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Report on Study

CGA 329351

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STUDY TITLE

Aqueous Photolysis of ^{14}C-CGA 329351 at pH 7 under Artificial Sunlight Conditions

DATA REQUIREMENT

Environmental Fate Subdivision N, EPA-540/9-82-021, Section 161-2: Photodegradation Studies in Water; US Environmental Protection Agency, October 18, 1982

AUTHOR

Dr. 5.1.2.e Wo

STUDY COMPLETED ON

December 20, 1995

PERFORMING LABORATORY

CIBA-GEIGY Limited
Crop Protection Division
Product Safety
Ecochemistry
CH-4002 Basle, Switzerland

LABORATORY PROJECT IDENTIFICATION

Project Number :95EH04

SPONSOR

CIBA-GEIGY Limited
Crop Protection Division
Product Safety
Ecochemistry
CH-4002 Basle, Switzerland

Total Number of Pages: 49

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE

Project	95EH04
Test Substance	[U-14]-C-phenyl-ring labelled CGA 329351
Study Director	Dr. [redacted]
Study Title	Aqueous Photolysis of ^{14}C-CGA 329351 at pH 7 under artificial Sunlight Conditions

This study was performed in compliance with Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986 {Verfahren und Grundsätze der Guten Laborpraxis (GLP) in der Schweiz, März 1986} issued by the Swiss Federal Department of Interior and the Intercantonal Office for the Control of Medicaments, Switzerland. These procedures are based on the OECD Principles of GLP adopted on May 12, 1981 by Decision of the OECD Council concerning the Mutual Acceptance of Data in the Assessment of Chemicals {C(81)30(Final)}.

December 20, 1995
Date

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Study Director
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Ecochemistry
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Quality Assurance Statement

Ciba-Geigy Ltd., GLP Quality Assurance Product Safety, 4002 Basel

Project	95EH04
Test Substance	CGA 329351
Study Title	Aqueous Photolysis of 14C-CGA 329351 at pH 7 under Artificial Sunlight Conditions
Study Director	Dr. 5.1.2.e Woo
QA-Inspector	5.1.2.e Woo

I hereby certify that the following Quality Assurance activities were performed:

Activity	Performed	Reported
Protocol Audit	June 16, 1995	June 16, 1995
Study Related Inspection	June 20, 1995	June 20, 1995
Facility Inspection	September 26, 1995	October 05, 1995
Final Report Audit	November 30, 1995	December 18, 1995

December 20, 1995
Date

5.1.2.e Woo
Inspector Quality Assurance

Form. QSSTAT12

GENERAL INFORMATION

Study Identification / Key Study Personnel

Guideline	The study was conducted to satisfy the: Environmental Fate Subdivision N, EPA-540/9-82-021, Section 161-2: Photodegradation Studies in Water; US Environmental Protection Agency, October 18, 1982 EC-Directive 91/414/EEC; Annex II, 7. Fate and Behaviour in the Environment; 7.2.1.2 Photochemical Degradation
Test Substance	Company Code: CGA 329351
Proposed Use	Herbicide
Project Number	95EH04
Sponsor	CIBA-GEIGY Limited Plant Protection Division CH-4002 Basle, Switzerland Product Safety Ecochemistry
represented by	Dr. 5.1.2.e Woo
Study Director[1]	Dr. CIBA-GEIGY Limited, Crop Protection Division Product Safety Ecochemistry CH-4002 Basle, Switzerland
Technical Personnel	5.1.2.e Woo (principal co-worker)
Testing Facilities	CIBA-GEIGY Limited, Crop Protection Division Product Safety Ecochemistry CH-4002 Basle, Switzerland
Supplier of unlabelled Test and Reference Substances	CIBA-GEIGY Limited, Crop Protection Division Product Safety Chemistry Support CH-4002 Basle, Switzerland Dr. 5.1.2.e Woo

[1] The job descriptions and the professional curricula vitae for all personnel participating in this study are archived in the testing facilities.

Supplier of the ^{14}C labelled Test Substance	CIBA-GEIGY Limited Crop Protection Division Research Services /Chemistry Support Isotope Laboratory CH-4002 Basle, Switzerland Dr. 5.1.2.e Woo
Testing Period	Study Initiation Date: June 13, 1995 Experimental Starting Date: June 19, 1995 Experimental Termination Date: July 7, 1995 Study Completion Date: December 20, 1995
Archives	Protocols, raw data, correspondence, and the final report are archived for at least ten years at: CIBA-GEIGY Limited, Crop Protection Division Product Safety Ecochemistry CH-4002 Basle, Switzerland
Quality Assurance	CIBA-GEIGY Limited, Crop Protection Division Product Safety/PS Services Quality Assurance CH-4002 Basle, Switzerland
represented by	Mrs. 5.1.2.e Woo
Integrity of the Study	No circumstances were observed affecting the integrity of the study.
Signature for the Report	December 20, 95 .... 5.1.2.e Woo .... Date: Dr. 5.1.2.e Woo (Author) CIBA-GEIGY Limited, Crop Protection Division Product Safety Ecochemistry CH-4002 Basle, Switzerland

LIST OF AMENDMENTS TO PROTOCOL

Amendment No.	Date	Concerning	Reason for Alteration
1	October 27, 1995	Unlabelled test substance	Update of data (batch, purity, exp. date)

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SUMMARY

The objectives of the aqueous photolysis study were to provide information on the rate of photolytic degradation of the test substance when introduced to aqueous systems, the pattern of formation and decline of photodegradation products and the nature of the major photodegradation products. For this purpose, 14C-CGA 329351, i.e. (R)-2-(N-(2,6-dimethyl-phenyl)-methoxyacetylamino)-propionic acid methyl ester labelled in the phenyl-ring was irradiated with a xenon arc light source at 25 °C under sterile conditions in aqueous buffer solution at a pH of 7. Control solutions were treated in the same way as irradiated solutions, except that they were kept in the dark. The concentration of the test substance in the buffer solution was 2.16 PPM.

The parent molecule was practically not broken down by light. After 240 hours (= 29.83 days 40°N or 30.92 days 30°N) of continuos exposure still 97.16 % of CGA 329351 were present. In unexposed samples (dark control) the amount of CGA 329351 was 96.30-98.61 % after 240 hours.

No half-life was calculated for light exposed and unexposed samples due to the insignificant degradation observed.

Besides the parent molecule only very minor amounts of degradates were observed (M1, M3-M6), ranging , on average, at the end of the study between 0.22 and 1.77 % of the radioactivity applied. Due to their insignificant amounts they were not further characterised.

In conclusion, phenyl-ring labelled CGA 329351 was not significantly degraded by light.

1. Introduction

The objectives of the aqueous photolysis study were to provide information on the rate of photolytic degradation of the test substance when introduced to aqueous systems, the pattern of formation and decline of photodegradation products and the nature of the major photodegradation products. For this purpose, ^{14}C-labelled test substance was irradiated with a xenon arc light source at 25 °C under sterile conditions in aqueous buffer solution at a pH of 7. Control solutions were treated in the same way as irradiated solutions, except that they were kept in the dark. The concentration of the test substance in the buffer solution did not exceed 50 % of its water solubility.

2. Materials and Methods

2.1. Chemicals

2.1.1. Unlabelled Test Substance [2]

Company Code	CGA 329351
Chemical Name (IUPAC)	(R)-2-(N-(2,6-dimethyl-phenyl)-methoxyacetylamino)-propionic acid methyl ester
CAS Registry Number	70630-17-0
Empirical Formula	$C_{15}H_{21}NO_4$
Molecular Weight	279.34 g/mole
Physical State at 20°C	viscous liquid
Specific rotation	α_D = -54.8°
Colour	colourless
Vapour Pressure at 20°C	2.9 x 10^{-3} Pa
Solubility (at 20 °C)	water: 26 g/l acetone: > 100% ethanol: > 100 % (w/v) acetonitrile: > 100 %

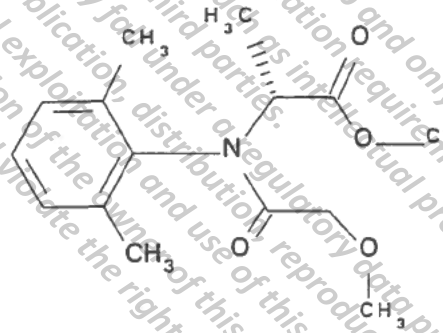
[2] Data taken from Ciba-Geigy Status Report, May 9, 1994.

Partition Coefficient n-octanol/water	log P_{OW} = 1.53 (flask-method)
pK_a-Value	< 0 (basic)
Stability	
Hydrolysis	pH 1-7 >200 days; pH 9 and 10: 115 and 12 days
Amount	0.05 g
Batch Number	AMS 758/101
Purity	99.4 %
Expiration Date	11/96
Storage Conditions	0-5 °C in the dark
Max. recommended Field Rate per Application	100 g a.i./ha
Toxicity	LD 50 rat: >200-500 mg/kg
Special Precautions	Routine hygiene procedures

2.1.2. Radiolabelled Test Substance

Structure/

(label: C-U-ring)



Amount	1.9 mg (=2.75 MBq)
Batch Number	MSR-II-89
Specific Activity	1.45 MBq/mg or 39.3 µCi/mg
Rad.-chem. purity	98.8 % as certified on data sheet (See also under.3.1)

Expiration Date 9/95

2.2. Test System

2.2.1. Aqueous System

The photolytic behaviour of the test substance was investigated in 15 ml aqueous solution buffered at pH 7 (0.01 M phosphate buffer) where the test compound is known to be hydrolytically stable. Since the starting concentration of the pesticide was less than 10-4 M, the buffers ionic strength did not exceed 0.01 M at which concentration buffer effects are negligible. The buffer was sterilised by filtration.

2.2.2. Photolysis vessels

Solutions were irradiated by xenon arc light in cylindrical vessels (sample volume = 15 ml; see Figure 2), constructed of borosilicate glass but covered with a quartz glass lid. The vessels were irradiated from above through their quartz glass lids. All vessels were fitted with inlet and outlet ports for collection of volatiles. A thermocouple was fitted to one vessel for temperature control. The vessels were irradiated in sockets of a water cooled steel tank (25 °C) placed within the photolysis apparatus (Figure 3).For the collection of volatiles all vessels were connected in line and ventilated with an air flow of about 5 ml/minute controlled by a peristaltic pump. Incoming air was passed through a water trap for humidification. The outlet air passed traps for absorbing volatiles in the sequence ethylene glycol and 2 N NaOH. Dark control solutions were exposed in the same type of vessels connected to an identical gas flow system and maintained in darkness at 25 °C temperature.

2.2.3. Irradiation equipment

The test solutions for exposure were irradiated in a "Suntest" accelerated exposure table unit, fitted with a xenon arc light source and filters to cut-off light of less than 290 nm wavelength (W.C. Heraeus GmbH, Hanau, Federal Republic of Germany; see Figure 1 and Figure 6).The spectral energy distribution (300-700 nm) of the light source, i.e. the incident light available at the water level in all vessels, and that of natural sunlight were recorded using a portable spectroradiometer, Model LI-1800, LICOR (Lincoln, NE 68504, USA). A holographic grating monochromator and a Teflon cosine diffuser either fixed or in combination with a glass fiber optic probe were used. For data collection and standardisation relative to sunlight the spectroradiometer was interfaced to a portable personal computer. The light intensities in the various exposure vessels at the beginning and end of the test is presented in Figure 5 showing a homogeneous distribution of light intensities in all exposure vessels of the corresponding Suntest apparatus.

2.3. Study procedures

2.3.1. Preparation of the test solution

The 14C-labelled test substance was dissolved in acetone (2.0 ml) transferred to a volumetric flask of 2 ml, the solvent completely evaporated under a gentle stream of nitrogen, the residual radioactivity dissolve in sterilised phosphate buffer and thereafter its accurate amount determined by liquid scintillation counting to be 1.854 mg. From this stock solution aliquots of 0.035 ml (=0.032 mg) were added to 15 ml of buffer solution thus giving a final concentration of the test compound of 2.16 mg/l. No co-solvents were used.

2.3.2. UV Spectra of Test Substance

The UV absorbency spectra of the final test solution at the beginning and end of the test were recorded using a Perkin-Elmer UV/VIS spectrophotometer model Lamda 15. The spectra are presented in Figure 7.

2.3.3. Number of test vessels

Per test, 14 treated vessels were set up (see also under allocation in Figure 4. In addition, one treated reserve (R1) and one untreated temperature control sample (TH1) were set up. Furthermore, 4 treated dark control samples, one treated reserve (R1) and one untreated temperature control sample (TH2) were set up.

2.3.4. Temperature Control

Irradiated test solutions in apparatus 1 and 2 were maintained at 25.79 +/- 0.15 and 25.03 +/-0.11 °C, respectively. The temperature of the dark control samples ranged from 24.63±0.27 °C. Temperatures were recorded automatically over the complete exposure period in intervals of 30 min. using the thermocouple probe in one of the vessels.

2.3.5. Sampling Times

Samples of two vessel each were taken for analysis directly after the treatment and after 28, 72, 100, 165, 196 and 240 hours of exposure to continuos light. The latter time interval corresponded to 29.83 days sunlight equivalents 40°N (Table 5) as calculated from the initial spectral energy distribution measurements (Table 4). The trapping solutions were taken for analysis and replaced by fresh solutions at all sampling times of the vessels.

2.4. Analyses

2.4.1. Test solutions

2.4.1.1. Control of pH

For each sample to be analysed, the solution pH was measured immediately after the samples were taken.

2.4.1.2. Sterility

Microbial infection of the solutions during application of a.i. and by ventilation of the system was avoided by adequate procedures and equipment. Subsamples of the final test solution were tested for possible microbial contamination at the end of the experiment using 10 ml of the 240 hours reserve sample (R1) made up with sterile water to 50 ml. In addition sterile water was tested as control. Furthermore the buffer solution prior to the start of the experiment was tested for sterility. The test was performed by using the total plate count method (Total Count Standard Medium, M-TGE broth, Millipore M00000P2T).

2.4.1.3. Sample Preparation

After exposure the radioactivity in the samples was determined by liquid scintillation counting and thereafter aliquots of the samples directly analysed by HPLC.

2.4.2. Trapping solutions

The radioactivity in the trapping solutions was determined by direct LSC. Since only negligible amounts of radioactivity were found in ethyleneglycol and NaOH traps no attempts to further characterise the radioactivity were made.

2.5. Analytical Methods

2.5.1. Measurement of Radioactivity

All measurements were performed at least in duplicate using a model 2200 CA Liquid Scintillation Spectrometer (Packard Instruments Company Inc., Downers Grove Ill., US). All measurements were corrected for background and counting efficiency. The following scintillatators were used:

Scintillator I: HiSafe-2®
(for measurement of aqueous and organic samples up to 0.8 ml in 10 ml scintillator I)

Scintillator II: Hionic Fluor®
(for measurement of NaOH samples up to 0.8 ml in 10 ml scintillator II)

2.5.2. High Performance Liquid Chromatography (HPLC)

The identification and quantification of CGA 329351 and its degradation products was performed with a Spectra Physics Liquid Chromatograph equipped with a Berthold radioactivity monitoring system and a Spectra Physics UV/visible detector.

HPLC-System:

Pump:	SP 8800
Auto sampler:	SP 8880
Integrator:	SP 4400
Oven:	SP 8792D
Column:	Nucleosil C 18, length: 25 cm, inner diameter: 4.6 mm, particle size: 5 µm.
Data System:	Spectra Physics Chromstation
Detectors:	UV/VIS: Spectra 200 RAM: Berthold LB 506 C-1 The detector was equipped with a 150 ml solid scintillator flow-cell (YG 150) and an Epson PC AX data system

The column was equipped with a Nucleosil 100S C-18 Guardcartridge K2 (Bischoff, Leonberg, FRG).

Operating conditions:

Mobile phases:	A: Acetonitrile B: Water Flow: 1 ml/min.
Oven:	30°C

Gradient Program:

Time (min.)	Mobile Phases (%)	
	A	B
0-3	25	75
3-10	25-->95	75-->5
10-14	95	5
14-15	95-->25	5-->75
15-22	25	75

Injector:	Injection volume: 100 µl
Detectors:	
UV/VIS:	Wavelength (nm) 210 (CGA 329351)
	Range 0.1 AUF's
RAM:	Control method YG-150

The following retention times were observed on HPLC:

Compound Code-Number	Retention Time (min.)
CGA 329351	12.97
5.1.1.c Woo [redacted]	2.02
5.1.1.c Woo [redacted]	2.62
5.1.1.c Woo [redacted]	11.17
5.1.1.c Woo [redacted]	12.43
5.1.1.c Woo [redacted]	13.38
M6	15.72

2.6. Calculations

2.6.1. Kinetics of Test Substance Decay

All data analyses were performed on a EPSON PC AX4s computer using the Microsoft Excel software. Values given refer to the radioactivity applied. An example is given in Appendix A. No half-lives were calculated due to the insignificant degradation of the test compound.

2.6.2. Relationship between artificial and natural Light Source

Since light in the wavelength range of 300 - 400 nm will be most significant for photodegradation of most chemicals this range was used for quantitative comparison of artificial light from the Suntest xenon arc and of natural sunlight at latitude 50°N. The mean of light intensity measurements at the beginning of the study at different positions under the two Suntest apparatus with coefficients of variation of 4.44 and 6.13 % was used (Table 4) for the calculation of the exposure time (see below). However, for visual comparison the corresponding spectra spanning the visible wavelength region and the near UV (300-800 nm) are shown in Figure 8.

For relation of the different light sources corrections were performed. The integral light intensities at 300-400 nm of the xenon arc sources of both apparatus were determined to be 54.66 and 49.81 Watt/m^2 (Table 4). The corresponding value of spring midday sunlight at 50°N equalled to 43.84 Watt/m^2 resulting in a ratios of intensities for apparatus 1 and 2 of r=1.247 and 1.14, respectively. Published data[3] show that spring light at 50°N corresponds to about 99 % (F=0.99) and to 102 % (F=1.02) of summer light at latitude 40°N and 30°N, respectively. Additionally, it is assumed that the average daily radiation intensity from the sun is about 75% of the peak intensity over a 12 hours period, whereas the radiation in the Suntest was constant in intensity and continuous over time. Therefore, the equivalent days (d) of latitude 30°N and 40°N summer can be calculated according to:

Apparatus:

1:	$d_{(30°N)}$ =	h*r*F/(0.75*12) = h*1.247*1.02/(0.75*12) =	h*0.1413[4]
	$d_{(40°N)}$ =	h*r*F/(0.75*12) = h*1.247*0.99/(0.75*12) =	h*0.1372
2:	$d_{(30°N)}$ =	h*r*F/(0.75*12) = h*1.14*1.02/(0.75*12) =	h*0.1292
	$d_{(40°N)}$ =	h*r*F/(0.75*12) = h*1.14*0.98/(0.75*12) =	h*0.1241

where is:

d= daylight equivalents of summer irradiation at latitude 30°N or 40°N
h= hours of irradiation in the Suntest apparatus
r= ratio of intensity of Suntest radiation to spring sunlight at latitude 50°N
F= correction for season (spring-->summer and latitude 50°N-->30°N or 50°N-->40°N)
0.75= correction for diurnal variation of natural sunlight
12= conversion of hours to days.

The actual calculated sunlight equivalents are shown in Table 5.

3. Results and Discussion

3.1. Purity and Stability of Test Substance

HPLC-analysis of the stock solution of CGA 329351 in aqueous medium prior to the treatment showed a purity of 97.47 (Figure 9).

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[3] For publication see under reference 1.

[4] Slight differences when calculating exposure days with the above given formula from data in Table 6 are due to on-line calculation of the data.

The stability of the test compound was proven by analysis of an aliquot of the stock solution of CGA 329351 left at room temperature during treatment. Its purity was still 97.42% (Figure 10) thus proving the stability of the test compound in the vehicle during treatment.

3.2. Recovery of Radioactivity

The recovery of radioactivity for light exposed and unexposed samples is shown in Table 1 and Table 3, respectively.

As shown in the tables the mean recovery of light exposed samples ranged from 96.67 to 99.86% of the radioactivity applied. For the dark control the recovery ranged from 98.51 to 100.14 %

By far the majority of the recovered radioactivity remained in the aqueous exposure solution. Only negligible amounts of radioactivity were trapped by ethyleneglycol and NaOH totally accounting after 240 hours of light exposure for 0.002 and 0.05 % of the radioactivity applied.

3.3. Rate of Degradation of the Parent Molecule

The results are given in Table 2, Table 3 and Figure 11.

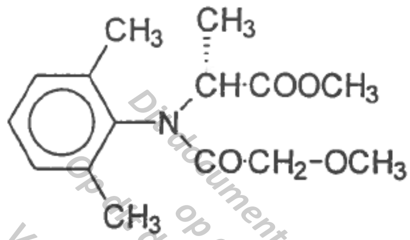
The parent molecule was practically not broken down by light. After 240 hours (= 29.83 days 40°N or 30.92 days 30°N) of continuos exposure still 97.16 % of CGA 329351 were present. In unexposed samples (dark control) the amount of CGA 329351 was 96.30-98.61 % after 240 hours.

No half-life was calculated for light exposed and unexposed samples due to the insignificant degradation observed.

3.4. Formation of Degradates

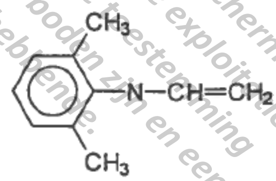
The identity of radioactivity isolated from photolysis solution as radioactive fraction "Parent" from sample 4A (100 hrs. exposure) and 7A (240 hrs. exposure) with the parent molecule was shown by comparing the mass spectra of the authentic reference compound, CGA 329351 on GC-MS after chemical ionisation with methane.

1. <u>*Reference Compound CGA 329351*</u>

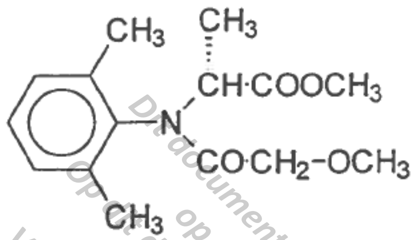


$C_{15}H_{21}NO_4$

DCI-MS/Methane (negative mode)

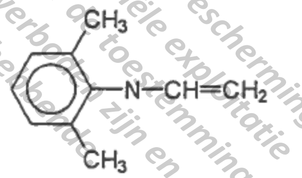
AMU	Rel. Intensity	Ion Registration	Assignments	Fragments
278	30	negative	M - H	
264	11	negative	M - 15	⇒ CH_3
263	23	negative	278 - 15	⇒ CH_3
246	100	negative	278 - 32	⇒ CH_3OH
233	8	negative	278 - 45	⇒ CH_3OCH_2
192	49	negative	M - 87	⇒ CH_3-CH-$COOCH_3$
191	12	negative	278 - 87	⇒ CH_3-CH-$COOCH_3$
174	13	negative	246 - 72	⇒ O=C=CH-OCH_3
146	8	negative	M - 133	= 

2. *Fraction from Sample 4A*



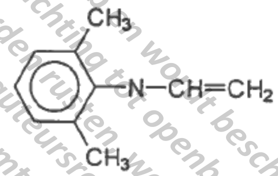
$C_{15}H_{21}NO_4$

DCI-MS/Methane (negative mode)

AMU	Rel. Intensity	Ion Registration	Assignments	Fragments
278	30	negative	M - H	
264	7	negative	M - 15	⇒ CH_3
263	21	negative	278 - 15	⇒ CH_3
246	100	negative	278 - 32	⇒ CH_3OH
233	21	negative	278 - 45	⇒ CH_3OCH_2
192	35	negative	M - 87	⇒ CH_3-CH-$COOCH_3$
191	40	negative	278 - 87	⇒ CH_3-CH-$COOCH_3$
174	9	negative	246 - 72	⇒ O=C=CH-OCH_3
146	26	negative	M - 133	= 

3. Fraction from Sample 7A

DCI-MS/Methane (negative mode)

AMU	Rel.Intensity	Ion Registration	Assignments	Fragments
278	29	negative	M - H	
264	8	negative	M - 15	⇒ CH_3
263	19	negative	278 - 15	⇒ CH_3
246	100	negative	278 - 32	⇒ CH_3OH
233	31	negative	278 - 45	⇒ CH_3OCH_2
192	34	negative	M - 87	⇒ CH_3-CH-$COOCH_3$
191	37	negative	278 - 87	⇒ CH_3-CH-$COOCH_3$
174	12	negative	246 - 72	⇒ O=C=CH-OCH_3
146	26	negative	M - 133	= 

Besides the parent molecule only very minor amounts of degradates were observed (M1, M3-M6), ranging , on average, at the end of the study between 0.22 and 1.77 % of the radioactivity applied (Table 2 and Table 3). Due to their insignificant amounts they were not further characterised.

4. Conclusions

In conclusion, phenyl-ring labelled CGA 329351 was not significantly degraded by light.

5. References

[1] [redacted], Laboratory Protocols for Evaluating the Fate of Organic Chemicals in Air and Water. EPS-600/3-82-022 EPA Contract 68-03-227. Updated figures were obtained according to [redacted] and [redacted], Brighton Crop Protection Conference, Pests and Diseases (1988).

Table 1: Balance of Radioactivity after Exposure of ^{14}C-CGA 329351 to artificial Light.

(Values given in % of the radioactivity applied)

Time (hrs. cont. light)	Days (40°N)	Aqueous Phase	CO2	Org. Volatiles	Recovery
0	0.00	98.31	0.000	0.000	98.31
28	3.82	98.49	0.005	0.000	98.50
72	9.82	96.14	0.018	0.000	96.15
100	12.43	98.71	0.019	0.000	98.73
165	20.51	101.13	0.029	0.000	101.16
196	24.36	98.05	0.033	0.001	98.08
240	29.83	99.20	0.038	0.002	99.24

Series B

Time (hrs. cont. light)	Days (40°N)	Aqueous Phase	CO2	Org. Volatiles	Recovery
0	0.00	98.86	0.000	0.000	98.86
28	3.82	97.30	0.005	0.000	97.30
72	9.82	97.17	0.018	0.000	97.19
100	12.43	98.57	0.018	0.000	98.59
165	20.51	98.53	0.029	0.000	98.56
196	24.36	97.38	0.033	0.001	97.42
240	29.83	98.61	0.038	0.002	98.65

Mean

Time (hrs. cont. light)	Days (40°N)	Aqueous Phase	CO2	Org. Volatiles	Recovery
0	0.00	98.58	0.00	0.00	98.58
28	3.82	97.89	0.01	0.00	97.90
72	9.82	96.65	0.02	0.00	96.67
100	12.43	98.64	0.02	0.00	98.66
165	20.51	99.83	0.03	0.00	99.86
196	24.36	97.72	0.03	0.00	97.75
240	29.83	98.90	0.04	0.00	98.94

Table 2: Amount of CGA 329351 and Pattern of Degradates in Light exposed Samples

Time (hrs. cont. light)	Days (40°N)	Degradate/Code-No.				
		CGA 329351	M1	M3	M4	M5
		Retention Time (min.)				
		12.97	2.02	11.17	12.43	13.38
		Identity/CGA-No.				
		329351	UK	UK	UK	UK
0	0.00	96.12	0.00	0.69	0.71	0.80
28	3.82	96.69	0.00	0.00	0.00	1.80
72	9.82	95.45	0.00	0.00	0.00	0.68
100	12.43	97.88	0.00	0.00	0.00	0.83
165	20.51	97.96	2.11	0.00	0.00	1.06
196	24.36	96.54	0.00	0.00	0.00	1.51
240	29.83	97.74	0.00	0.00	0.00	1.46

Series B

Time (hrs. cont. light)	Days (40°N)	Degradate/Code-No.				
		Parent	M1	M3	M4	M5
		Retention Time (min.)				
		12.97	2.02	11.17	12.43	13.38
		Identity/CGA-No.				
		329351	UK	UK	UK	UK
0	0.00	98.14	0.00	0.00	0.00	0.72
28	3.82	96.39	0.00	0.00	0.00	0.90
72	9.82	95.67	0.00	0.00	0.00	1.50
100	12.43	97.06	0.84	0.00	0.00	0.67
165	20.51	95.42	1.11	0.00	0.00	1.99
196	24.36	96.18	0.44	0.00	0.00	0.76
240	29.83	96.57	0.98	0.00	0.00	1.06

Mean

Time (hrs. cont. light)	Days (40°N)	Degradate/Code-No.				
		CGA 329351	M1	M3	M4	M5
		Retention Time (min.)				
		12.97	2.02	11.17	12.43	13.38
		Identity/CGA-No.				
		329351	UK	UK	UK	UK
0	0.00	97.13	0.00	0.34	0.35	0.76
28	3.82	96.54	0.00	0.00	0.00	1.35
72	9.82	95.56	0.00	0.00	0.00	1.09
100	12.43	97.47	0.42	0.00	0.00	0.75
165	20.51	96.69	1.61	0.00	0.00	1.53
196	24.36	96.36	0.22	0.00	0.00	1.13
240	29.83	97.16	0.49	0.00	0.00	1.26

Table 3: Balance of Radioactivity and Pattern of Degradates of unexposed Samples (Dark Controls)

Balance of Radioactivity

Time (hrs.)	Aqueous Phase	CO2	Org. Volatiles	Recovery
100	98.92	0.006	0.001	98.92
100	99.05	0.006	0.001	99.06
240	100.10	0.036	0.005	100.14
240	98.47	0.036	0.005	98.51

Pattern of Degradates

Time (hrs.)	Degradate/Code-No.			
	Parent	M1	M5	M6
	Retention Time (min.)			
	12.97	2.02	13.38	15.72
	Identity/CGA-No.			
	329351	UK	UK	UK
100	96.78	0.94	1.20	0.00
100	96.09	1.19	1.77	0.00
240	98.61	0.00	1.49	0.00
240	96.30	0.00	1.21	0.96

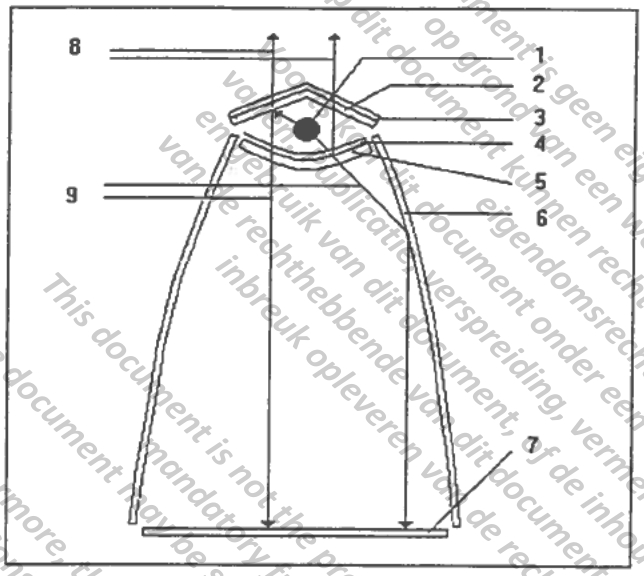
Table 4: Distribution of Light Intensities in Suntest Apparatus compared with natural Sunlight in Basel.

Apparatus	Sample	Date	Position Suntest	Energy (Watt/m2)	Date	Energy (Watt/m2)
1	2A	16.06.1995	11	54,66	29.06.1995	51,78
	2B		12	51,93		48,76
	3A		13	57,83		54,94
	3B		14	54,21		51,06
	TH1		15	58,40		55,25
Average 2A-3B				54,66		51,64
STD				2,43		2,55
%				4,44		4,94
r				1,25		
F 40°N				0,99		
F 30°N				1,02		
2	4A	16.06.1995	1	45,28	29.06.1995	43,84
	4B		2	44,72		42,92
	5A		3	51,63		47,47
	5B		4	48,88		46,01
	6A		5	52,48		49,41
	6B		6	50,57		48,16
	7A		7	53,62		50,84
	7B		8	51,16		48,81
	R1		9	49,96		47,47
	TH2		10	47,46		44,67
Average 4A-R1				49,81		47,18
STD				3,06		2,74
%				6,13		5,82
r				1,14		
F 40°N				0,98		
F 30°N				1,02		
Basel Sunlight	1	20.06.1995		44,01		44,01
	2	20.06.1995		43,67		43,67
Average				43,84		43,84

Table 5: Duration of Irradiation Period for each Test Vessel.

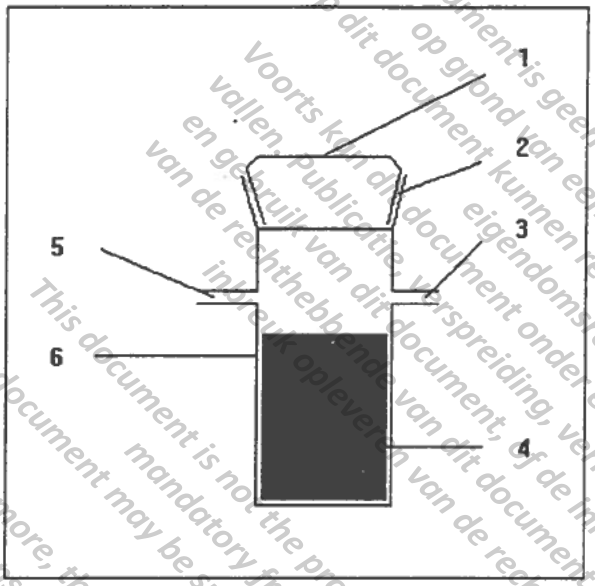
Sample-No.	Suntest Apparatus	Exposed to Light	Exposure Time (hrs.)	Calendar days	Days 30°N	Days 40°N
0A/0B	-	No	0	0.00	0.00	0.00
2A/B	1	Yes	28	2.33	3.96	3.82
3A/B	1	Yes	72	6.00	10.18	9.82
4A/B	2	Yes	100	8.33	12.89	12.43
5A/B	2	Yes	165	13.75	21.26	20.51
6A/B	2	Yes	196	16.33	25.25	24.36
7A/B	2	Yes	240	20.00	30.92	29.83

Figure 1: Optical System of Heraeus Suntest Apparatus



1. Xenon burner
2. Ultra violet mirror
3. Light mirror
4. Supplementary , special UV-filter
5. Quartz glass with selectively reflecting coating
6. Parabolic reflector
7. Specimen plane
8. Infra-red radiation
9. UV-radiation and visible light

Figure 2: Aqueous Photolysis Vessel



1. Borosilicate lid
2. Teflon sleeve
3. Air outlet
4. Photolysis solution
5. Air inlet
6. Photolysis vessel

Figure 3: Aqueous Photolysis System

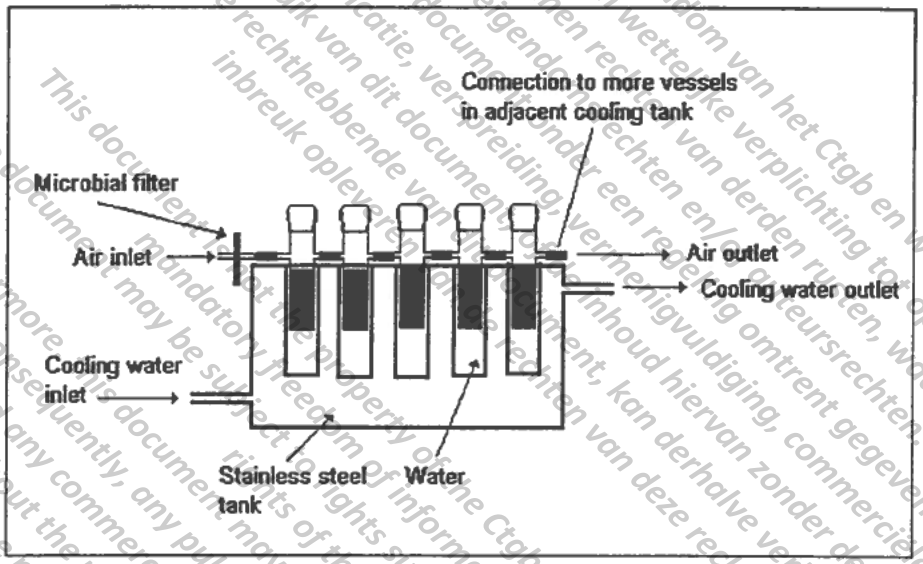
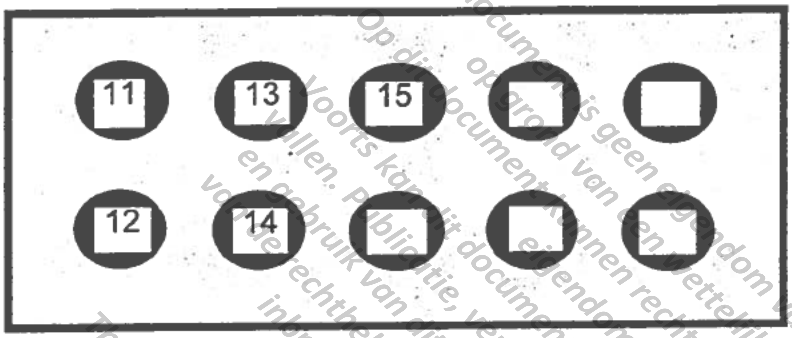
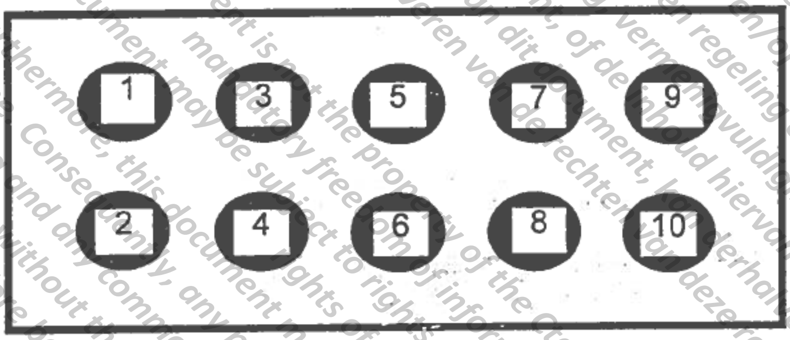


Figure 4: Sample Allocation in Suntest Apparatus

Sample Position in Apparatus 1



Sample Position in Apparatus 2



Apparatus	Sample	Position	Apparatus	Sample	Position
1	2A	11	2	4A	1
	2B	12		4B	2
	3A	13		5A	3
	3B	14		5B	4
	TH1	15		6A	5
				6B	6
				7A	7
				7B	8
				R1	9
				TH2	10

TH: Thermocouple

Figure 5: Distribution of Light Intensities in Exposure Vessels at the Beginning and End of the Study

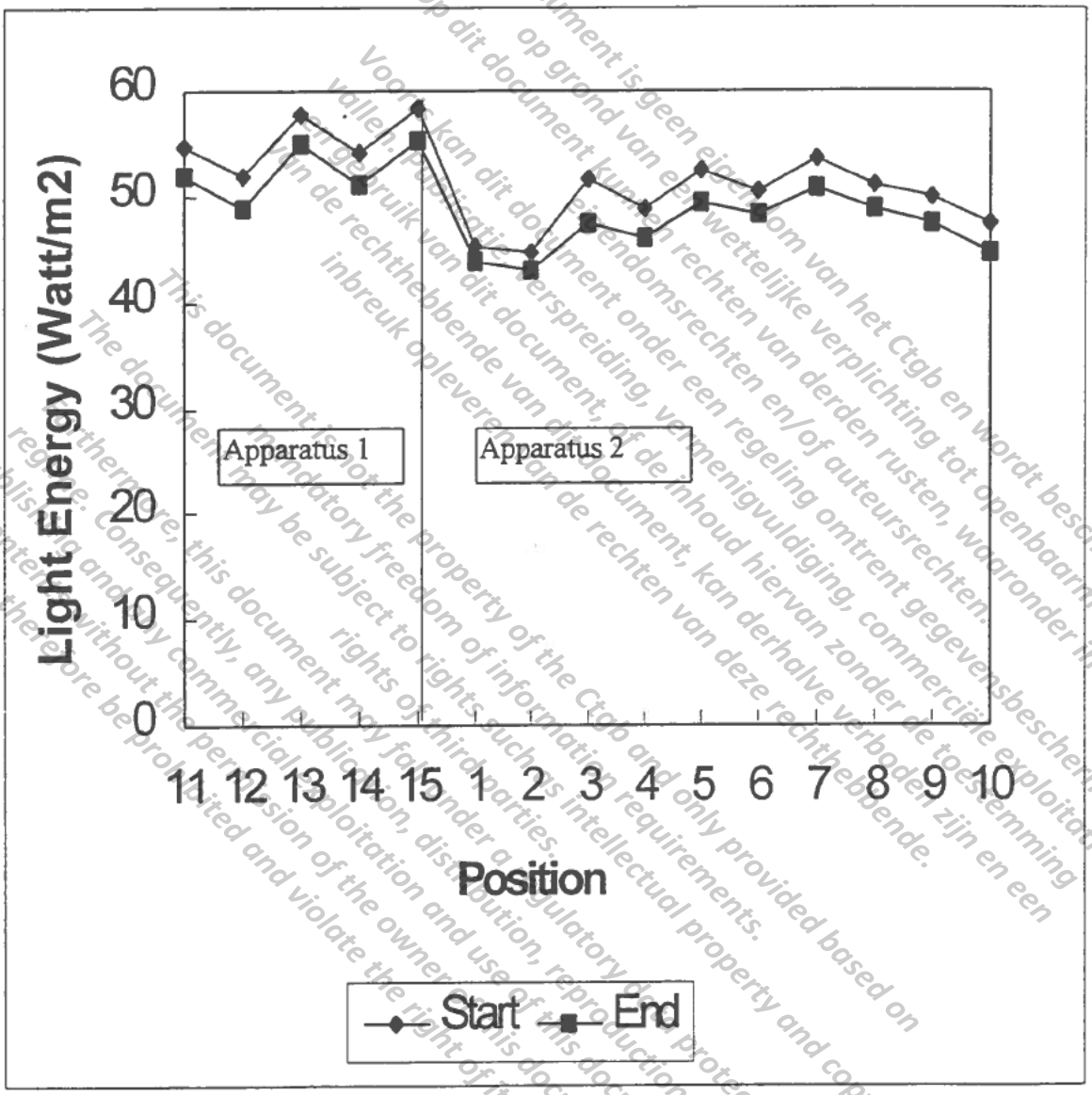
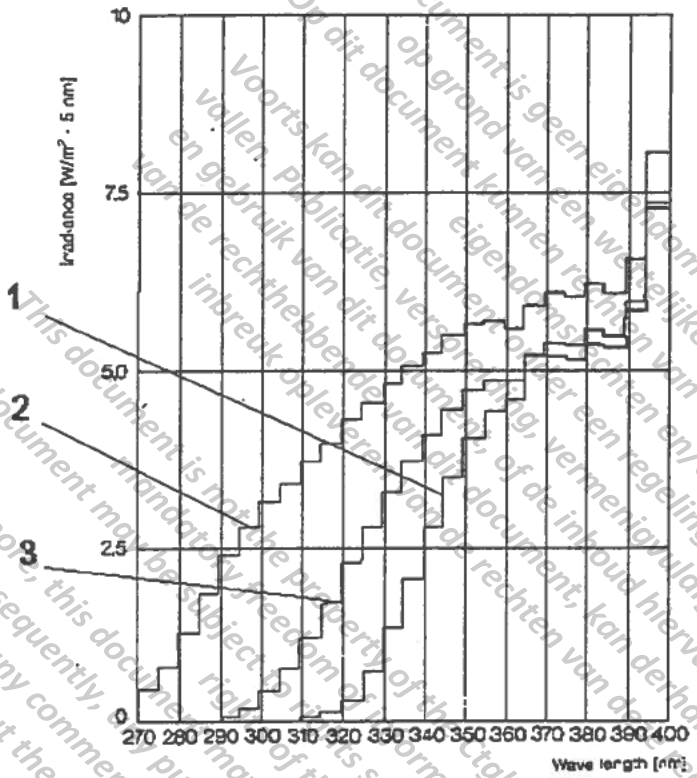


Figure 6: UV-Radiation from 270 to 400 nm of the Xenon Arc Burner in the Suntest Apparatus

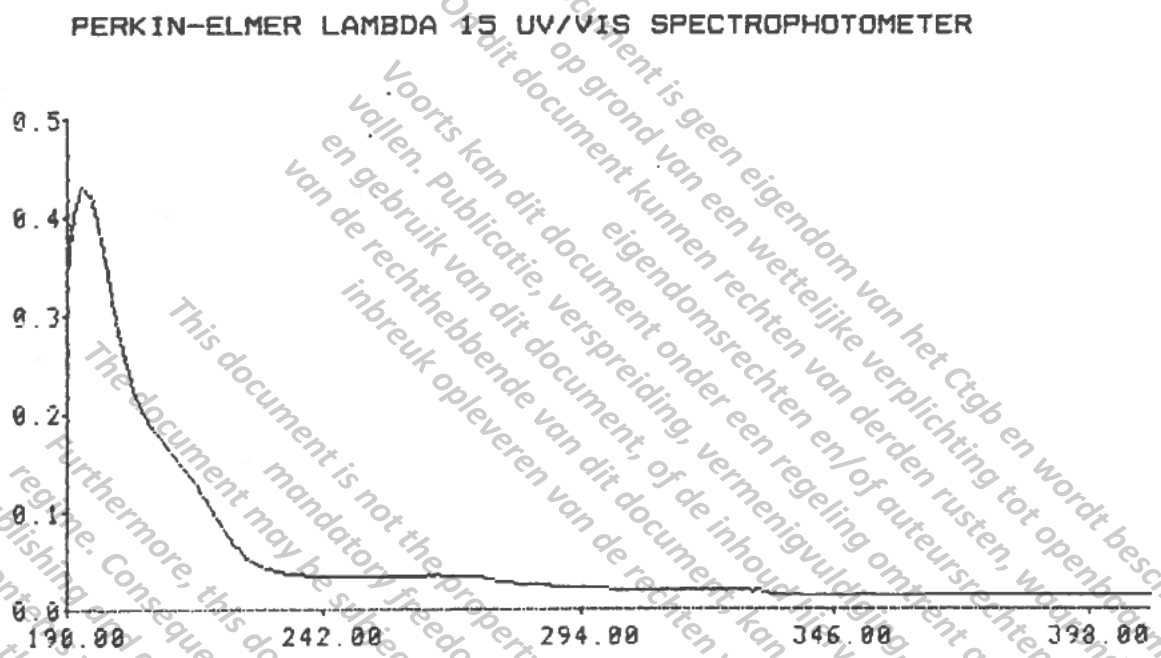


1. Irradiation energy of the Xenon arc lamp without filters
2. Irradiation energy of the Xenon arc lamp with window glass filter (not installed)
3. Irradiation energy of the Xenon arc lamp with UV-filter No. 56009561 simulating sunlight outdoors; UV-edge at about 290 nm (installed).

Figure 7: Absorption Spectrum of Test Solution of CGA 329351 in Phosphate Buffer pH 7 at Start and End of the Experiment

(Measuring conditions: cell: 1 cm , slit: 2 nm, scan speed: 120 nm/min..)

Start of Experiment



End of Experiment

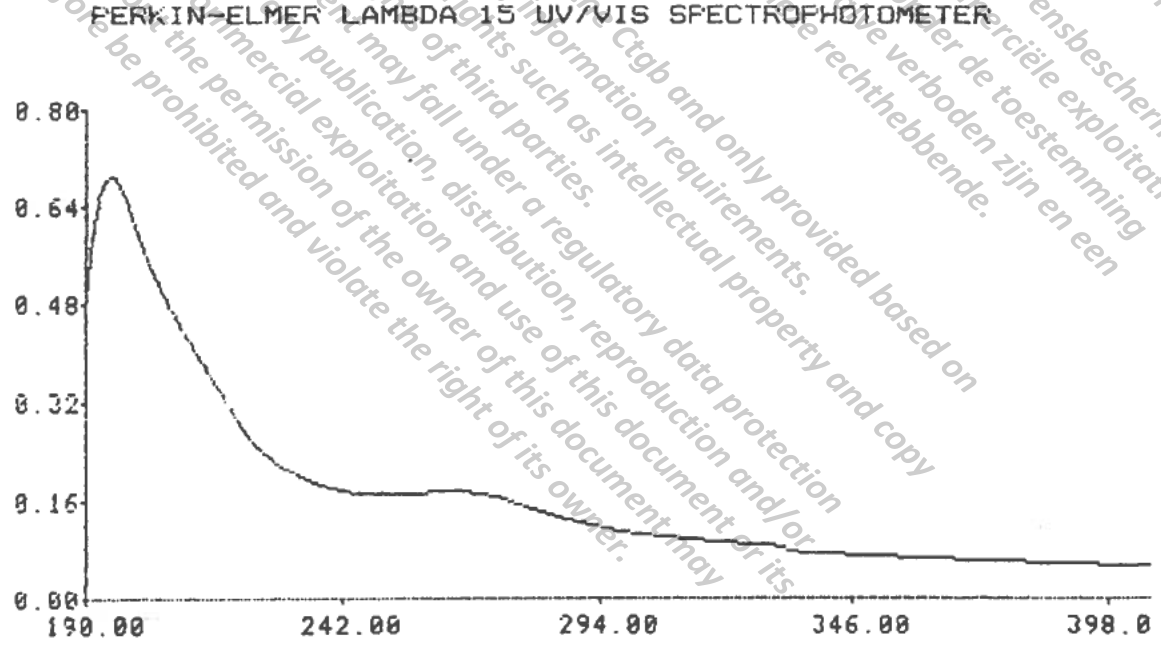
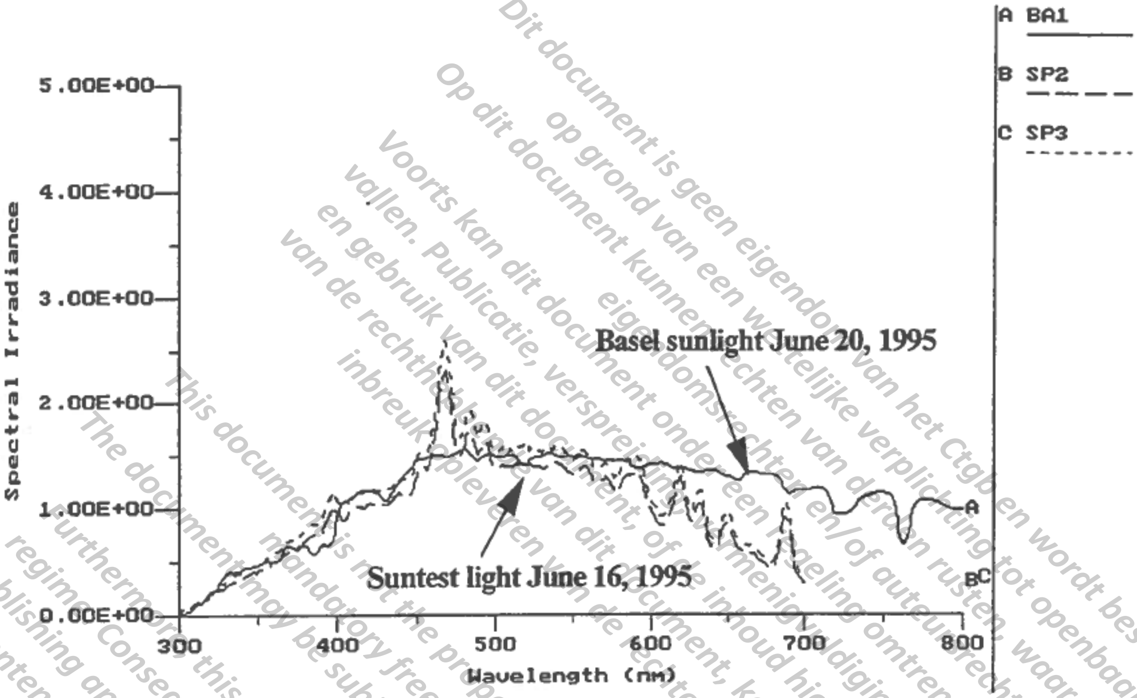


Figure 8: Emission Spectra of Suntest Light and Basle Spring Sunlight at the Beginning (A) and End (B) of the Study.

A: Suntest: Position 2/3; 16.6.1995



<u>*B: Suntest : Position 2/3; 29.6.1995*</u>

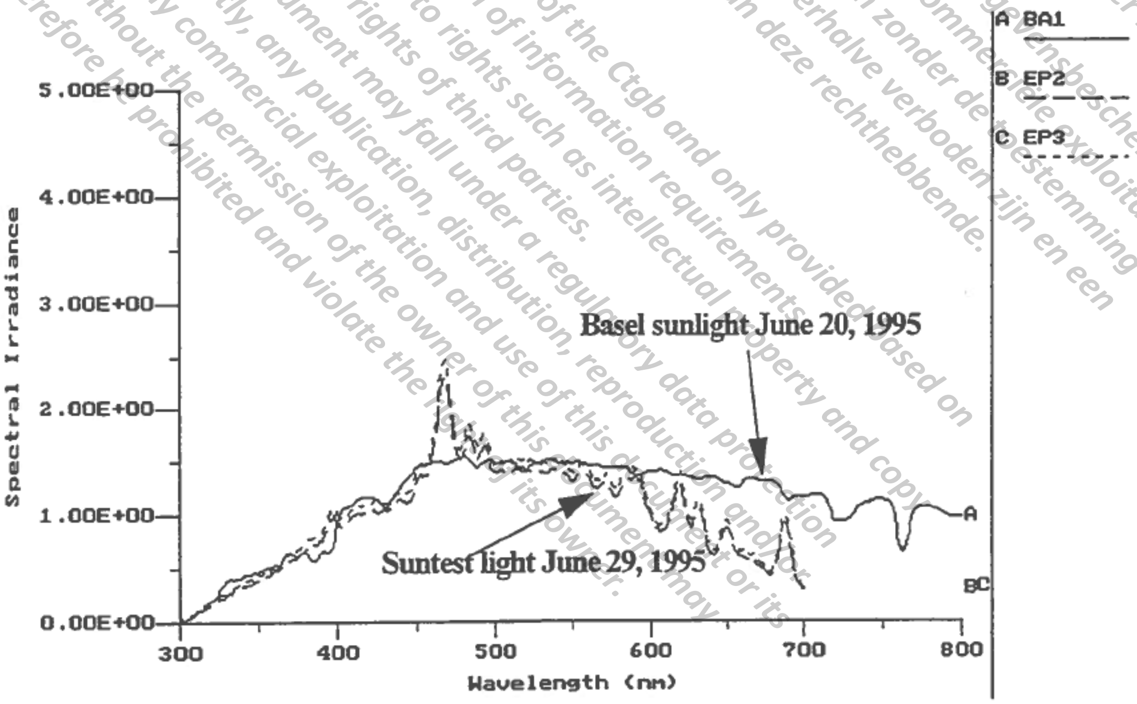
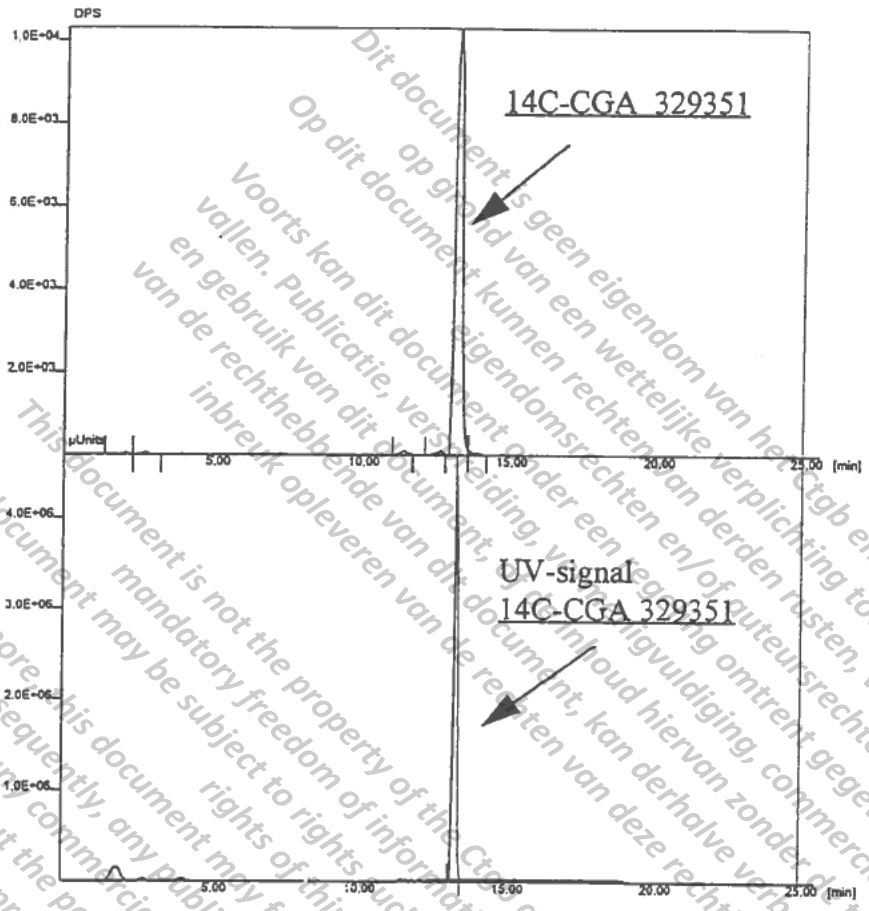


Figure 9: Purity of Phenyl-ring labelled CGA 329351

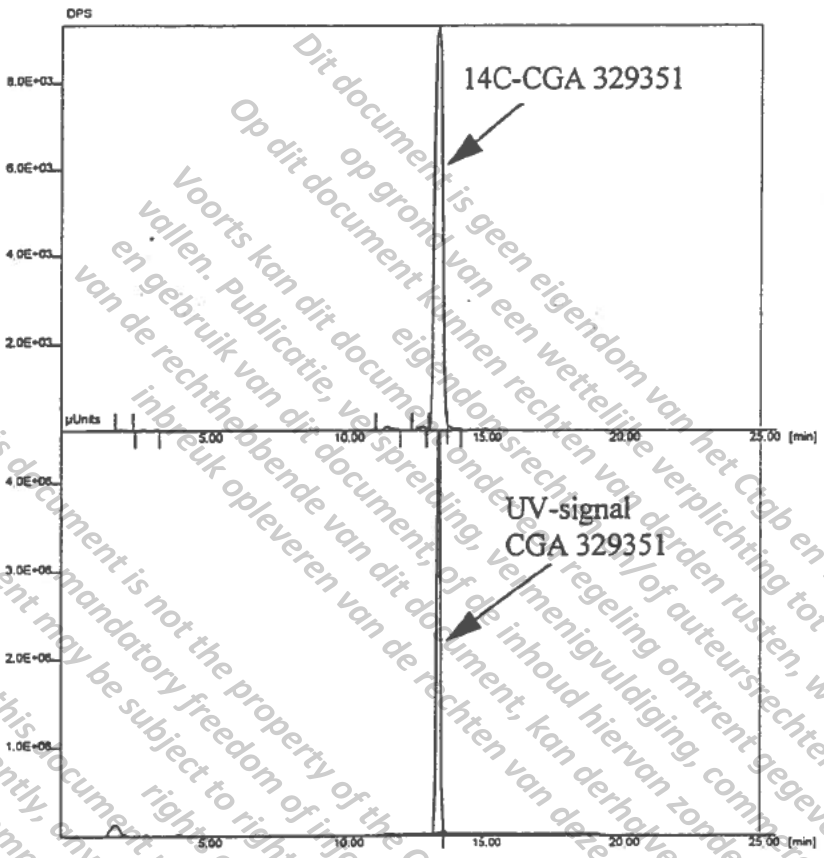


File name : PURIT001.CH2 User : 14C
Curr. Date : 23-Oct-95 10:27:18
Acqu. Date : 15-Jun-95 15:32:30
Info :
Purity 14C-CGA 329351/0.1 ml = 235760 dpm
Control Method : YG_150

#	Name	Rt	DPM	%Area
1	M1	2.02	29	0.19
2	M2	2.62	30	0.20
3	M3	11.17	132	0.86
4	M4	12.43	104	0.68
5	CGA329351	12.97	14999	97.47
6	M5	13.38	94	0.61

Total Area of Peak = 15388.81

Figure 10: Stability of Phenyl-ring labelled CGA 329351



File name : STAB_001.CH2 User : 14C
Curr. Date : 23-Oct-95 10:45:20
Acqu. Date : 19-Jun-95 14:28:20
Info :
Stability/14C-Stock CGA 329351/0.1 ml = 239464 dpm
Control Method : YG_150

#	Name	Rt	DPM	%Area
1	M1	2.20	34	0.22
2	M2	2.78	20	0.23
3	M3	11.25	139	0.92
4	M4	12.53	112	0.74
5	CGA 329351	13.03	14778	97.42
6	Unknown	13.40	87	0.58

Total Area of Peak = 15169.16

Figure 11: Decline of [U-^{14}C]-Phenyl -ring labelled CGA 329351 after Exposure to artificial Light.

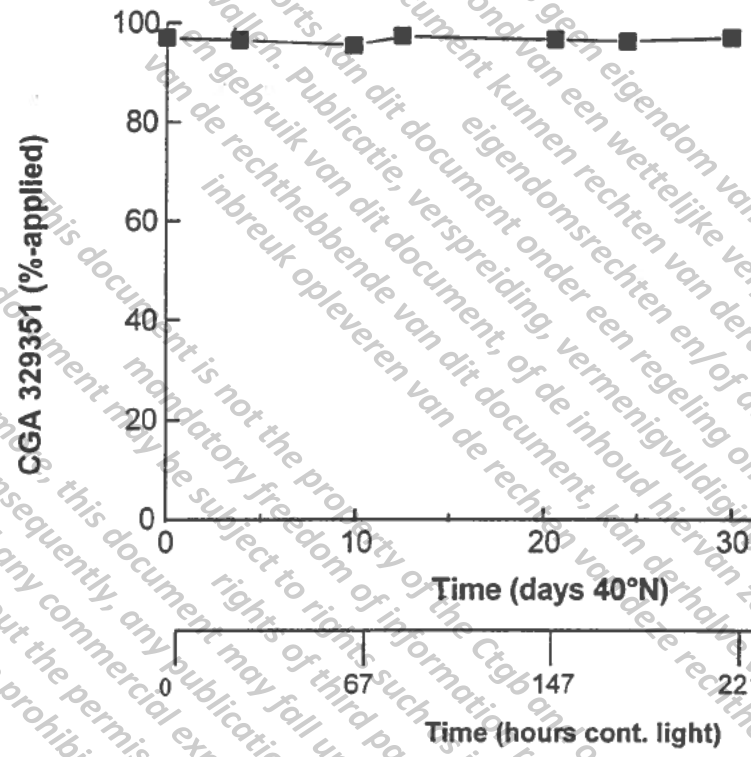
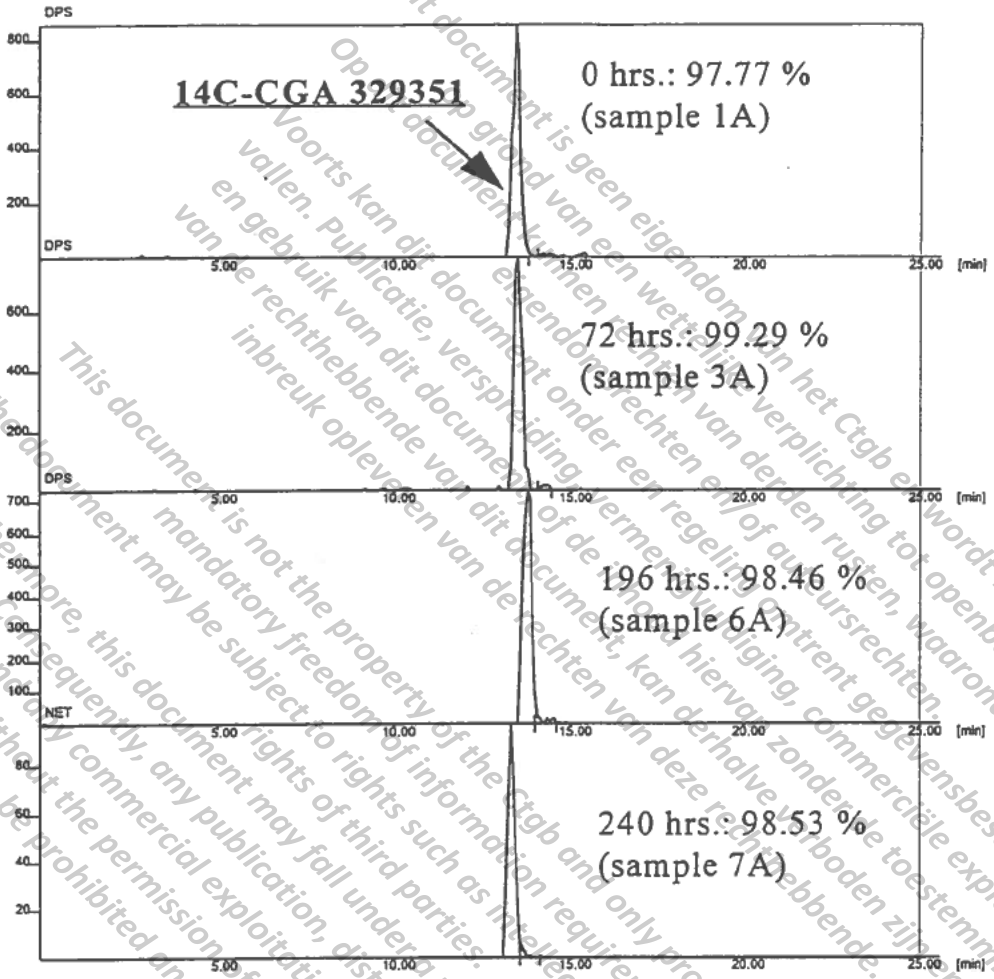


Figure 12: Pattern of Radioactivity in aqueous Phase of Samples exposed for various Times to artificial Light.

(HPLC of photolysis solution after 0, 72, 196 and 240 hours of exposure.)



HPLC Data (Berthold Sample 3A)

File name : SAMPA003.CH2 User : 14C Curr. Date : 26-Oct-95 16:19:22
Acqu. Date : 22-Jun-95 11:51:50
Info :
Sample 3A/72 Std. belichtet/0.1 ml = 17560 dpm
Control Method : YG_150
Bkg (Cpm) = 18
Efficiency % = 75

#	Name	Rt	DPM	%Area
1	CGA329351	13.18	9526	99.29
2	M5	14.02	68	0.71

Total Area of Peak = 9594.67

Figure 13: Pattern of Radioactivity in aqueous Phase of Samples exposed in the Dark.

(HPLC of photolysis solution after 100 and 240 hours exposure in the Dark.)

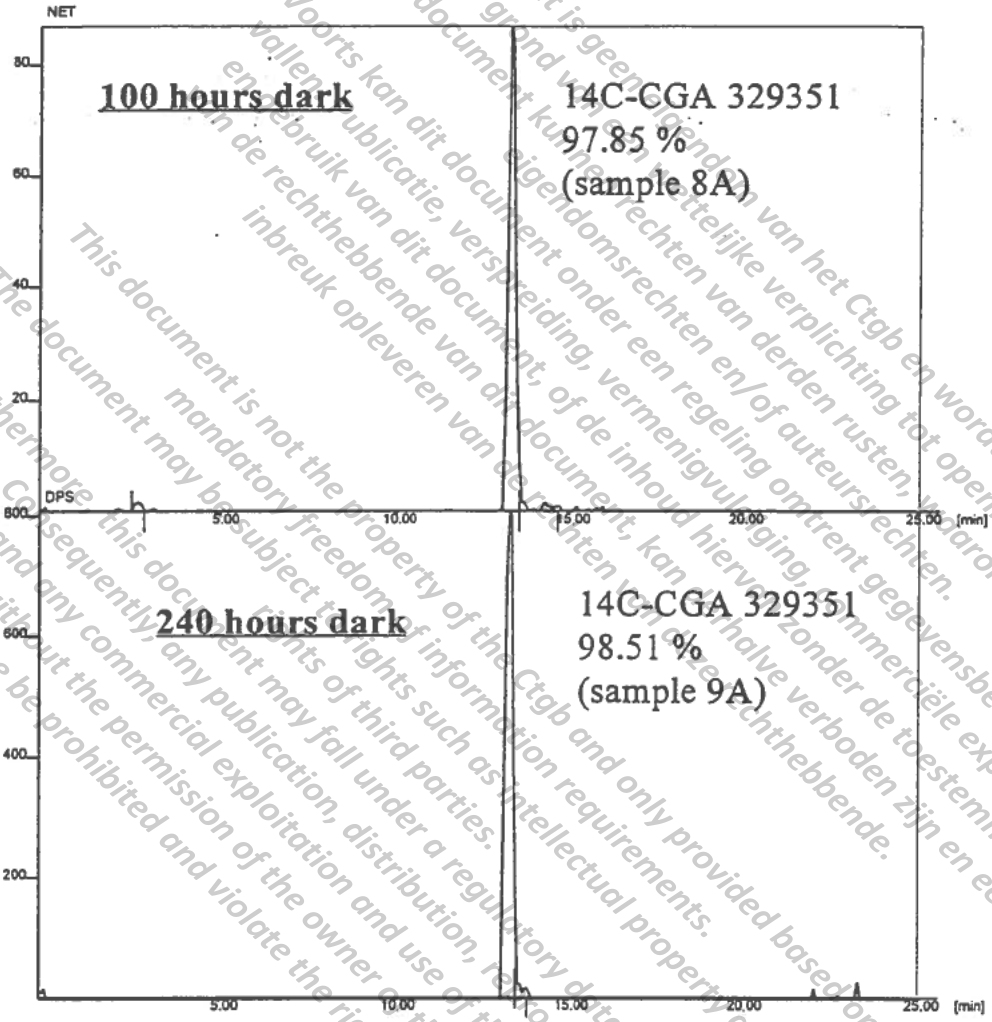


Figure 14: Mass Spectrum of Reference CGA 329351 (negative DCI-MS/methane

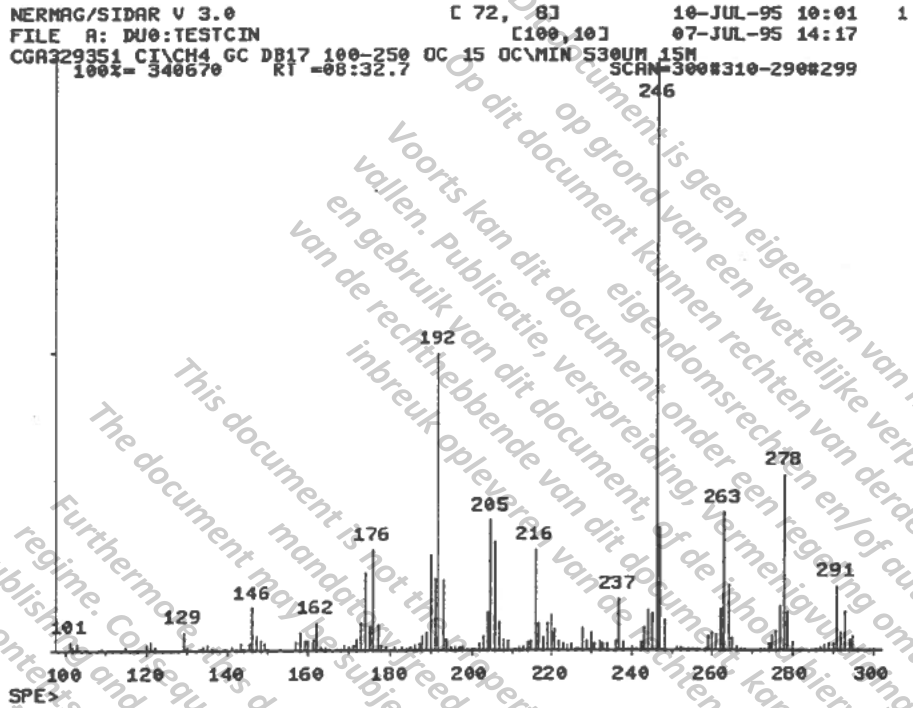


Figure 15: Mass Spectrum of Sample 4A (negative DCI-MS/methane)

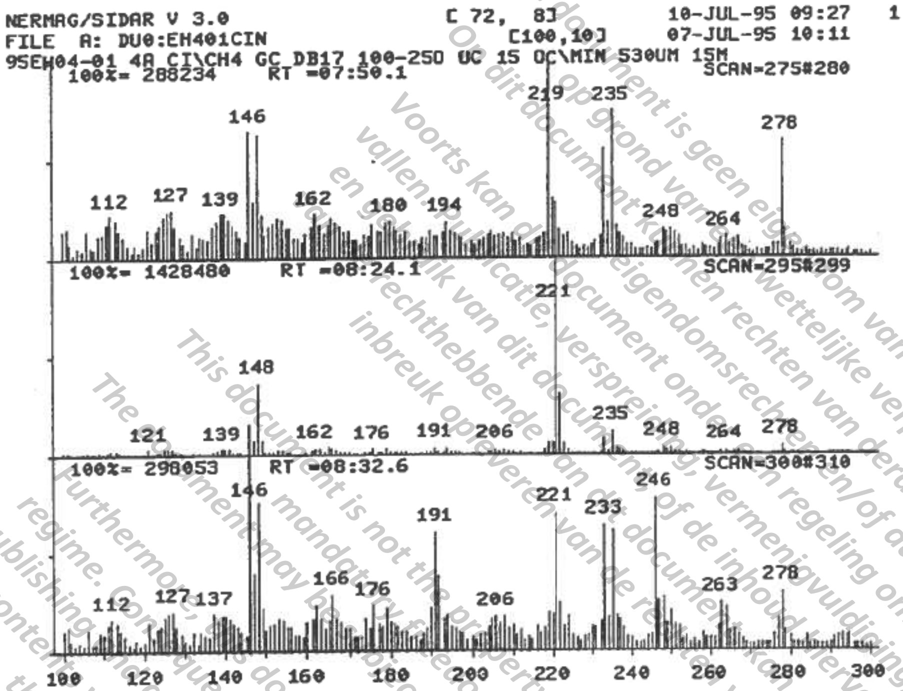


Figure 16: Mass Spectrum of Sample 7A (negative DCI-MS/methane)

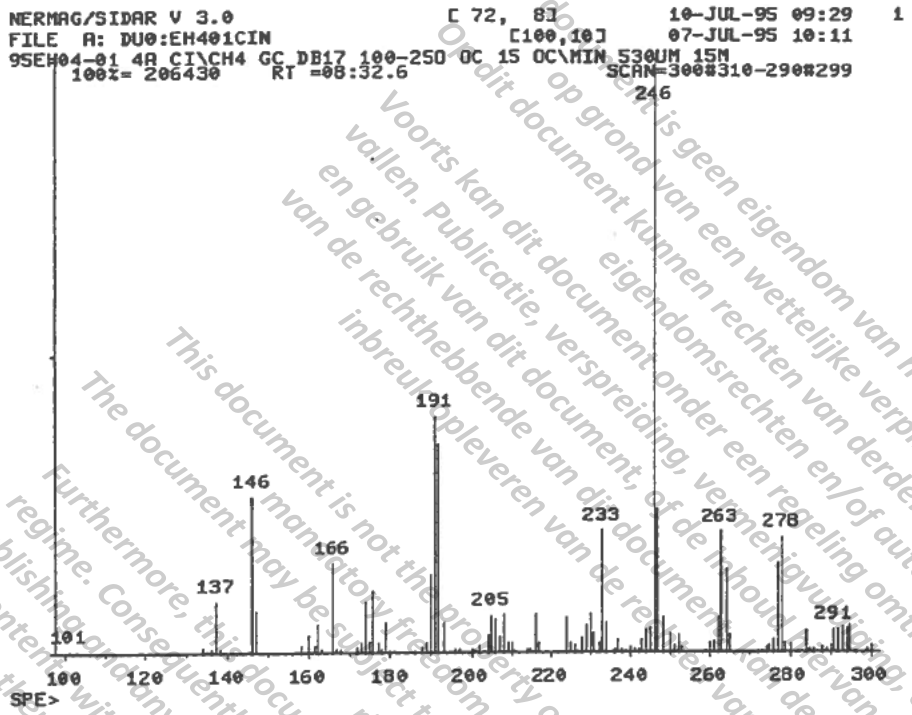
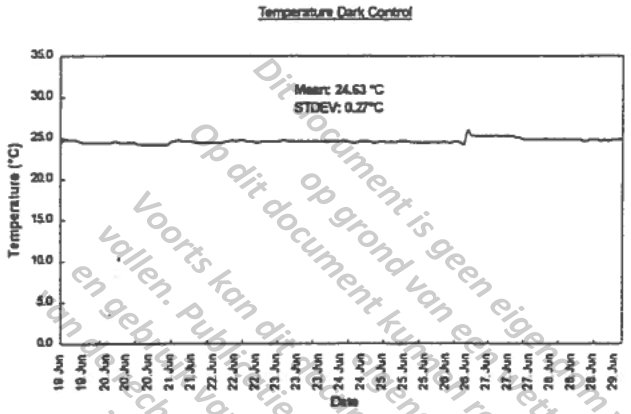
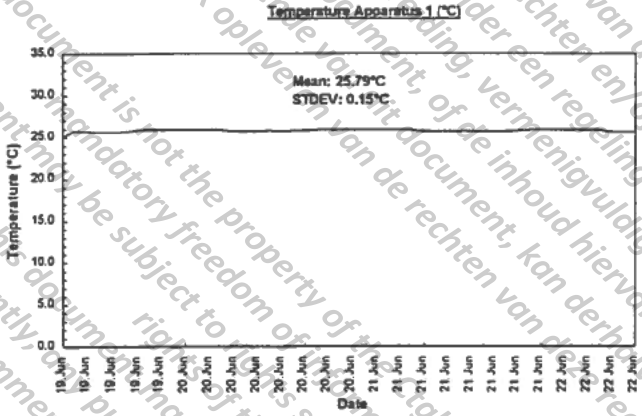


Figure 17: Temperature in Exposure Vessels of Suntest Apparatus

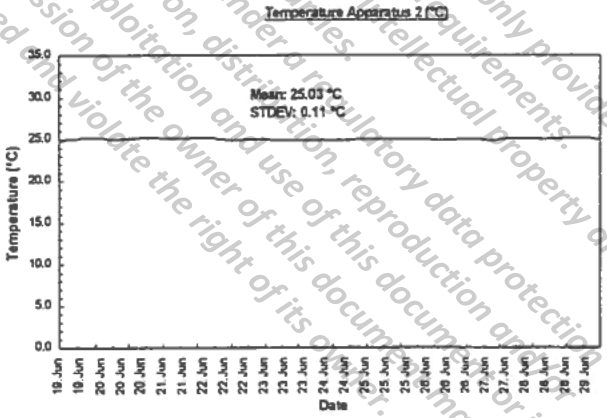
Dark Control



Suntest Apparatus 1



Suntest Apparatus 2



APPENDIX A: Representative Data

Analytical Data

Measurement of Radioactivity

(Sample 3A;72 hrs.; 25°C; Series A)

Work-up of Samples

Page 1

General Data

Project :		95EH04		spez.Act (dpm/mg) :		87246000
Sample :		3A		dpm applied.:		2831255
Substance:	CGA	329351		mg applied. :		0.032
Batch :		MSR-II-89		Buffer ml :		15.00
Applied		19.06.95 11,00		Start pH :		7.05
Sampled		22.06.95 11,00		End pH :		7.12
Exposure		3.00	Cal days	Datum		
		72.00	hrs.	Visum		

Measurement of Radioactivity

Samples transferred to graduated tube. Volume and and pH determined and aliquot submitted to LSC and HPLC.

Sample No	Sample ml	Total ml	Sample dpm	Total dpm	Applied. %	Counter
3A	0.50	15.50	87802	2721862	96.14	7

HPLC-ANALYSIS OF PHOTOLYSIS SOLUTION Page

(Results of RAM; Berthold)

HPLC-No.:	6 (Figure 12; Report)
TLC-No. :	none
Project :	95EH04

Sample-No./	Degradate/Code-No.				
Time (hrs.)	Parent	M1	M3	M4	M5
3A	Retention Time (min.)				
72	12.97	2.02	11.17	12.43	13.38
	Identity/CGA-No.				
	329351	UK	UK	UK	UK
	99.29	0.00	0.00	0.00	0.71

HPLC-ANALYSIS OF PHOTOLYSIS SOLUTION

(Results in %-applied)

Sample-No./	Degradate/Code-No.				
Time (hrs.)	Parent	M1	M3	M4	M5
3A	Retention Time (min.)				
72	12.97	2.02	11.17	12.43	13.38
	Identity/CGA-No.				
	329351	UK	UK	UK	UK
Total	95.45	0.00	0.00	0.00	0.68

Sum :	96.14
Check :	96.14

Balance

	Total dpm	Normal.. %	Appliz. %	Analyse
3A	2721862	99.98	96.14	HPLC 6
Volatiles. NaOH	521	0.02	0.02	
Volatiles Ethyl. Glycol	0	0.00	0.00	
Backwash	0	0.00	0.00	
Total	2722383	100.00	96.15	

Calculation e.g. amount CGA 329351 sample 3A, 72 hrs.:

Radioactivity in aqueous phase: 96.14 % applied
Result of HPLC for CGA 329351: 99.29 % ROI (Region of Interest)
CGA 329351 (in %-applied): (96.14*99.29)/100 = **95.45**%

APPENDIX B: Limit of Detection and Quantitation

Calculation of Limit of Detection and Quantitation

Limit of Detection and Quantitation

(Sample 3A;72 hrs.; 25°C; Series A)

Limit of Detection for HPLC

(Figure 12)

Specific radioactivity (dpm/mg)	87246000
Amount applied (mg)	0.032
Amount applied (dpm)	2831255
Volume of buffer (ml)	15.00
Concentration in Buffer (mg/l)	2.16

	cpm (HPLC)	% applied
Background:	18	
Parent molecule:	9526	95.45
DL = 95.45/9526*15 =		0.18

(DL: Detection Limit)

	%-applied	Parent equiv. (mg)	mg/l Buffer (ppm)
Background HPLC (DL):	0.18	0.0001	0.00000
Limit of Detection (2XDL):	0.36	0.0001	0.00770
Limit of Quantitation (3xDL):	0.54	0.0002	0.01154

Limit of Detection and Quantitation for Liquid Scintillation Counter (LSC: 7)

	ml	dpm	Total dpm	%-applied	Parent equiv (mg)	mg/l Buffe (ppm)
Sample size measured (ml)	0.50					
Buffer Volume (ml)	15.50					
Background LSC (BGK LSC) :		16	496	0.02	0.000006	0.00038
Limit of Detection (2*BGK):		32	992	0.04	0.000011	0.00076
Limit of Quantitation (3*BGK):		48	1488	0.05	0.000017	0.00114