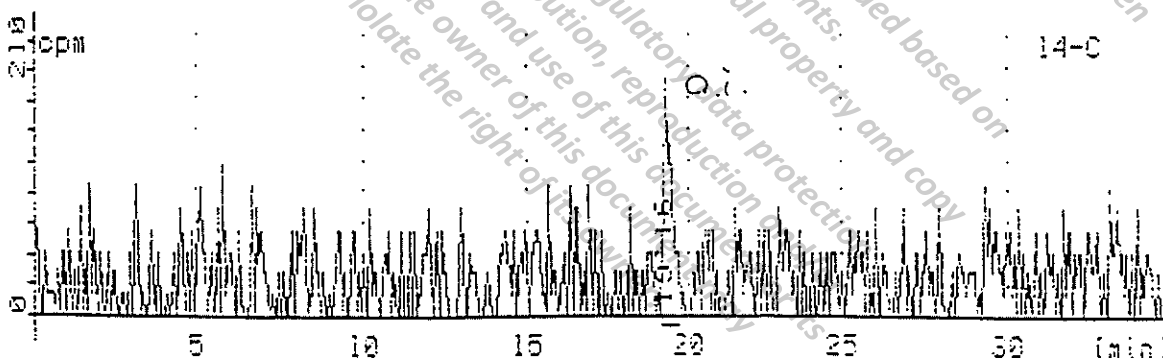
app. 10d

Determination and detection limits for the a.i.
in percolate sampling by HPLC

a) Chromatogram of the determination limit ($0.05 \mu\text{g}/\text{l}$)



b) Chromatogram of the detection limit ($0.01 \mu\text{g}/\text{l}$)



app. 11a

Percolate investigation, 1st year
Results of lysimeter 15

date of sampling	radioactivity in the percolate			¹⁴ CO ₂ rep. [ug/l]	not extracted = loss 3) n.i. [ug/l]	e x t r a c t e d		
	[1] [kBq/l]	[ug/l]	[ug/l]			metabolites CGA 108906 [ug/l]	CGA 62826 [ug/l]	active ingredient [ug/l]
July 02,92	-1)							
Aug. 03,92	22.1	-	-					
Sep. 03,92	-1)							
Oct. 05,92	-1)							
Nov. 02,92	20.8	2.79	1.39	0.02	a 0.06 b 0.49	0.13 0.05	1.18 0.79	- 0.05
Nov. 19,92	24.5	4.72	2.35	0.02	a 0.02 b 0.46	0.32 0.06	1.96 1.78	0.03 0.03
Dec. 08,92	32.4	4.99	2.48	0.02	a - b 0.06	0.35 0.29	2.12 2.11	- -
Dec. 18,92	10.6	14.13	7.03	0.09	a 0.60 b 0.09	0.52 0.81	5.83 6.05	- -
Jan. 04,93	7.2	15.53	7.73	0.12	a 0.30 b 0.18	0.77 0.79	6.54 6.64	- -
Jan. 18,93	14.6	13.27	6.60	0.11	a 0.03 b -	0.75 0.70	5.71 5.89	- -
Feb. 01,93	12.1	13.68	6.81	0.14	a - b -	1.29 1.31	5.47 5.36	- -
Feb. 15,93	7.4	13.66	6.80	0.16	a 0.02 b 0.04	0.70 0.81	5.92 5.79	- -
March 01,93	16.6	13.47	6.70	0.21	a 0.52 b 0.65	0.84 0.71	5.13 5.13	- -
March 16,93	21.0	14.23	7.08	0.20	a 0.42 b 0.21	0.52 0.54	5.94 6.13	- -
April 06,93	17.5	14.32	7.12	0.25	a - b -	0.69 0.59	6.27 6.37	- -
April 19,93	6.0	14.25	7.09	0.22	a 0.47 b 0.78	0.64 0.50	5.75 5.57	- -
May 03,93	6.6	12.84	6.39	0.27	a - b -	0.63 0.58	5.59 5.67	- -
May 19,93	9.6	11.58	5.76	0.49	a - b -	0.52 0.49	4.91 4.92	- -
June 02,93	6.6	10.47	5.21	0.51	a - b -	0.49 0.51	4.42 4.45	- -

1) percolate volume < 3l
2) calculated as a.i. equivalents on the base of the spec. radioactivity (2.01 kBq/μg)
3) n.i. not identified
"- " below detection limit of 0.01 μg/l

app. 11b

Percolate investigation, 1st year

Results of lysimeter 16

date of sampling	radioactivity in the percolate			¹⁴ CO ₂ rep. [ug/l]	not extracted = loss n.i. 3) [ug/l]	e x t r a c t e d		
	[1] [kBq/l]	[ug/l]	[ug/l]			metabolites CGA 108906 [ug/l]	CGA 62826 [ug/l]	active ingredient [ug/l]
July 02,92	-1)							
Aug. 03,92	15.7	-	-					
Sep. 03,92	-1)							
Oct. 05,92	-1)							
Nov. 02,92	23.7	1.01	0.50	0.02	a 0.03 b 0.03	0.05 0.01	0.40 0.43	- -
Nov. 19,92	24.2	3.54	1.76	0.02	a 0.05 b 0.08	0.44 0.47	1.25 1.19	- -
Dec. 08,92	32.5	4.26	2.12	0.03	a 0.05 b 0.05	0.71 0.69	1.32 1.56	- -
Dec. 18,92	12.9	10.30	5.12	0.08	a 0.10 b 0.02	1.59 1.54	3.35 3.48	- -
Jan. 04,93	8.6	11.36	5.65	0.12	a 1.00 b 0.29	1.50 1.86	3.04 3.38	- -
Jan. 18,93	14.4	11.90	5.92	0.18	a 0.11 b 0.17	1.86 1.89	3.77 3.68	- -
Feb. 01,93	14.5	11.89	5.92	0.16	a 0.15 b 0.15	1.76 1.71	3.85 4.07	- -
Feb. 15,93	7.9	11.82	5.88	0.17	a 0.17 b 0.02	1.23 1.30	4.52 4.40	- -
March 01,93	17.2	10.75	5.35	0.20	a 0.15 b 0.35	0.81 0.70	4.19 4.10	- -
March 16,93	23.9	9.38	4.67	0.19	a 0.45 b 0.55	0.68 0.57	3.35 3.35	- -
April 06,93	16.7	8.33	4.14	0.18	a 0.18 b 0.09	0.65 0.72	3.13 3.15	- -
April 19,93	7.1	7.83	3.90	0.19	a 0.03 b 0.16	0.69 0.59	2.98 2.95	- -
May 03,93	-1)							
May 19,93	14.5	6.80	3.38	0.25	a 0.06 b 0.05	0.46 0.45	2.62 2.64	- -
June 02,93	8.5	6.21	3.09	0.22	a 0.06 b 0.26	0.41 0.33	2.40 2.29	- -

1) percolate volume < 3l
 2) calculated as a.i. equivalents on the base of the spec. radioactivity
 3) (2.01 kBq/μg)
 n.i. not identified
 "-" below detection limit of 0.01 μg/l.

app. 11c

Percolate analysis 1st year/total

	lysimeter 15			lysimeter 16		
radioactivity applied	58807.9 kBq			53184.3 kBq		
total amount of percolate	235.6 l			242.3 l		
¹⁴ C-carbonate	kBq	% ²⁾	µg/l ³⁾	kBq	% ²⁾	µg/l ³⁾
	63.1	0.11	0.13	57.7	0.11	0.12
loss: not extractable not identified	78.3	0.13	0.17	69.0	0.13	0.14
CGA 108906	225.6	0.38	0.48	369.0	0.69	0.76
CGA 62826	1828.8	3.11	3.86	1179.9	2.22	2.42
active ingredient	2.8	<0.01	0.01	-	-	-
total activity ¹⁾	2198.6	3.73	4.65	1675.6	3.15	3.44

- 1) ¹⁴C-balance sheet
- 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

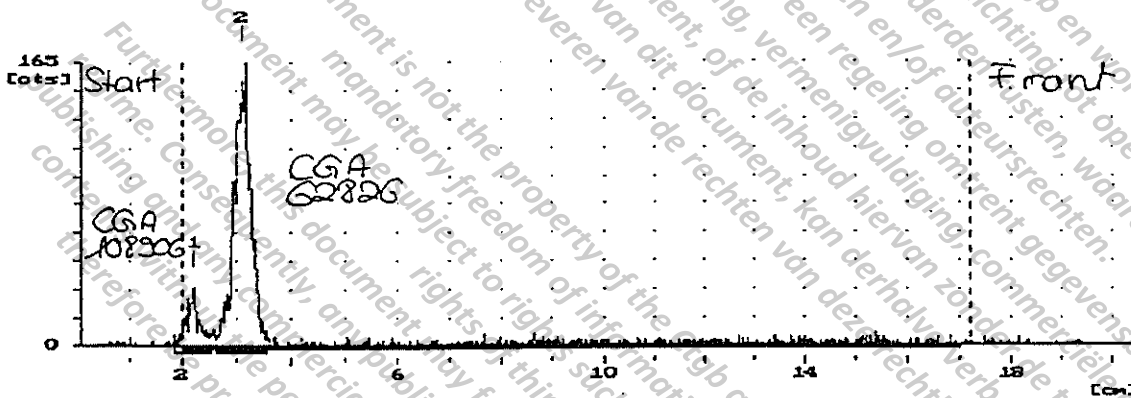
app. 12a

Percolate investigation, 1st year

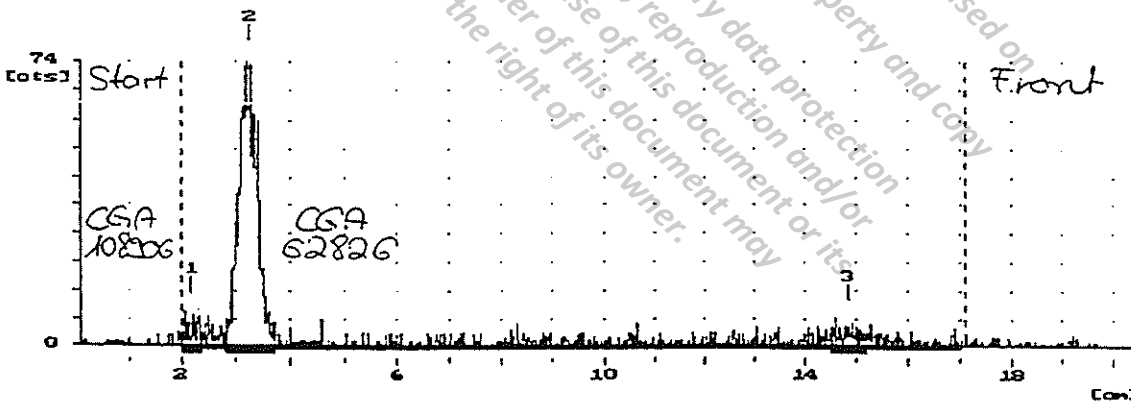
Examples of TLC analyses

Solvent : chloroform : carbon tetrachloride : ethanol = 3:1:1
Plate : silicagel 60F₂₅₄

a) Chromatogram of lysimeter 15, percolate of November 02, 1992



b) Chromatogram of lysimeter 16, percolate of November 02, 1992

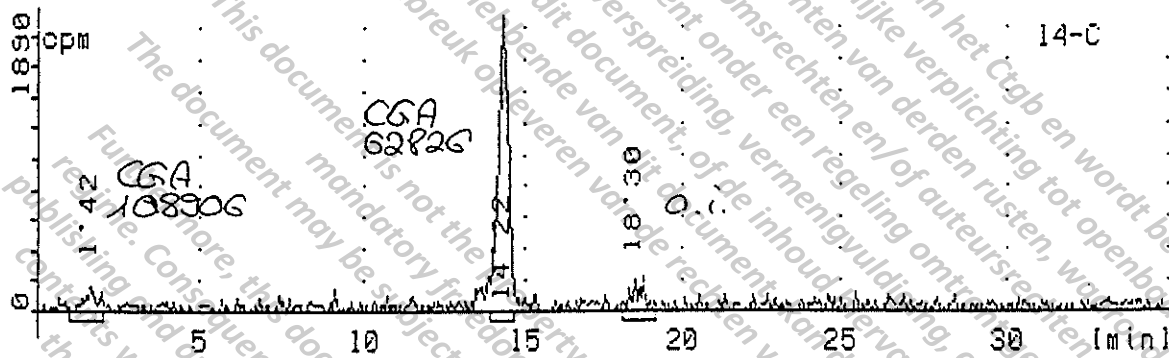


app. 12b

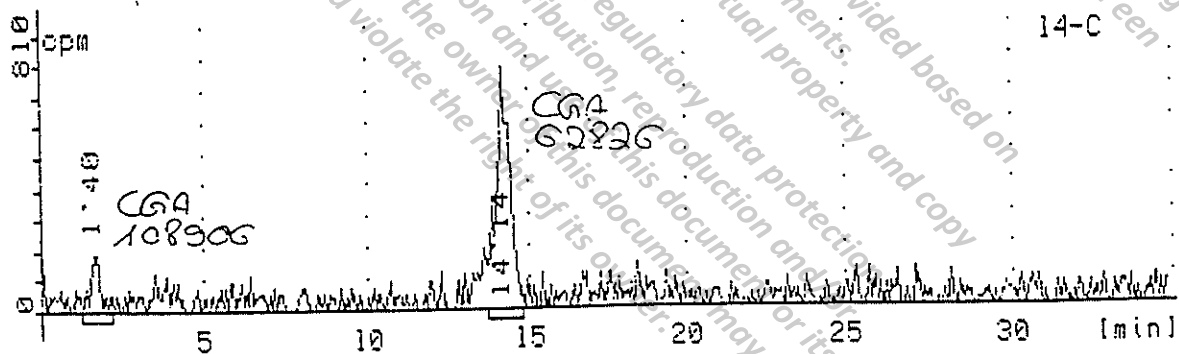
Percolate investigation, 1st year

Examples of HPLC analyses

a) Chromatogram of lysimeter 15, percolate of November 02, 1992



b) Chromatogram of lysimeter 16, percolate of November 02, 1992



app. 13a

Plant investigation, 1st year

¹⁴C-balance sheet

lysimeter 15

plant fraction	dry weight g	kBq	% ¹⁾	mg/kg ³⁾
leaves	105.0	1795.6	3.05	8.51
wood	71.4	43.6	0.07	0.30

lysimeter 16

plant fraction	dry weight g	kBq	% ²⁾	mg/kg ³⁾
leaves	120.0	1501.4	2.82	6.20
wood	73.6	22.2	0.04	0.15

- 1) radioactivity applied (58807.9 kBq) = 100 %
- 2) radioactivity applied (53184.3 kBq) = 100 %
- 3) calculated on the base of the spec. radioactivity of the a.i.
(2010.0 kBq/mg)

app. 13b

Extraction scheme, grapes

Homogenize 100 g of grapes for 5 minutes in 100 ml
acetonitrile/water (8:2; vol/vol)

Centrifuge for 5 minutes (5000 rpm) and filtrate

Repeat the procedure twice using 50 ml of acetonitrile/water (8:2; vol/vol) and
combine the liquid phases

Evaporate to the water phase

Clean up the water phase using a activated C₁₈-column

Determine radioactivity in the eluated water

Dessicate the C₁₈-column for 30 min using nitrogen

Eluate the column with 4 x 500 µl methanol

Concentrate the extract to 1 ml for HPLC and TLC analysis

app. 13c

Grape extraction balance, 1st year
lysimeter 15 and 16

lysimeter	15				16			
¹⁴ C applied	58807.9 kBq				53184.3 kBq			
grape weight	0.992 kg				0.523 kg			
100g extracted								
methanol extract	kBq	μg/kg ¹⁾	% ²⁾	% ³⁾	kBq	μg/kg ¹⁾	% ²⁾	% ³⁾
a.i.	106.88	531.74	61.25	1.80	62.54	311.14	58.51	0.62
metabolites								
CGA 108906	3.94	19.60	2.26	0.07	2.81	13.98	2.63	0.03
CGA 62826	11.49	57.16	6.58	0.19	6.24	31.04	5.84	0.06
not identified metabolites	17.02	84.68	9.75	0.29	7.83	38.96	7.33	0.08
total ¹⁴ C extracted	139.33	693.18	79.84	2.35	79.42	395.12	74.31	0.79
not extracted solid phase	16.26	80.90	9.32	0.27	8.54	42.49	7.99	0.08
not extracted fluid phase	18.92	94.13	10.84	0.32	18.92	94.13	17.70	0.19
total	174.51	868.21	100.00	2.94	106.88	531.74	100.00	1.06

- 1) calculated on the base of the spec. radioactivity of the a.i. (2.01 kBq/μg)
- 2) radioactivity in the grape sample = 100 %
- 3) extraction results extrapolated to the total grape weight and related to the radioactivity applied (100%)

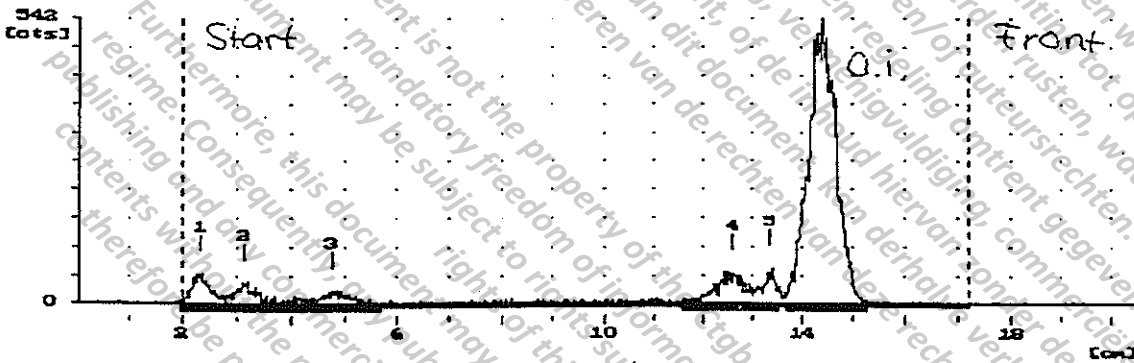
app. 14a

Grape investigation, 1st year

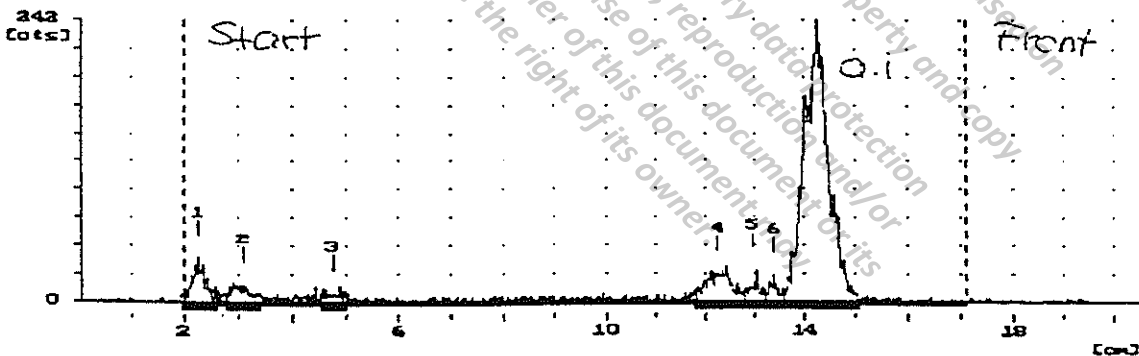
Examples of TLC analyses

Solvent : chloroform : carbon tetrachloride : ethanol = 3:1:1
Plate : silicagel 60F₂₅₄

a) Chromatogram of lysimeter 15, grapes of September 22, 1992



b) Chromatogram of lysimeter 16, grapes of September 22, 1992

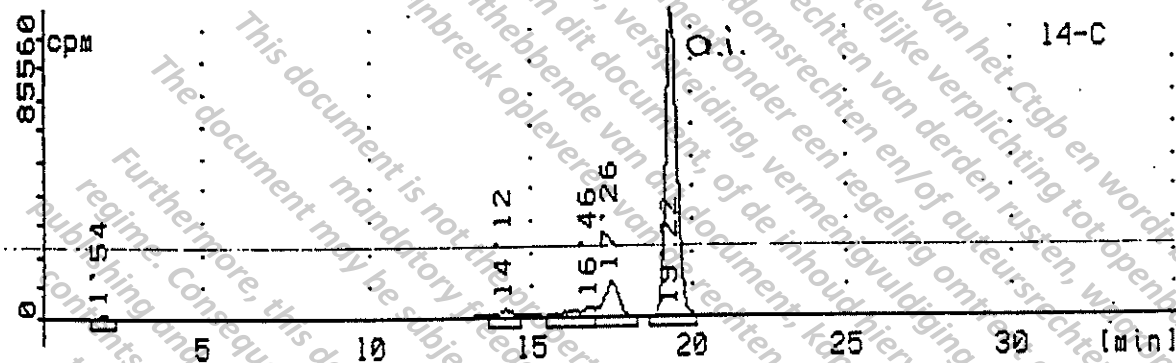


app. 14b

Grape investigation, 1st year

Examples of HPLC analyses

a) Chromatogram of lysimeter 15, grapes of September 22, 1992



b) Chromatogram of lysimeter 16, grapes of September 22, 1992

