REPORT

Study Title:

DETERMINATION OF ^{14}C-EMAMECTIN B1A (^{14}C-NOA 426007) PHOTOLYSIS QUANTUM YIELD

Data Requirement/Test Guidelines:

OECD Guideline for Testing of Chemicals, draft document, August 2000
JMAFF Agchem Test Guidelines 12 Nohsan N. 8147, 24 November 2000, revised 26 June 2001: Photodegradation in water (2-6-2)
EPA-540/9-82-021, Section 161-2, October 18, 1982
EPA 540/09-90-078, December 1989

Study Director:

Dr. R. Phaff

Study Completion Date:

May 31, 2005

Test Facility:

RCC Ltd
Environmental Chemistry & Pharmanalytics
CH-4452 Itingen/Switzerland

Sponsor:

Syngenta Ltd.
Jealott's Hill International Research Centre
Bracknell
Berkshire RG 42 6EY
United Kingdom

Identification:

RCC Study No.: 856664
Syngenta Study No. T002561-04

RCC

GLP CERTIFICATE

The Swiss GLP Monitoring Authorities

Swiss Federal Office of Public Health

Swiss Agency for the Environment, Forests and Landscape

swissmedic Swissmedic Swiss Agency for Therapeutic Products

Statement of GLP Compliance

It is hereby confirmed that

during the period of November 18 – 22, 2002

the following Test Facilities of

RCC Ltd
4452 Itingen
Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for Therapeutic Products and the Swiss Agency for the Environment, Forests and Landscape with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facilities	**Areas of expertise ***
- Toxicology	TOX, ACC, MUT, OTH (Safety Pharmacology)
- Environmental Chemistry and Pharmanalytics	ACC, ECT, ENF, EMN, PCT, RES, OTH (Animal metabolism)

The inspections were performed in agreement with the OECD Guidelines for National GLP Inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice [RS 813.016.5] at the time they were inspected.

Federal Office of Public Health
The Director

Bern, March 2003

Prof. Th. Zeltner

* TOX = Toxicology ; ACC = Analytical and Clinical Chemistry ; ECT = Environmental toxicity on aquatic and terrestrial organisms ; ENF = Behaviour in water, soil and air. Bioaccumulation ; EMN = Studies on effects on mesocosms and natural ecosystems; MUT = Mutagenicity; PCT = Physical-chemical testing ; RES = Residue studies ; OTH = Other, to be specified.

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

RCC Study Number:	856664
Test Item:	emamectin
Study Director:	Dr. R. Phaff
Study Title:	Determination of ^{14}C-Emamectin B1a (^{14}C-NOA 426007) Photolysis Quantum Yield

This study was conducted in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

There were no circumstances that may have affected the quality or integrity of the data.

Study Director: Dr. R. Phaff

.....

Date: May 31, 2005

SIGNATURES

Study Director: Dr. R. Phaff

.....

Date: May 31, 2005

Management: for Dr. U. Morgenroth

.....

Date: May 31. 2005

QUALITY ASSURANCE

RCC Ltd, Environmental Chemistry & Pharmanalytics, CH-4452 Itingen / Switzerland

STATEMENT

RCC Study Number: 856664

Test Item: emamectin

Study Director: Dr. R. Phaff

Study Title: Determination of ^{14}C-Emamectin B1a (^{14}C-NOA 426007) Photolysis Quantum Yield

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were periodically inspected. The study plan and this report were audited by the Quality Assurance. The dates are given below:

Dates and Types of QA Inspections		Dates of Reports to the Study Director and to the Management
November 03, 2004	Study Plan	November 03, 2004
January 04, 2005	Application	January 04, 2005
April 26, 2005	Final report	April 26, 2005

This statement also confirms that this final report reflects the raw data.

Quality Assurance: Ms. I. Wiedeking

Date: May 31, 2005

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PREFACE

GENERAL

Study Title:	Determination of ^{14}C-Emamectin B1a (^{14}C-NOA 426007) photolysis quantum yield
Sponsor:	Syngenta Ltd. Jealott's Hill International Research Centre Bracknell Berkshire RG 42 6EY United Kingdom
Study Monitor:	Dr. G. Nicollier Syngenta Crop Protection AG Global Environmental Safety Assessments/ Ecochemistry 4002 Basel/Switzerland
Test Facility:	RCC Ltd Environmental Chemistry & Pharmanalytics Zelgliweg 1 CH-4452 Itingen / Switzerland

RESPONSIBILITIES

Study Director:	Dr. R. Phaff
Deputy Study Director:	Dr. A. Mamouni
Technical Coordinator:	Ms. A. Kurscheidt
Head of RCC Quality Assurance:	Mrs. Iris Wüthrich

SCHEDULE

Experimental Starting Date:	November 9, 2004
Experimental Completion Date:	April 01, 2005
Study Completion Date:	May 31, 2005

ARCHIVING

RCC Ltd, CH-4452 Itingen/Switzerland will retain the study plan, raw data, a sample of the unlabelled test item and the final report of the present study for at least ten years. No data will be discarded without the sponsor's consent.

DATA REQUIREMENT / TEST GUIDELINES

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

- OECD Guideline for Testing of Chemicals, Proposal for a new Guideline: Phototransformation of Chemicals in Water – Direct and Indirect Photolysis, Draft Document, August 2000.
  GCPF/ECPA –Comments on the Draft Proposal for a New OECD Test Guideline on: "Phototransformation of Chemicals in Water – Direct and Indirect Photolysis", 30 October 2000.

- JMAFF Agchem Test Guidelines 12 Nohsan N. 8147, 24 November 2000, revised 26 June 2001: Photodegradation in water (2-6-2)

- Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, EPA-540/9-82-021, Section 161-2: Photodegradation Studies in Water, U.S. Environmental Protection Agency, October 18, 1982.

- FIFRA Accelerated Reregistration, Phase 3 Technical Guidance, EPA 540/09-90-078, December 1989.

SUMMARY OF STUDY PLAN AMENDMENTS

First Amendment to Study Plan

Concerning:	Alteration:	Reason:
Schedule	New experimental completion date	Additional analyses performed

SUMMARY

The rate of photochemical degradation of ^{14}C-emamectin and its subsequent quantum yield have been determined under simulated sunlight in aqueous buffer solution at pH 7.

The test item was irradiated using a "Suntest" apparatus equipped with a 1.8 kW xenon arc lamp. Filters were used to cut off ultraviolet light with a wavelength below 290 nm. For a representative range (300 nm to 400 nm) of the whole visual light spectrum, the intensity of light was determined to be 47.9 W/m^2 at the surface of the photo-degradation vessels. The intensity of the lamp was similar to the intensity of natural daylight with vertical incidence at temperate climates in June 2004 (50.4 W/m^2, measured in Itingen/Switzerland, latitude 50° N).

Individual samples at an initial concentration of 0.91 mg ^{14}C-emamectin per liter sterile buffer solution at pH 7 were prepared.

Samples were continuously irradiated for a period of 24 hours at a mean temperature of 25.3 ± 0.3 °C. Duplicate irradiated samples were taken for analysis at suitable intervals. Dark control samples were incubated similarly to the irradiated samples but in the dark. All samples were submitted to radiochemical quantification by liquid scintillation counting and chromatographic analysis by high performance liquid chromatography and thin layer chromatography. Total mean recoveries during the study were 100.8% ± 1.7% and 100.8% ± 0.2% of the applied radioactivity for irradiated and dark control samples, respectively.

^{14}C-emamectin was found to be very rapidly photolysed in sterile pH 7 buffer solution. Within 24 hours of irradiation, the test item decreased from 99.3% to 44.6% of the applied radioactivity (mean values).

The number of photons reaching the test solution was determined under the same conditions by actinometry. p-Nitroanisole (PNA) was used as actinometer.

The quantum yield of emamectin was determined to be 1.44 10^{-2} molecules degraded photon^{-1}. This value was used to calculate the environmental half lives of photochemical degradation in water at different latitudes and seasons, using the GC Solar computer program.

Theoretical lifetime (days)*	emamectin			
	Spring	Summer	Fall	Winter
Latitude 30° N	1.49	1.32	2.13	2.95
Latitude 40° N	1.63	1.35	2.81	4.69
Latitude 50° N	1.88	1.42	4.27	9.48

* Conditions: Pure water close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values.

These results demonstrate that emamectin is very rapidly photodegraded in the aquatic environment.

1 PURPOSE

The aims of the study were to provide information on the rate of photolytic degradation of the test item in aqueous systems to calculate the quantum yield. For this purpose, ^{14}C-emamectin B1a (^{14}C-NOA 426007) was irradiated with a Xenon arc light source at 25 ± 1 °C under sterile conditions in aqueous buffer solution at pH 7 where the compound is stable to hydrolysis. Dark control solutions were treated in the same way as the irradiated solutions, except that they were protected from light.

The quantum yield [Φ] (i.e. number of molecules undergoing phototransformation per number of photons absorbed by these molecules) of the disappearance of ^{14}C-emamectin B1a by direct photolysis in aqueous solution was determined. The quantum yield obtained was then used to estimate the lifetime of the chemical in an aquatic environment.

2 MATERIAL AND METHODS

2.1 TEST ITEM

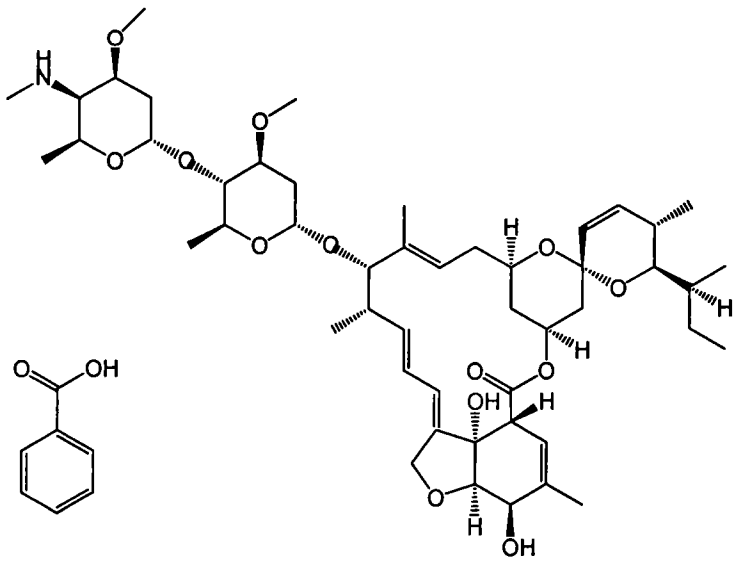
All data on the test item as supplied by the Sponsor.

2.1.1 Unlabelled Test Item

The unlabelled test item was only used for co-chromatography purposes.

Test Item: NOA 426007

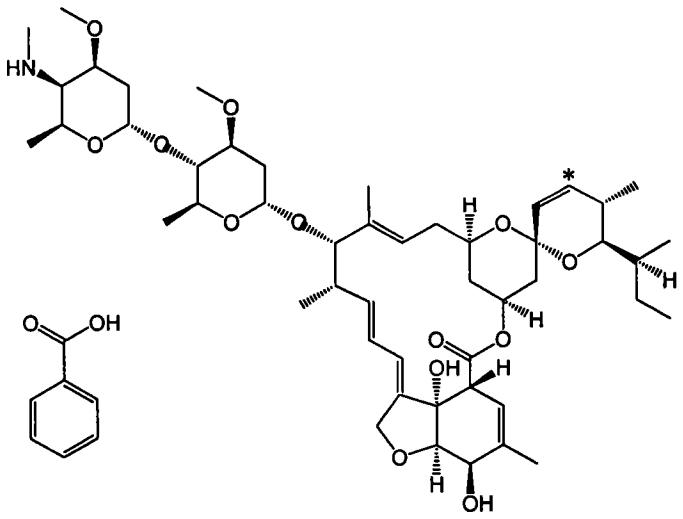
Structural Formula:



Molecular Formula: emamectin Benzoate B1a: $C_{49}H_{75}NO_{13}\cdot C_7H_6O_{22}$

Lot No.:	CDC-XIV-86-1
CAS RN No.:	138511-97-4
Molecular weight:	emamectin Benzoate B1a: 1008.2
Water solubility:	not available
Purity:	97.6%
Re-analysis Date:	January 31, 2006
Stability:	Stable under standard conditions
Storage:	-20°C
Safety Precautions:	special hygienic procedures according to Syngenta safety data sheets.

2.1.2 ^{14}C-Labelled Test Item

Chemical Name CA:	Avermectin A1a, 5-O-demethyl-4"-(methylamino)-23-^{14}C, (4"R)-, benzoate (salt)
Syngenta Code:	[23-^{14}C]-NOA 426007
Structural Formula: * position of labelling	
Lot No.:	WFH-XI-3
Spec. Radioactivity:	2.2126 MBq/mg
Radiochemical Purity:	97.8%; re-determined to be 99.0% before use.
Storage at RCC:	-20°C

Expiry Date:	Not needed since the purity was re-determined before use.
Stability:	It was checked by HPLC using an aliquot of the application solution before and after treatment.
Safety Precautions:	Routine hygienic procedures according to the Swiss Legislation on Radiological Protection (Switzerland, Ordinance of June 22, 1994) and special hygienic procedures according to Syngenta safety data sheets.

2.2 EXPERIMENTAL SET-UP

2.2.1 Test System

Photolysis was performed using cylindrical vessels (diameter 5.8 cm, sample volume 50 ml, corresponding to an aqueous layer of about 2 cm, see Figure 1) placed under the photolysis apparatus and cooled by means of a water jacket connected to a cooling water bath. The vessels were constructed entirely of Pyrex glass and covered with quartz glass plates previously sterilised by rinsing with an ethanol/water solution (70:30; v/v) prior to use.

In addition to the irradiated reaction vessels, duplicate reaction vessels containing 15 ml test solution were incubated under identical conditions but in the dark (control samples).

2.2.2 Test Solutions

Deionised water was further purified using an ELGA water purifier unit, which is a totally enclosed automatic unit producing ultra pure water. This water was used for the preparation of the buffer and actinometer solutions.

Buffer Solution

The following buffer solution was used:

0.210 g potassium dihydrogen phosphate (KH_2PO_4; Fluka 60220) and 0.321 g di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$; Merck 1.06580.0500) were added to a measuring flask and diluted to 2 litres with purified water.

To minimise the process of microbial degradation during incubation, the buffer solution was sterilised by autoclaving for about 30 minutes at 121 °C.

The pH of the buffer solution was re-measured after sterilisation (pH of 6.99).

Actinometry

For the determination of the number of photons entering the test solution, the p-nitroanisole (PNA) actinometer was used (Sections 2.3 and 2.4). The PNA actinometer is one of the actinometers recommended by EPA and OECD. The actinometer is a solution of p-nitroanisole and pyridine in water. The rate of degradation of p-nitroanisole depends on the concentration of pyridine in the solution. This dependence is given by following equation:

$\Phi act = 0.437 \cdot [Pyr] + 0.000282$

2.2.3 Light Source

The study was performed in a "Suntest CPS, Original Hanau" apparatus (Heraeus, Germany), equipped with a 1.8 kW xenon burner and UV filter system (Figure 1).

Xenon Burner:	Max. 765 W/m^2 at max. UV filtering (lambda < 800 nm) with irradiance between 400 W/m^2 and 765 W/m^2 to a pre-set value.
Filters:	UV filter with a 290 nm cut-off to simulate natural sunlight.
Exposure Area:	Approximately 500 cm^2 total area
Vessel:	Exposed area 26.42 cm^2; 50 ml solution
Light Intensity:	Determined with a LI-1800 spectrophotometer at the surface of the irradiated solution (see Section 2.4).

The spectral energy distribution of the Xenon burner measured from 300 to 800 nm, is presented in Figure 2.

2.3 PREPARATION AND APPLICATION OF THE TEST ITEM AND THE ACTINOMETER

Test Item Application Solution

An aliquot (750 µl) of the radiolabelled test item, as delivered by the sponsor (solution in ethanol), was transferred to a 5 ml measuring flask and the ethanol evaporated under a stream of nitrogen. The volume was made up to the mark with acetonitrile (application solution). The total amount of ^{14}C-emamectin present in this solution was determined by liquid scintillation counting (LSC). Based on the total radioactivity measured 60'569'500 dpm and the specific activity of 2.2126 MBq/mg, the concentration of the test item in the solution was calculated to be about 0.091 mg ^{14}C-emamectin / ml.

Actinometer Solution

A stock solution of p-nitroanisole (PNA) was prepared by dissolving 30.63 mg PNA in 20 ml acetonitrile in a volumetric flask. An aliquot of 1 ml of the stock solution was transferred to a 10 ml volumetric flask and the volume made up to the mark with acetonitrile (dilution A).

A volume of 2 ml of this solution was transferred to a 2 L volumetric flask. Thereafter, 1582 mg of pyridine were added and the volume was made up to the mark with purified water. The actinometer solution contained 0.153 mg PNA / L ($1 \cdot 10^{-6}$ M), 791 µg pyridine / L (0.01 M), and 0.1% acetonitrile. The quantum yield of this actinometer solution is 0.004652 molecules degraded photon $^{-1}$.

Application Procedure

Aliquots of 50 ml sterile pH 7 buffer solution were transferred to the photolysis vessels, followed by 500 µl application solution. The initial amount applied (100% applied value) corresponded to 0.91 mg/l.

For the dark control samples, aliquots of 15 ml pH 7 buffer solution and 150 µl application solution were combined in the test vessels.

Following application, all solutions were mixed thoroughly.

For the actinometry part, 50 ml of the actinometer solution were transferred to the photolysis vessels.

2.4 TEST SYSTEM MAINTENANCE AND SAMPLING

Experimental Conditions and Monitoring

The temperature of the test solutions was maintained at 25°C by means of a refrigerated circulator (JULABO F25). The actual temperature in the test solutions was continuously recorded by a data logger.

The intensity of light was measured with a LI-1800 spectrophotometer (Li-Cor Inc./USA) prior to and at the end of irradiation for all four positions under the Suntest.

Sampling Intervals

Duplicate samples were taken after 0, 1.5, 3, 5, 6.5, 10 and 24 hours of continuous irradiation. Since emamectin is known to be stable to hydrolysis, duplicate dark control samples were taken only after 24 hours of incubation.
At each sampling interval, the corresponding samples were analysed as described in Section 2.6.

Volatiles

Since in a preliminary test no volatile radioactivity was detected, no collection of volatiles was performed.

Sterility

The sterility of the buffer solution was determined at the start of the irradiation/incubation periods (Section 2.5). Due to the short incubation period (1 day), there was no need for determination of the sterility of the test solutions at the end of the study.

Actinometer Solution

The actinometer solution was irradiated in the same way as emamectin but for only 100 minutes. Aliquots (200 µl) of the actinometer solution were taken every 20 minutes and analysed by HPLC (Section 2.6.2).

2.5 SAMPLE PREPARATION / PROCESSING

After irradiation, the test item solution was removed and the photolysis vessel rinsed with about 5 ml acetonitrile and combined with the aqueous solution. The volume of the sample was adjusted to about 100 ml with acetonitrile and the radioactivity measured by LSC. Thereafter, the sample was analysed by HPLC and TLC.

The dark control solutions were worked-up in the same way as the irradiated solutions except that the end volume was about 30 ml.

pH Measurements

The pH-value of the test samples (Table 2) was measured with a pH-meter pH330i (WTW, Weilheim/Germany).

Sterility of the Test Solutions

The sterility of the treated buffer solution was checked by plate counts at the start of the irradiation/incubation period.

Aliquots (0.5 ml) of the test solution were uniformly distributed onto the surface of agar plates (Merck, CASO-Agar Caseinpepton-Sojamehlpepton-Agar for Microbiology Merckoplate®) and then incubated at room temperature for up to 6 days. In addition, a positive control (1 ml tap water) and a negative control (1 ml sterile water) were incubated under the same conditions. The colonies that developed on these plates were counted.

2.6 SAMPLE ANALYSIS

2.6.1 Radiocarbon Determination Procedures (LSC)

The quantity of radioactivity was determined by Packard liquid scintillation counters equipped with efficiency and luminescence correction options (TRI-CARB 2500TR, 2550TR, 2700TR, 2900TR or Quantasmart). Quench curves were prepared twice a year and the measurements were automatically corrected. One set of vials from Packard each containing different known radioactivity was measured every two weeks. The result was greater than 95% of their nominal radioactivity. Furthermore, the efficiency of the counters was checked once a day by measurement of a Packard-vial containing a known amount of radioactivity and also compared to its radioactivity. All measurements were performed at least in duplicate, corrected for background, and counted for up to 5 minutes.

The following scintillation mixture was used:

IRGA SAFE Plus (Packard Instruments Comp., Meriden CT, USA)

Measurement of Application and Test Solutions

Aliquots of up to 1 ml from the application and test solutions were measured in 10 ml of the scintillation mixture.

2.6.2 High Performance Liquid Chromatography (HPLC)

HPLC was used as the primary analytical method. The following HPLC conditions were used:

Instruments

Pump:	Merck-Hitachi L-6200, L-6200A or L-7100
Autosampler:	Merck-Hitachi AS-2000, AS-2000A or L-7200
UV-detector:	Merck-Hitachi L-4000 or L-7400
^{14}C-detector:	Packard Flow scintillation analyser, A500 or 500TR
Software used:	FLO-ONE for Windows Analysis, Version 3.6.1, Packard Instrument Co. Inc.

Chromatographic Method 1

For quantification of emamectin.

Pre-column:	Lichrosphere 100 C18, 4 x 4 mm, 5 µm	
Column:	Zorbax RX-C18, 250 mm x 4.6 mm, 5 µm	
Column Temperature:	Ambient	
Mobile Phase:	Solvent A:	0.1% Trifluoroacetic acid in water
	Solvent B:	0.1% Trifluoroacetic acid in acetonitrile

Gradient:

Time (min)	0	5	60	65	66	80
Solvent A (%)	80	80	5	5	80	80
Solvent B (%)	20	20	95	95	20	20

Emamectin had a typical retention time of about 41 minutes.

Chromatographic Method 2

For quantification of p-nitroanisole (PNA)

Pre-column: Lichrosphere 100 C18, 4 x 4 mm, 5 µm
Column: Kromasil 100 C18, 250 mm x 4.6 mm, 5 µm
Column Temperature: Ambient
Mobile Phase: Solvent A: 0.1% Water
Solvent B: 0.1% Acetonitrile

Gradient:

Time (min)	0	3	15	20	20.1	30
Solvent A (%)	80	80	0	0	80	80
Solvent B (%)	20	20	100	100	20	20

p-Nitroanisole had a typical retention time of about 12 minutes.

2.6.3 Thin-Layer Chromatography (TLC)

Two-dimensional TLC (2D-TLC) was used as secondary method to confirm the results obtained by HPLC. 2D-TLC was performed on pre-coated plates (20 cm x 20 cm, layer thickness 0.25 mm) of silica gel 60 F_{254} (SG, normal phase) or RP-18 (RP, reversed phase).

Samples were mixed with the unlabelled test item (dissolved in acetonitrile) and the mixture was applied to the plate (about 2 mm band) by using a Linomat. The plates were developed with chamber saturation of 15 min.

The following solvent systems (SS) were used in the study:

SS 1:	Tetrahydrofuran/methanol/formic acid/water	(60:35:1:4; v/v/v/v)	SG
SS 2:	Chloroform/methanol/water	(75:18:2; v/v/v)	SG
SS 3:	Ethyl acetate/toluene/i-propanol	(70:21:4; v/v/v)	SG
SS 5:	Dioxane/water	(9:1; v/v)	RP
SS 6:	Acetonitrile/water	(9:1; v/v)	RP

SG: Silica Gel
RP: Reversed phase

Detection

The unlabelled test item was visualised by UV-light at a wavelength of 254 nm.

Typical Rf-values for the non-labelled reference item in the different solvent systems are shown below:

Rf-value	SS1	SS2	SS3	SS5	SS6
emamectin	0.53	0.37	0.00	0.70	0.29

Phosphor Imager

All TLC plates were submitted to phosphor imaging. The phosphor imaging was performed on a Fuji BAS 1000. The phosphor imager detects radioactive areas on TLC plates by exposure against a stored phosphor imaging plate. The imaging plate has an image-sensing layer made up of photostimulable phosphor crystals ($BaFBr:Eu^{+}$). When exposed, this sensing layer accumulates and stores the irradiated radioactive energy. Following exposure, the imaging plate was inserted into an image reading unit for scanning with a laser beam which causes the emission of luminescence in proportion to the adsorbed radiation intensity. The luminescence is detected by a photomultiplier tube and converted to electrical signals.

2.6.4 Light Absorption

Absorption spectra were measured with a Perkin Elmer UV/VIS Spectrophotometer Lambda 6. UV-absorption spectra (200 to 400 nm) of emamectin in methanol at a concentration of 52 mg/l and of the actinometer solution in water with 2% acetonitrile at a concentration of 3.06 µg/ml are presented in Figure 3.

2.7 CALCULATIONS

2.7.1 Sample Calculations

The radioactivity detected in the aqueous solutions was expressed in % of the initial radioactivity applied.

$$\text{Percent of applied} = \frac{\text{total dpm in sample}}{\text{initial dpm in sample}} \cdot 100\%$$

In order to express the radioactivity in concentration of parent item equivalents (mg) per unit aqueous solution (l) the following equation was used:

$$\text{Concentration mg/l} = \frac{\text{total dpm in sample}}{SR \cdot CF} \cdot 1000$$

Where:

SR	=	Specific radioactivity:	2.2126 MBq/mg
CF	=	Conversion factor:	$6.0 \cdot 10^7$ dpm/MBq
1000	=	Conversion factor:	ml to l

2.7.2 Limit of Detection and Quantitation

The limits of detection and quantitation are given in Appendix I.

2.7.3 Half-life

The rate of decrease of emamectin in the irradiated samples could be satisfactorily described by linear first-order reaction kinetics.

$$C = C_0 \exp(-k \cdot t)$$

Where:

C	=	Amount of emamectin at any irradiation time (mg/l)
C_0	=	Amount of emamectin at time 0 (mg/l)
t	=	Incubation time (days)
k	=	First-order rate constant ($days^{-1}$)

The experimental data were analysed by linear regression applying the equation above using the program Origin 6.1 (Technical Graphics and Data Analysis in Windows™; MicroCal Software Inc., Northhampton, MA 01060/USA).

The DT-50 value was obtained by using:

$$DT\text{-}50 = \frac{\ln(2)}{k}$$

2.7.4 Quantum Yield Φ

The calculation of the quantum yield was performed according to the method described in the ECETOC Technical Report No. 12 [3]. The arithmetic operations were based on the equations and abbreviations summarised below and in Appendix II. The photolysis quantum yield is defined as:

$$\Phi = \frac{\Delta M\,(t)}{P_{abs}\,(t)}$$

with $\Delta M\,(t)$	=	Number of photo reacted molecules within time t
and $P_{abs}\,(t)$	=	Number of absorbed photons in the same time.

The rate of disappearance of emamectin was calculated by applying linear first-order reaction kinetics (Section 2.7.3).

The number of molecules degraded within time t was calculated by:

$$\Delta M(t) = M_0 - M(t)$$

M_0 is the number of test item molecules at time 0.

Determination of P_{abs} (t)

When incident light of intensity P_{inc} (λ) (or photons P_{inc} (λ)) at a wavelength λ (nm) passes through a filter or other absorbing medium with absorbency E (λ) at a wavelength λ, the total number of absorbed photons P_{abs} per unit surface is calculated from:

$$\sum_{\lambda} P_{abs}(\lambda) = \sum_{\lambda} P_{inc}(\lambda) - \sum_{\lambda} P_{trans}(\lambda)$$

P_{abs} = Absorbed photons or light intensity
P_{inc} = Incident photons or light intensity
P_{trans} = Transmitted (or non-absorbed) photons or light intensity

Details of calculation of the P_{abs} (λ) are given in Appendix II.

2.7.5 Environmental Lifetime

The quantum yield value was used for the estimation of the half-life of emamectin in an aquatic environment at different latitudes. The theoretical half-life was calculated by considering the direct phototransformation for midday sunlight conditions, in the top millimetres of natural aquatic systems.

The real half-life was calculated by considering the following factors which determine the sunlight intensities available for direct photolysis:

- Those which determine the solar light intensity incident upon the upper layer of the water.
- Those which determine the penetration of light into water and the absorption of light by organic matter dissolved in water (e.g. river water).

For the calculation of the expected half-life of emamectin, the computer program GCSOLAR from EPA (Version 1.20, July 1999) [6] was used. The conditions used were direct photolysis in pure water close to the surface (0-5 mm depth), clear sky and typical ozone concentrations in the atmosphere. Additionally, the obtained quantum yield value and the absorption data of a 52 mg emamectin/l solution in methanol were used.

The absorption spectrum of a typical river water sample (River Rhine) was used in the computer program GCSOLAR from EPA (Version 1.20, July 1999) to calculate the environmental half-life in natural water down to 30 cm depth. The same atmospheric conditions as above were chosen. The calculations were performed for all seasons at latitude 40° N.

3 RESULTS AND DISCUSSION

3.1 PHYSICAL CONDITIONS

The radiochemical purity of ^{14}C-emamectin in the application solution was determined by HPLC to be 99.0% before treatment (Figure 5). After treatment, the content of the test item was still 97.9%, thereby indicating its stability in the application solution during the treatment procedure (Figure 5).

The pH at the start of the photolysis was measured to be 6.99 and 7.02 for the replicates. After 24 hours of irradiation the pH was 7.03 and 7.06. For the dark control samples, the pH was 7.00 and 7.05 after 24 hours of incubation proving that the pH remained constant during the entire incubation period.

No colonies of bacteria formed in the test solution at study start thereby proving its sterility. Due to the short irradiation/ incubation time, no sterility test was performed at study end.

The light intensity of the apparatus was measured by photometry (Figure 2). The exact intensity of the light acting on the test solutions was measured by actinometry (Appendix II). The mean radiant flux incident on the receiving surface (irradiance) in the range of 300 nm to 400 nm was 47.9 W/m² (Table 5). This value was similar when compared to the light intensity of 50.4 W/m² for natural daylight during summer with vertical incidence of the sun on a clear, cloudless day (measured in Itingen/Switzerland, latitude 50° N, in June 2004; Figure 2).

The mean temperature during the study was 25.3 ± 0.3 °C.

3.2 MATERIAL BALANCE

The results obtained are presented in percent of applied radioactivity and mg parent equivalents/L in Table 1.
The total mean recoveries from the irradiated and dark control samples during the study were 100.8% ± 1.7% and 100.8% ± 0.2% of the applied radioactivity, respectively.

3.3 PHOTODEGRADATION OF ^{14}C-EMAMECTIN

The results are shown in Table 2 (irradiated) and Table 3 (dark control). HPLC chromatograms are presented in Figure 6 to Figure 9. The HPLC results were confirmed by 2D-TLC analysis (see Figure 10 to Figure 13). A graphical representation showing the degradation of ^{14}C-emamectin is shown in Figure 4.

^{14}C-emamectin was rapidly photo-degraded in pH7 buffer solution. Under irradiation the amount of ^{14}C-emamectin decreased from 99.3% initially to 44.6% within 24 hours (Table 2). Besides the test item, a large number of radioactive fractions were detected.

No degradation of ^{14}C-emamectin was observed in the samples incubated under the same conditions but in the dark. ^{14}C-emamectin represented 99.3% and 97.8% of the applied radioactivity at the start and end of the experiment, respectively (Table 3).

3.4 KINETICS OF PHOTODEGRADATION

The rate of photolytic degradation of ^{14}C-emamectin was described using a first-order reaction kinetic model (see Figure 4). The experimental half-life (DT_{50}) was calculated to be 21 hours (or 0.89 days).

3.5 QUANTUM YIELD

For the calculation of the quantum yield of emamectin, a Microsoft Excel based worksheet was used. Details of the calculation of the quantum yield are presented in Section 2.7.4 and in Appendix II. The quantum yield of emamectin was determined to be:

$$\Phi \text{ (emamectin)} = 1.44 \cdot 10^{-2} \text{ molecules degraded photon}^{-1}$$

3.6 ESTIMATED HALF-LIVES IN THE ENVIRONMENT

For the estimation of the environmental half-lives of emamectin, the computer program GCSOLAR from EPA [6] was used. Direct photolysis at the surface of pure water was assumed at 30° N, 40° N and 50° N of latitude. The following half-life values were obtained:

Theoretical lifetime (days)*	emamectin			
	Spring	Summer	Fall	Winter
Latitude 30° N	1.49	1.32	2.13	2.95
Latitude 40° N	1.63	1.35	2.81	4.69
Latitude 50° N	1.88	1.42	4.27	9.48

* Conditions: Pure water close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values.

The absorption spectra of a typical river water sample (River Rhine) were used in CGSOLAR to calculate the environmental lifetime in natural water down to 30 cm depth. The same atmospheric conditions as above were chosen. The calculations were performed for latitude 40° N.

Theoretical lifetime (days) in river water	emamectin			
	Spring	Summer	Fall	Winter
Depth 0 cm	1.63	1.35	2.81	4.69
Depth 10 cm	1.78	1.47	3.06	5.10
Depth 20 cm	1.93	1.60	3.33	5.53
Depth 30 cm	2.08	1.73	3.60	5.97

4 CONCLUSION

The rate of photochemical degradation of emamectin was determined in aqueous buffer solution at pH 7 with simulated sunlight. A "Suntest" half-life of 21 hours of continuous irradiation was calculated using first order kinetics. No degradation was observed in the dark control samples. A quantum yield for the photochemical reaction was determined to be $1.44 \cdot 10^{-2}$ molecules degraded $photon^{-1}$.

Using the quantum yield, the half-lives of emamectin under aqueous conditions at latitudes between 30° N and 50° N were calculated and shown to range from 1.32 to 9.48 days depending on the latitude and season.

It can be concluded that emamectin is very rapidly photochemically degraded in aqueous systems under natural sunlight.

5 REFERENCES

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[7] Leifer A, The Kinetics of Environmental Aquatic Photochemistry, Theory and Practice. ACS Professional Reference Book, 1988.

6 TABLES

Table 1: Balance of radioactivity in the irradiated (top and upper middle) and dark control (lower middle and bottom) samples. Values expressed as % of applied radioactivity and mg parent equivalents/l.

Irradiated

Irradiated (% applied) Sterile buffer (pH 7)	Sample	Irradiation Time in hours								
		0	1.5	3	5	6.5	10	24	Mean	SD
Concentration in sample	A	102.0	103.2	101.5	99.3	103.1	97.5	100.4	101.0	1.9
	B	100.0	103.2	102.1	99.9	101.4	98.6	98.8	100.6	1.6
	mean	**101.0**	**103.2**	**101.8**	**99.6**	**102.3**	**98.1**	**99.6**	**100.8**	**1.7**

Irradiated (mg p.e./l) Sterile buffer (pH 7)	Sample	Irradiation Time in hours								
		0	1.5	3	5	6.5	10	24	Mean	SD
Concentration in sample	A	0.931	0.941	0.926	0.906	0.941	0.890	0.916	0.922	0.017
	B	0.913	0.942	0.932	0.911	0.925	0.900	0.901	0.918	0.015
	mean	**0.922**	**0.941**	**0.929**	**0.909**	**0.933**	**0.895**	**0.909**	**0.920**	**0.015**

Dark control

Dark control (% applied) Sterile buffer (pH 7)	Sample	Irradiation Time in hours			
		0	24	Mean	SD
Radioactivity in sample	A	102.0	99.8	100.9	1.1
	B	100.0	101.2	100.6	0.6
	mean	**101.0**	**100.5**	**100.8**	**0.2**

Dark control (mg p.e./l) Sterile buffer (pH 7)	Sample	Irradiation Time in hours			
		0	24	Mean	SD
Concentration in sample	A	0.931	0.911	0.921	0.010
	B	0.913	0.924	0.918	0.005
	mean	**0.922**	**0.917**	**0.919**	**0.002**

SD: Standard deviation

Table 2: Degradation pattern in the irradiated samples treated with ^{14}C-emamectin. Values are expressed as % of applied radioactivity (top) and in mg parent equivalents/l (bottom).

Irradiated pH 7 buffer Pattern (% applied)	Sample	Irradiation Time in Hours						
		0	1.5	3	5	6.5	10	24
emamectin	A	100.6	96.9	86.0	85.7	86.3	68.1	45.0
	B	98.0	96.3	87.4	86.5	89.4	70.1	44.2
	Mean	**99.3**	**96.6**	**86.7**	**86.1**	**87.9**	**69.1**	**44.6**
Others	A	1.3	6.2	15.4	13.6	16.9	29.5	55.4
	B	2.0	6.9	14.7	13.4	12.0	28.6	54.6
	Mean	**1.7**	**6.6**	**15.1**	**13.5**	**14.4**	**29.0**	**55.0**
TOTAL	A	**102.0**	**103.2**	**101.5**	**99.3**	**103.1**	**97.5**	**100.4**
	B	**100.0**	**103.2**	**102.1**	**99.9**	**101.4**	**98.6**	**98.8**

Irradiated pH 7 buffer Pattern (mg/l)	Sample	Irradiation Time in Hours						
		0	1.5	3	5	6.5	10	24
emamectin	A	0.918	0.885	0.785	0.782	0.787	0.621	0.411
	B	0.895	0.879	0.797	0.789	0.816	0.639	0.403
	Mean	**0.906**	**0.882**	**0.791**	**0.785**	**0.802**	**0.630**	**0.407**

Table 3: Amount of ^{14}C-emamectin in the dark control samples expressed as % of applied radioactivity (top) and as mg parent equivalents/l (bottom).

Dark control pH 7 buffer Pattern (% applied)	Sample	Irradiation Time in Hours	
		0	24
emamectin	A	100.6	97.1
	B	98.0	98.5
	Mean	**99.3**	**97.8**
Others	A	1.3	2.7
	B	2.0	2.7
	Mean	**1.7**	**2.7**
TOTAL	A	**102.0**	**99.8**
	B	**100.0**	**101.2**

Dark control pH 7 buffer Pattern (mg/l)	Sample	Irradiation Time in Hours	
		0	24
emamectin	A	0.918	0.886
	B	0.895	0.899
	Mean	**0.906**	**0.892**

Table 4: Comparison of 2D-TLC and HPLC.

Interval	Name of fraction	2D-TLC (% ROI)	HPLC (% peak)
0 h A	Emamectin others	95.6 4.4	98.7 1.3
5 h A	Emamectin others	88.4 11.6	86.3 13.7

ROI: Region of Interest

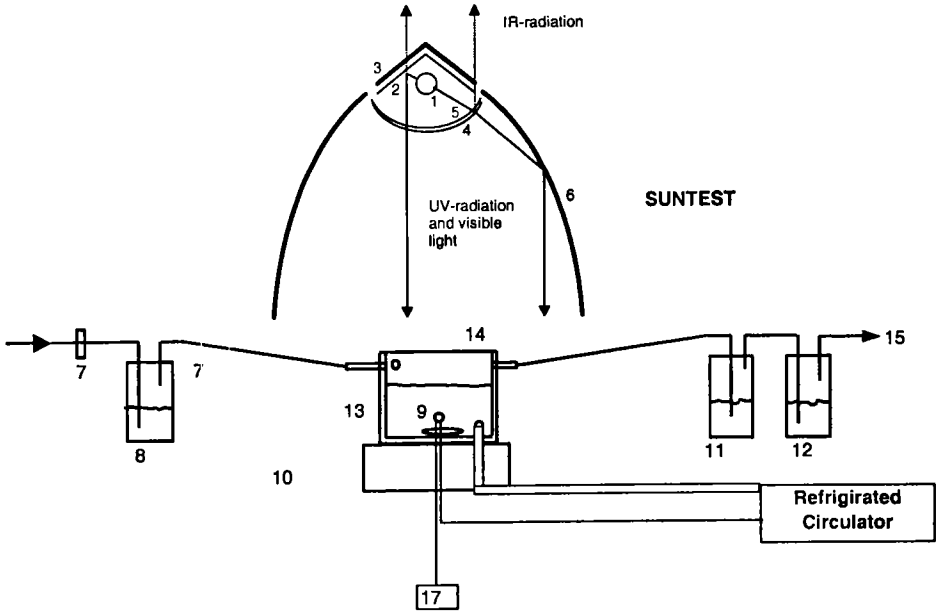
Table 5: Intensity of the Suntest apparatus and Sunlight at the test facility (47.5°N).

Position in Suntest Apparatus	Integral Irradiance in the Wavelength Range of 300 - 400 nm			Ratio* of Light Intensities "r"
	Start (Watt/m^2)	End (Watt/m^2)	Mean Start and End (Watt/m^2)	
1	43.38	46.12	44.75	0.89
2	52.79	55.95	54.37	1.08
3	44.01	46.47	45.24	0.90
4	44.84	49.93	47.39	0.94
Average	**46.26**	**49.62**	**47.94**	**0.95**
STD	**4.40**	**4.56**	**4.44**	**0.09**
%	**9.51**	**9.19**	**9.26**	**9.26**
Sunlight June 2004	**50.41**			

* Suntest to sunlight.

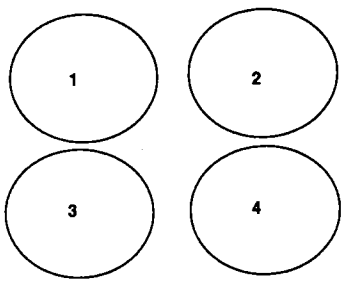
7 FIGURES

Figure 1: Aqueous photolysis apparatus for ^{14}C-emamectin.



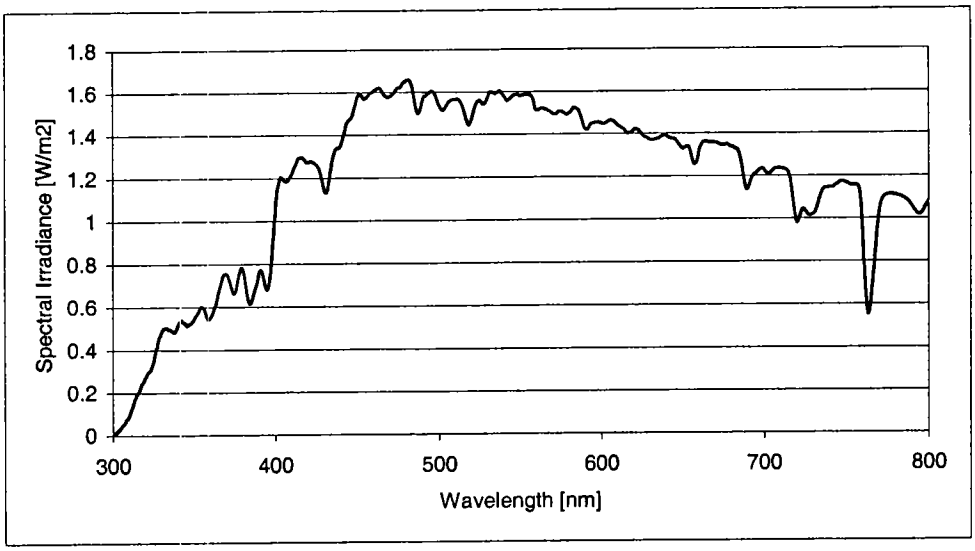
1 Xenon burner
2 UV mirror
3 Light mirror
4 Quartz glass dish with selective reflecting coating
5 Supplementry filter made of special UV glass
6 Parabolic reflector
7 Filter to sterilize the incoming air
8 Sterile bidistilled water to moisten the incoming air
9 Septum for sample collection
10 Magnetic stirrer
11 50 ml 2 N NaOH*
12 50 ml ethylene glycol*
13 Incubation vessel with double glass wall connected to a waterbath for thermoregulation
14 Quartz glass plate
15 Vacuum purnp
16 Temperature controller
17 Temperature logger

Positions of four samples under Suntest



* Based on the results of a pre-test a volatile trapping was not necessary since no volatile compounds were formed.

Figure 2: Comparison of the spectral energy distribution of the sun radiation in Itingen (top; RCC Ltd. facility in June 2004, 47.5° N, 7.8° E) and distribution of the "Suntest" apparatus (bottom).



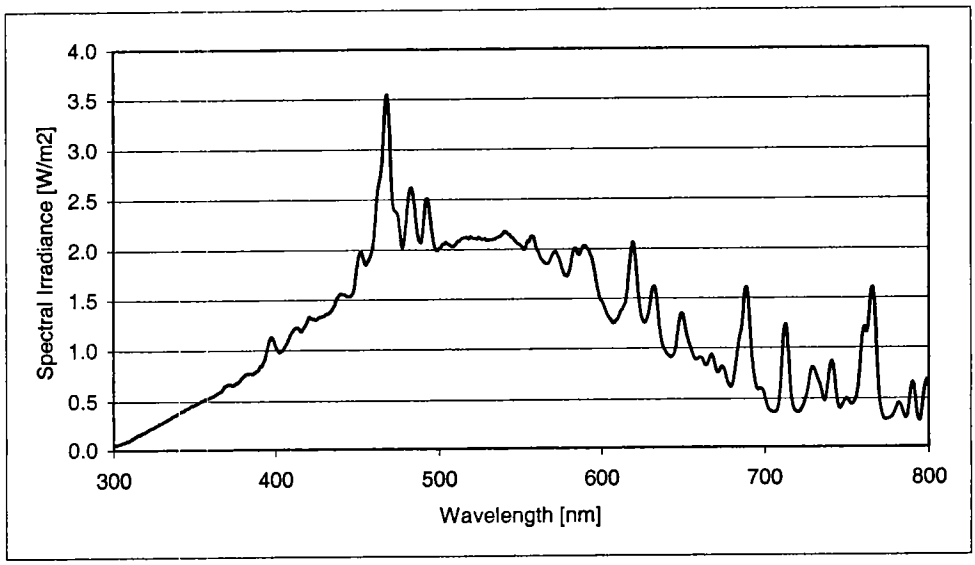
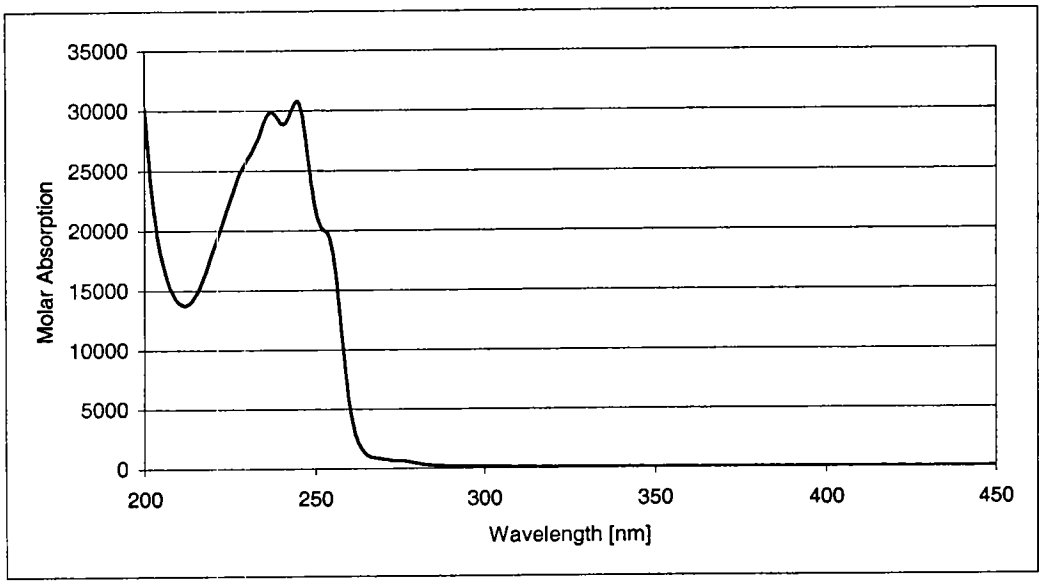


Figure 3: Absorption spectra of emamectin (52 mg/l) in methanol (top) and of p-nitroanisole (3.06 µg/ml) in water with 2% acetonitrile (bottom).



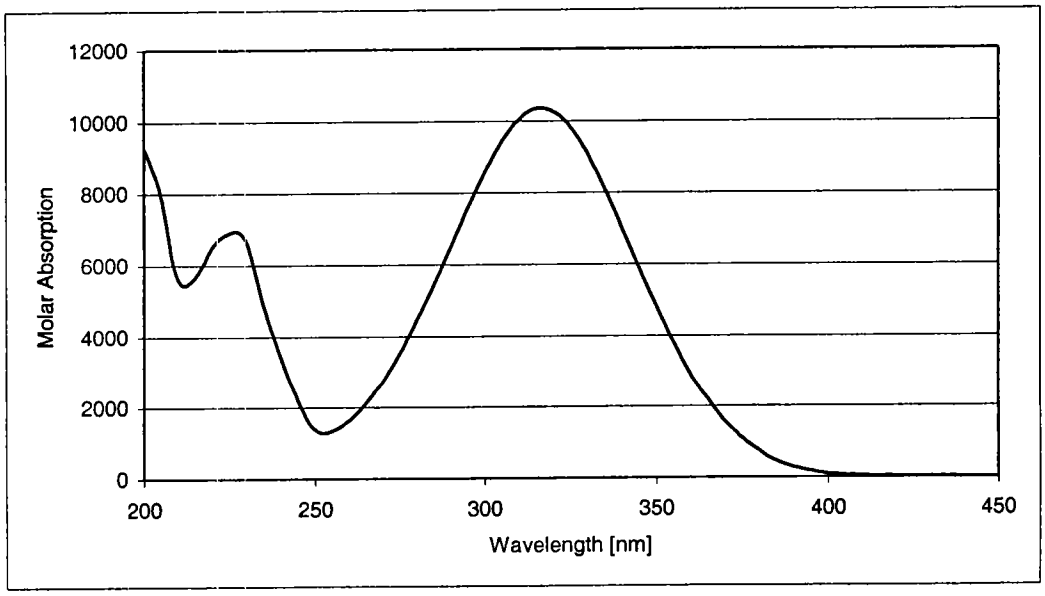


Figure 4: Degradation of ^{14}C-emamectin in pH 7 buffer solution.

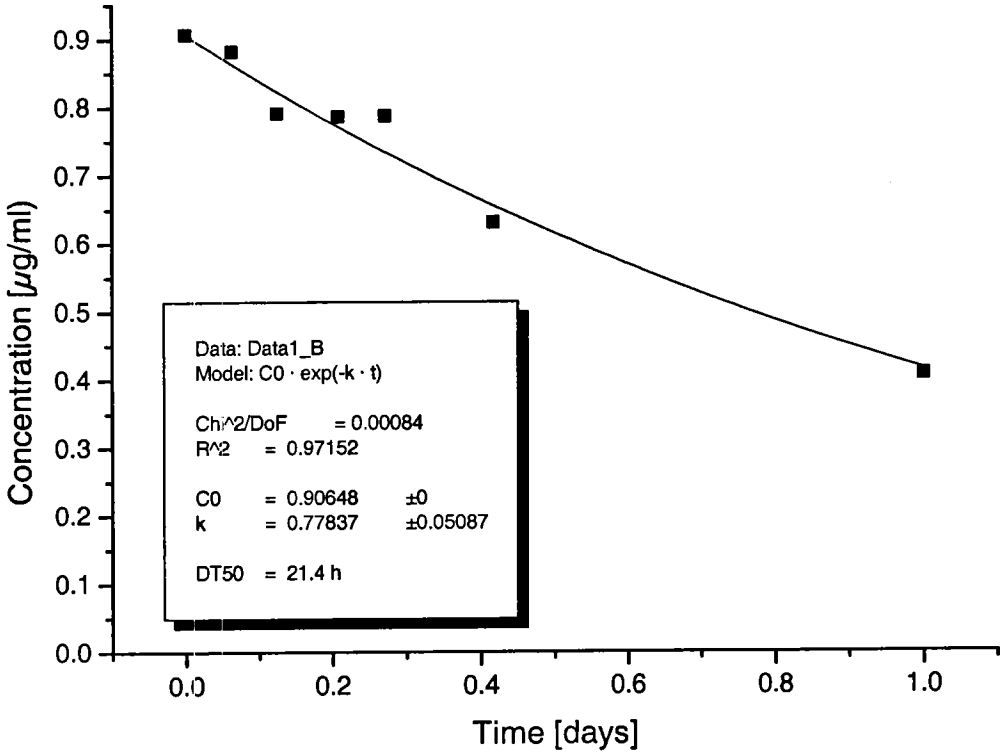
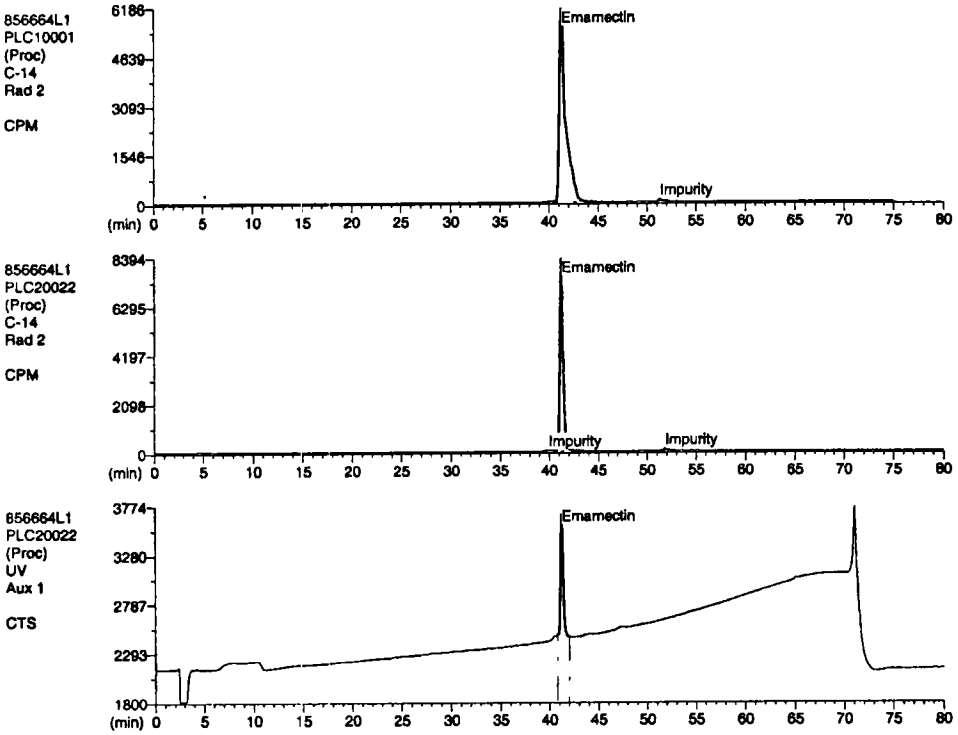


Figure 5: HPLC chromatogram of the radiochemical purity of ^{14}C-labelled emamectin before (top) and after (middle) application and co-chromatography with reference compound (bottom).



Peak Report: PLC10001 Channel 2 C-14 (CPM)

Pk #	Name	Ret. Time Mins	Start Time Mins	Net Area CPM	Stop Time Mins	% Peaks	Height CPM
1	Emamectin	41.20	39.30	48342	44.80	98.98	6186
2	Impurity	51.30	51.00	498	52.30	1.02	90
				48840			

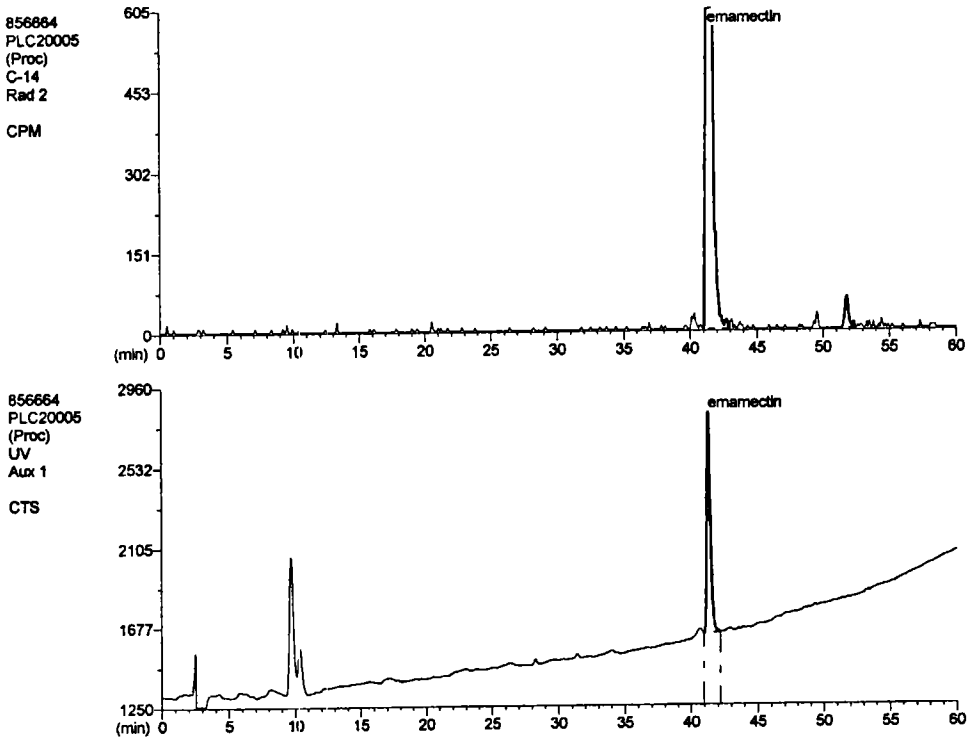
Total Run Area: 49980

Peak Report: PLC20022 Channel 2 C-14 (CPM)

Pk #	Name	Ret. Time Mins	Start Time Mins	Net Area CPM	Stop Time Mins	% Peaks	Height CPM
1	Impurity	39.90	39.30	360	40.70	1.05	48
2	Emamectin	41.20	40.70	33672	43.50	97.86	8394
3	Impurity	51.80	51.30	378	52.90	1.10	102
				34410			

Total Run Area: 35426

Figure 6: HPLC chromatogram of the day 0 sample, replicate A (top). Corresponding UV co-chromatography with unlabelled emamectin (bottom).

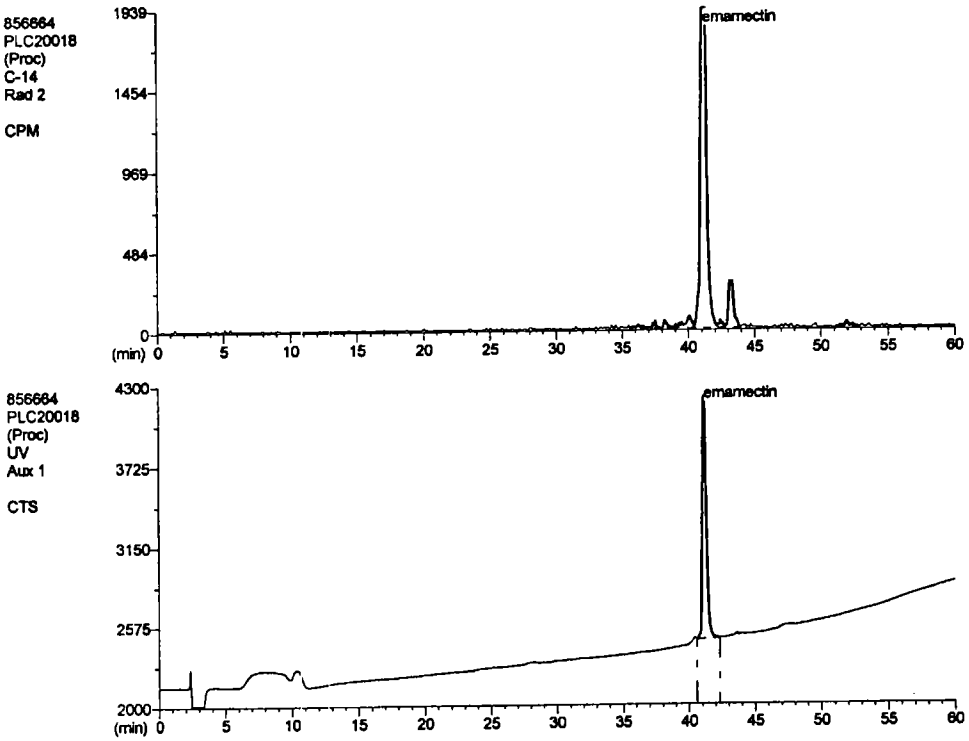


Peak Report: PLC20005 Channel 2 C-14 (CPM)

Pk #	Name	Ret. Time Mins	Start Time Mins	Net Area CPM	Stop Time Mins	% Peaks	Height CPM
1	emamectin	41.30	41.00	15294	42.90	98.68	4038
2		51.80	51.40	204	52.40	1.32	60
				15498			

Total Run Area: 16304

Figure 7: HPLC chromatogram of the 5 hours sample, replicate A (top). Corresponding UV co-chromatography with unlabelled emamectin (bottom).

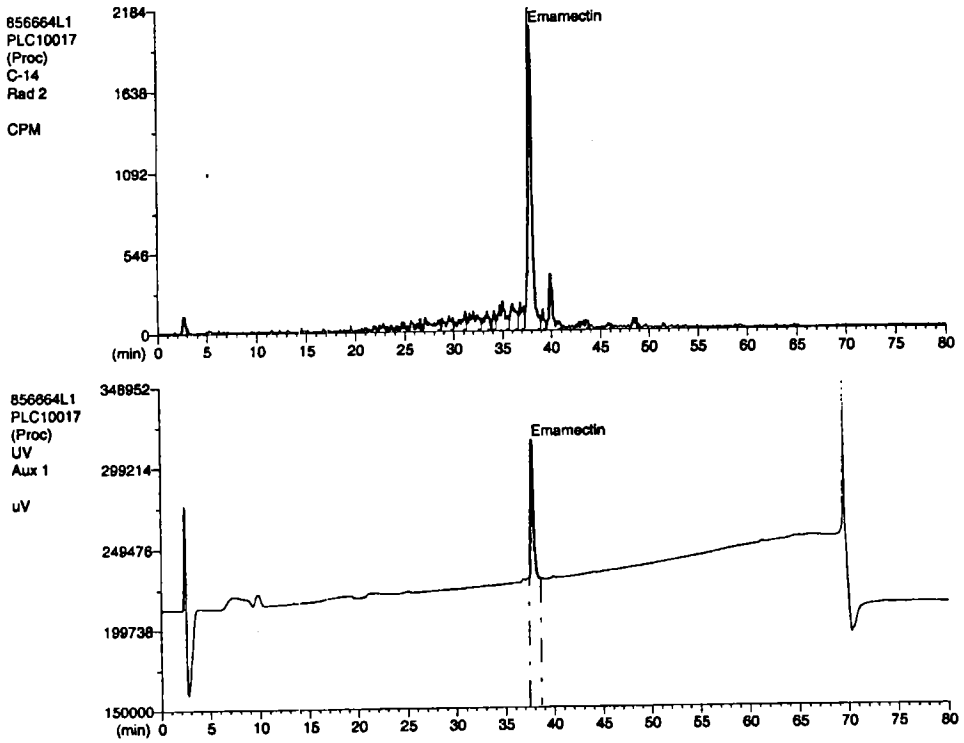


Peak Report: PLC20018 Channel 2 C-14 (CPM)

Pk #	Name	Ret. Time Mins	Start Time Mins	Net Area CPM	Stop Time Mins	% Peaks	Height CPM
1		37.50	35.60	240	37.90	1.38	48
2		38.20	38.00	150	38.90	0.86	48
3		40.10	38.90	468	40.40	2.70	72
4	emamectin	41.10	40.40	14982	42.70	86.28	3438
5		43.10	42.70	1356	43.90	7.81	282
6		51.90	51.10	168	52.80	0.97	36
				17364			

Total Run Area: 18484

Figure 8: HPLC chromatogram of the 24 hours sample, replicate A (top). Corresponding UV co-chromatography with unlabelled emamectin (bottom).

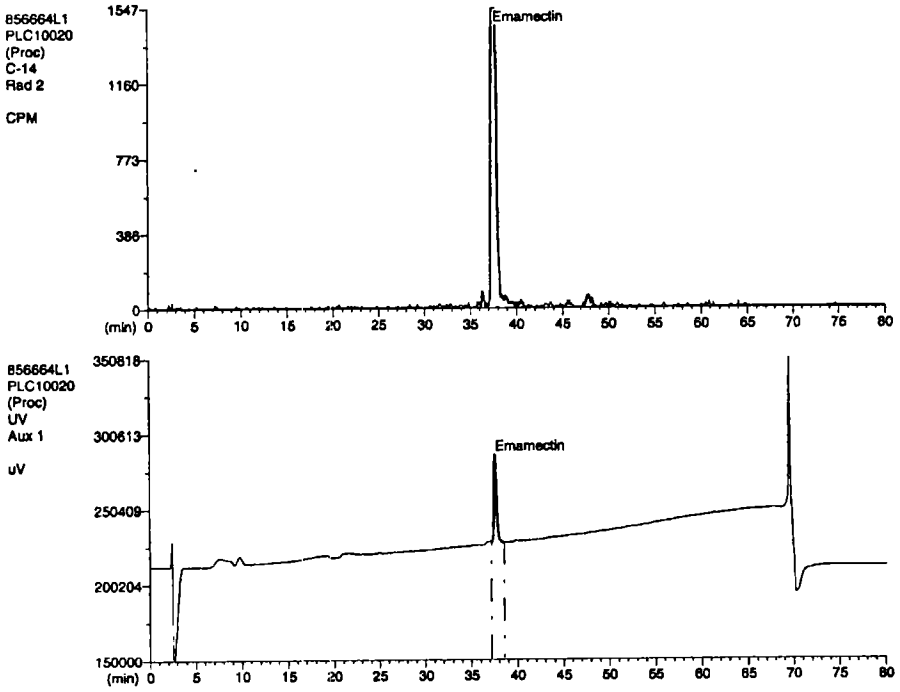


Peak Report: PLC10017 Channel 2 C-14 (CPM)

Pk #	Name	Ret. Time Mins	Start Time Mins	Net Area CPM	Stop Time Mins	% Peaks	Height CPM
1		2.60	2.40	380	3.10	1.62	110
2		22.80	20.80	408	23.30	1.73	48
3		24.70	23.30	408	25.40	1.73	56
4		26.60	25.40	504	26.90	2.14	80
5		27.10	26.90	832	28.70	3.54	88
6		29.50	28.70	760	30.00	3.23	96
7		31.20	30.00	824	31.30	3.50	128
8		32.00	31.30	1192	32.80	5.07	128
9		33.40	32.80	704	33.80	2.99	128
10		34.10	33.80	416	34.30	1.77	128
11		35.00	34.30	1528	35.70	6.50	192
12		36.00	35.70	1192	36.60	5.07	176
13		36.80	36.60	920	37.30	3.91	184
14	emamectin	37.80	37.30	10552	38.90	44.86	2184
15		39.10	38.90	384	39.50	1.63	136
16		39.90	39.50	1624	41.10	6.90	376
17		43.50	42.30	440	43.90	1.87	56
18		45.80	45.00	168	46.20	0.71	32
19		48.40	48.10	288	49.10	1.22	64
				23524			

Total Run Area: 24922

Figure 9: HPLC chromatogram of the 24 hours dark control sample, replicate A (top). Corresponding UV co-chromatography with unlabelled emamectin (bottom).



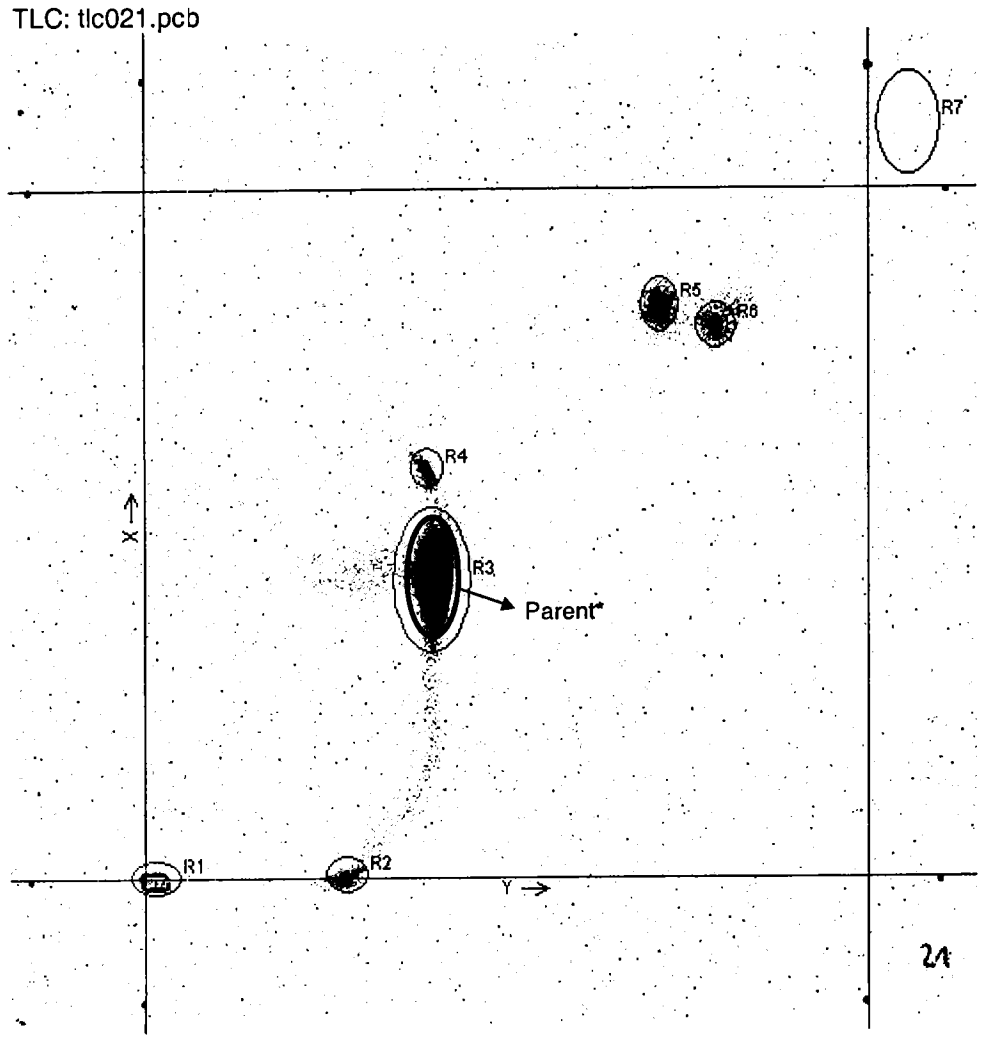
Peak Report: PLC10020 Channel 2 C-14 (CPM)

Pk #	Name	Ret. Time Mins	Start Time Mins	Net Area CPM	Stop Time Mins	% Peaks	Height CPM
1		36.30	35.70	288	36.90	1.05	80
2	Emamectin	37.50	36.90	26648	40.10	97.28	6448
3		40.50	40.10	112	40.80	0.41	32
4		47.70	47.20	344	48.40	1.26	56
				27392			

Total Run Area: 28628

Figure 10: 2D-TLC chromatogram of the day 0 sample, replicate A.

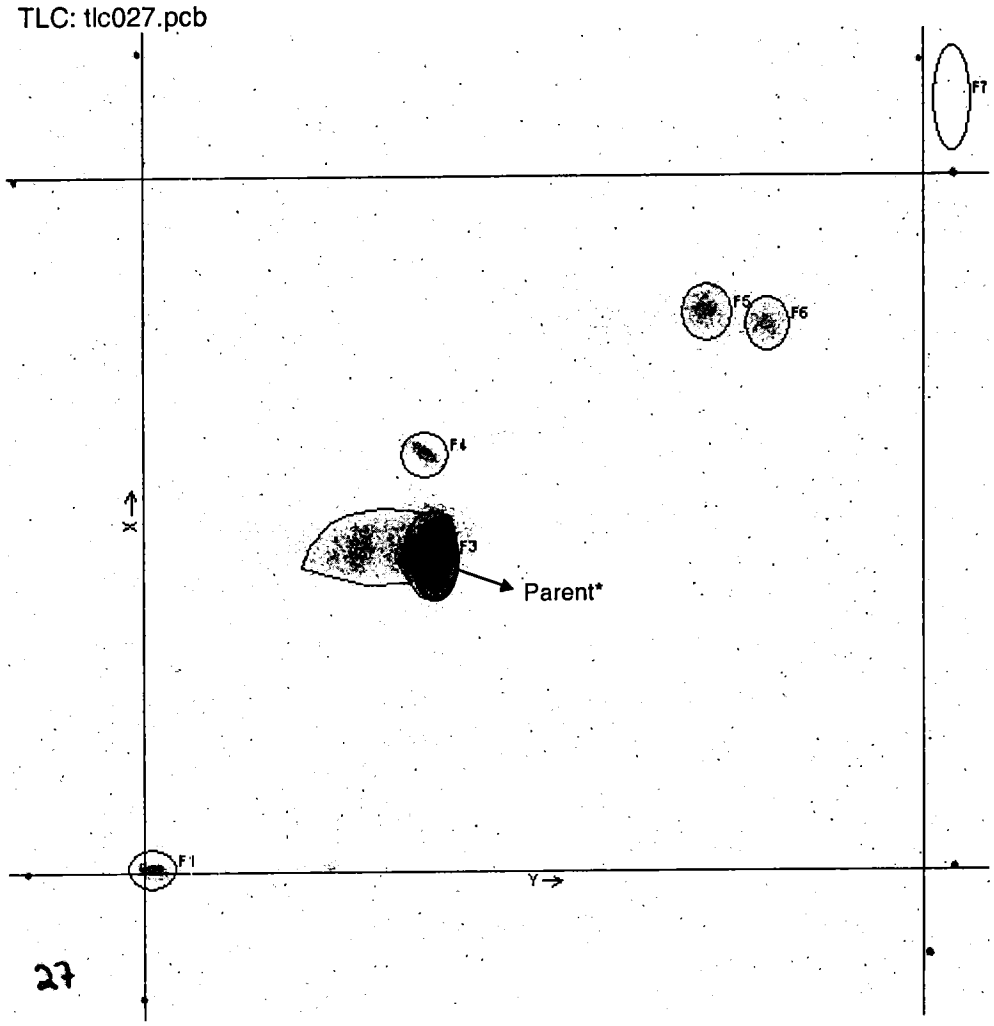
x: SS1
y: SS2
*: Co-chromatography with reference item.



No	Name	Area [mm²]	RF-X	RF-Y	PSL	%(PSL)	PSL-Bkg	%(PSL-Bkg)
1		59.68	--	0.01	278.01	0.91	255.33	0.84
2		52.60	0.00	0.28	226.21	0.74	206.22	0.68
3	emamectin	376.00	0.44	0.40	29178.70	95.26	29035.84	95.59
4		45.00	0.59	0.39	164.94	0.54	147.85	0.49
5		72.56	0.83	0.71	490.77	1.60	463.20	1.52
6		63.24	0.80	0.79	290.77	0.95	266.74	0.88
7	Bkg	218.32	--	--	82.95	0.27	0.00	0.00
-	Sum	669.08	--	--	30629.40	100.00	30375.18	100.00
-	Ttl	40101.60	--	--	48257.47	157.55	33020.94	108.71

Figure 11: 2D-TLC chromatogram of the sample irradiated for 5 hours, replicate A.

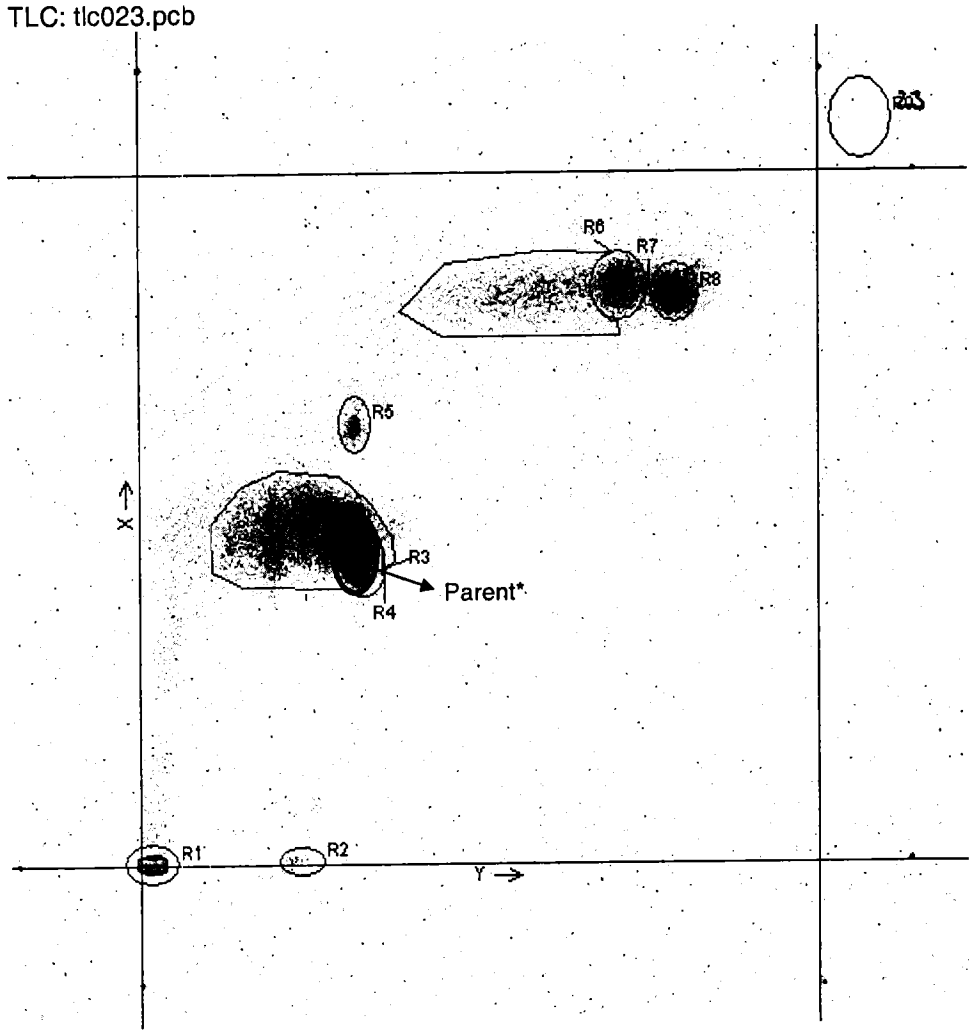
x: SS1
y: SS2
*: Co-chromatography with reference item.



No	Name	Area [mm²]	RF-X	RF-Y	PSL	%(PSL)	PSL-Bkg	%(PSL-Bkg)
1		65.76	0.01	0.01	335.80	1.33	300.05	1.21
2		264.84	0.47	0.29	1736.41	6.89	1592.42	6.42
3	emamectin	165.00	0.45	0.37	22005.70	87.31	21915.99	88.38
4		76.28	0.60	0.36	271.90	1.08	230.43	0.93
5		100.24	0.81	0.72	510.67	2.03	456.17	1.84
6		80.08	0.79	0.80	345.01	1.37	301.47	1.22
7	Bkg	135.04	--	--	73.42	0.29	0.00	0.00
-	Sum	752.20	--	--	25205.49	100.00	24796.52	100.00

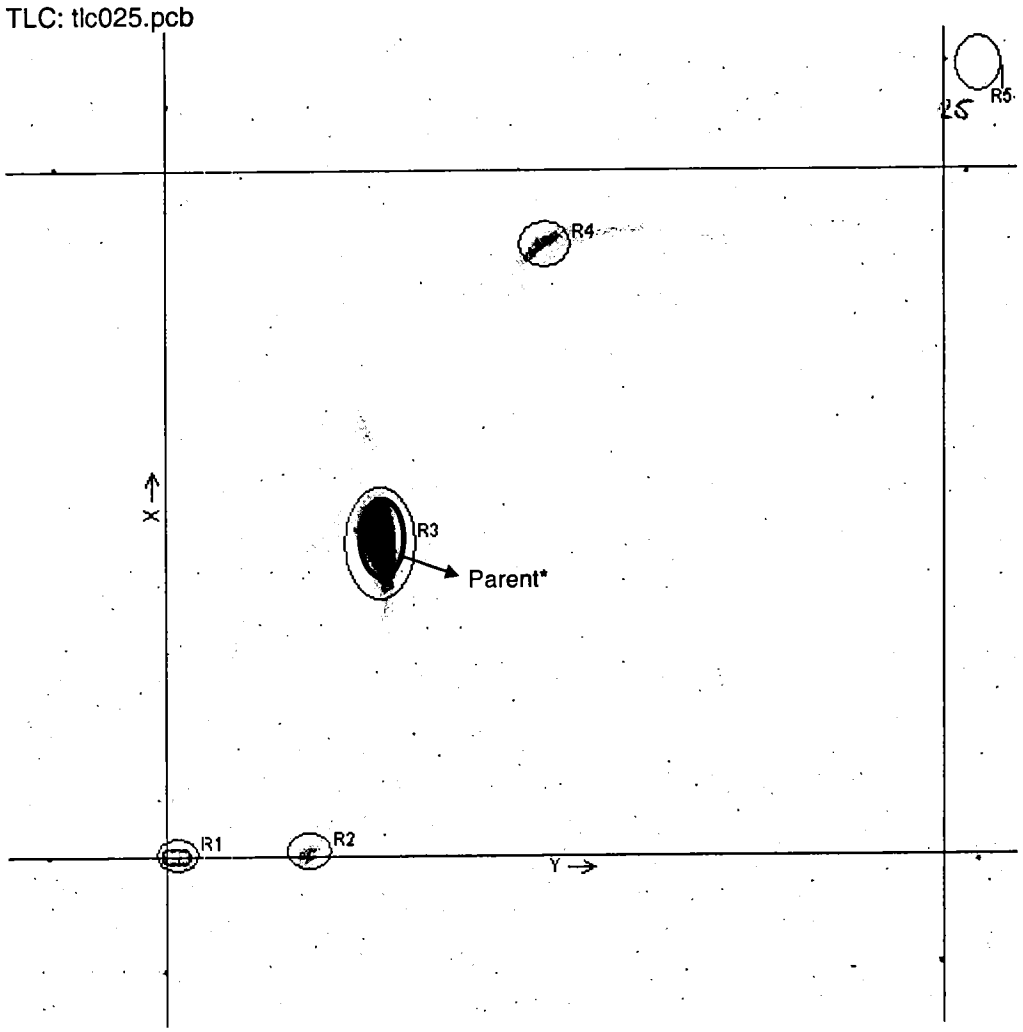
Figure 12: 2D-TLC chromatogram of the sample irradiated for 24 hours, replicate A.

x: SS1
y: SS2
*: Co-chromatography with reference item.



No	Name	Area [mm²]	RF-X	RF-Y	PSL	%(PSL)	PSL-Bkg	%(PSL-Bkg)
1		73.28	0.01	0.02	474.30	2.85	453.07	2.81
2		45.08	0.01	0.23	81.50	0.49	68.45	0.42
3	emamectin	591.36	0.49	0.23	4068.00	24.48	3896.73	24.18
4		140.72	0.46	0.31	7984.55	48.04	7943.79	49.29
5		64.40	0.64	0.32	214.88	1.29	196.23	1.22
6		583.64	0.82	0.56	1517.84	9.13	1348.81	8.37
7		134.72	0.84	0.71	1149.34	6.92	1110.32	6.89
8		97.56	0.83	0.79	1128.83	6.79	1100.57	6.83
9	Bkg	168.40	--	--	48.77	0.29	0.00	0.00
-	Sum	1730.76	--	--	16619.24	100.00	16117.98	100.00

Figure 13: 2D-TLC chromatogram of the dark control sample incubated for 24 hours, replicate A.



No	Name	Area [mm²]	RF-X	RF-Y	PSL	%(PSL)	PSL-Bkg	%(PSL-Bkg)
1		47.44	0.00	0.01	199.77	1.74	196.49	1.71
2		55.24	0.01	0.18	107.12	0.93	103.30	0.90
3	emamectin	267.72	0.47	0.27	11015.50	95.76	10996.99	95.86
4		81.16	0.89	0.49	181.34	1.58	175.73	1.53
5	Bkg	87.32	--	--	6.04	0.05	0.00	0.00
-	Sum	451.56	--	--	11503.73	100.00	11472.51	100.00

*: Co-chromatography with reference item.

APPENDIX I

LIMITS OF DETECTION AND QUANTITATION
VALIDATION OF HPLC METHOD

LIMITS OF DETECTION FOR LSC

General Data

Project	RCC No. 856664
Sample i.d.	24 hours, duplicate A
	Irradiated

Specific radioactivity	(MBq/mg)	2.2126
	(μCi/mg)	59.800
Specific radioactivity	(dpm/mg)	132'756'000
Amount applied per sample	(dpm)	6'662'718
Amount measured Aq. Phase	(dpm)	6'690'356
Amount measured Aq. Phase	(%)	100.4
Volume of Aq. Phase	(ml)	94.0

Limit of Detection and Quantitation for Liquid Scintillation Counting

Photolysis Solution

	Size	dpm	Total dpm	%-applied	Parent equiv. (mg)	Concentration (mg/l)
Sample size measured (ml)	0.5					
Volume of Aq. Phase	94.0					
BG (Background LSC) :		18	3384	0.0508	0.000025	0.00027
LD (Limit of Detection ;2x BG)		36	6768	0.1016	0.000051	0.00054
LQ (Limit of Quantitation ;3xBG)		54	10152	0.1524	0.000076	0.00081

LIMITS OF DETECTION FOR HPLC

Irradiated, 24 hours, Duplicate A

HPLC Data

HPLC-No	PLC 10017	**Background (cpm)**	12
Project	RCC 856664	**Flow (ml/min)**	1
Sample i.d.	Irradiated	**Efficiency (%)**	70.4
Sample	Duplicate A	**Actual Cell volume (ml)**	0.17
Day	24 hours	**Volume of Flow cell (void; ml)**	0.5
DPM injected	24911	**Flow scintillator (ml/min)**	2
Injection vol. (µl)	350		
Date	17.03.2005		

Irradiated, 24 hours, duplicate A; BG: 12 cpm (Cf. Figure 8)

Chromatogram Report

Peak Report: PLC10017 Channel 2 C-14 (CPM)

Pk #	Name	Ret. Time Mins	Start Time Mins	Net Area CPM	Stop Time Mins	% Peaks	Height CPM
1		2.60	2.40	380	3.10	1.62	110
2		22.80	20.80	408	23.30	1.73	48
3		24.70	23.30	408	25.40	1.73	56
4		26.60	25.40	504	26.90	2.14	80
5		27.10	26.90	832	28.70	3.54	88
6		29.50	28.70	760	30.00	3.23	96
7		31.20	30.00	824	31.30	3.50	128
8		32.00	31.30	1192	32.80	5.07	128
9		33.40	32.80	704	33.80	2.99	128
10		34.10	33.80	416	34.30	1.77	128
11		35.00	34.30	1528	35.70	6.50	192
12		36.00	35.70	1192	36.60	5.07	176
13		36.80	36.60	920	37.30	3.91	184
14	emamectin	37.80	37.30	10552	38.90	44.86	2184
15		39.10	38.90	384	39.50	1.63	136
16		39.90	39.50	1624	41.10	6.90	376
17		43.50	42.30	440	43.90	1.87	56
18		45.80	45.00	168	46.20	0.71	32
19		48.40	48.10	288	49.10	1.22	64
				23524			

Total Run Area: 24922

Peak Background and Significance of Peak on HPLC

Formulas:

1. Background of Peaks in counts (cts)

$$\text{Bkg} \times \text{Peak width}$$

2. Background of Peaks in DPM

$$\frac{\text{CTS (Peak Bkg)} \times \text{Flow (ml/min)} \times 100}{\text{Efficiency (\%)} \times \text{Cell vol. (ml)}}$$

Peak Background

Peak Background

Peak_No	Peak Name	Xstart	Xend	Peak-width (min.)
1	unknown	*2.4*	*3.1*	0.70
2	unknown	*20.8*	*23.3*	2.50
3	unknown	*23.3*	*25.4*	2.10
4	unknown	*25.4*	*26.9*	1.50
5	unknown	*26.9*	*28.7*	1.80
6	unknown	*28.7*	*30*	1.30
7	unknown	*30.0*	*31.3*	1.30
8	unknown	*31.3*	*32.8*	1.50
9	unknown	*32.8*	*33.8*	1.00
10	unknown	*33.8*	*34.3*	0.50
11	unknown	*34.3*	*35.7*	1.40
12	unknown	*35.7*	*36.6*	0.90
13	Emamectin	*36.6*	*37.3*	0.70
14	unknown	*37.3*	*38.9*	1.60
15	unknown	*38.9*	*39.5*	0.60
16	unknown	*39.5*	*41.1*	1.60
17	unknown	*42.3*	*43.9*	1.60
18	unknown	*45.0*	*46.2*	1.20
19	unknown	*48.1*	*49.1*	1.00

Peak-No	Peak Name	Peak-width (min.)	BG (cts)	BG (dpm)
1	unknown	0.70	8	72
2	unknown	2.50	30	256
3	unknown	2.10	25	215
4	unknown	1.50	18	153
5	unknown	1.80	22	184
6	unknown	1.30	16	133
7	unknown	1.30	16	133
8	unknown	1.50	18	153
9	unknown	1.00	12	102
10	unknown	0.50	6	51
11	unknown	1.40	17	143
12	unknown	0.90	11	92
13	Emamectin	0.70	8	72
14	unknown	1.60	19	164
15	unknown	0.60	7	61
16	unknown	1.60	19	164
17	unknown	1.60	19	164
18	unknown	1.20	14	123
19	unknown	1.00	12	102

Peak Significance

Peak-No	Peak Name	DPM (Peak corr.)	Ratio Peak versus BG		Significance	Comment
				(*)		
1	unknown	540	7.54	8.54	>=LQ	name and percent-value
2	unknown	579	2.27	3.27	>=LQ	name and percent-value
3	unknown	579	2.70	3.70	>=LQ	name and percent-value
4	unknown	716	4.67	5.67	>=LQ	name and percent-value
5	unknown	1181	6.42	7.42	>=LQ	name and percent-value
6	unknown	1079	8.12	9.12	>=LQ	name and percent-value
7	unknown	1170	8.80	9.80	>=LQ	name and percent-value
8	unknown	1692	11.04	12.04	>=LQ	name and percent-value
9	unknown	1000	9.78	10.78	>=LQ	name and percent-value
10	unknown	591	11.56	12.56	>=LQ	name and percent-value
11	unknown	2170	15.16	16.16	>=LQ	name and percent-value
12	unknown	1692	18.40	19.40	>=LQ	name and percent-value
13	Emamectin	1306	18.25	19.25	>=LQ	name and percent-value
14	unknown	14982	91.60	92.60	>=LQ	name and percent-value
15	unknown	545	8.89	9.89	>=LQ	name and percent-value
16	unknown	2306	14.10	15.10	>=LQ	name and percent-value
17	unknown	625	3.82	4.82	>=LQ	name and percent-value
18	unknown	239	1.94	2.94	>LD <LQ	only name
19	unknown	409	4.00	5.00	>=LQ	name and percent-value

LQ: Limit of Quantitation: 3x Bkg
LD: Limit of Detection: 2x Bkg
(*): Increase by "1", since Peak DPM were already corrected by Background

Limits of Detection and Quantitation

General data

Specific Radioactivity (μC/mg):	59.800
Specific Radioactivity (MBq/mg):	2.2126
Specific radioactivity (dpm/mg)	132756000
Amount applied (mg)	0.0502
Amount applied (dpm)	6662718
Amount in Aq. Phase (dpm)	6690356
Amount in Aq. Phase (%)	100.41
Concentration (mg/l)	0.534
Amount of Water (l)	0.094

Percentages of Peaks and Background (BG) (top) and LD and LQ (bottom)

Peak-No	Peak Name	Peak			Background	
		DPM	%-ROI	%-applied	DPM	%-applied
1	unknown	540	1.62	1.62	72	0.22
2	unknown	579	1.73	1.74	256	0.77
3	unknown	579	1.73	1.74	215	0.65
4	unknown	716	2.14	2.15	153	0.46
5	unknown	1181	3.54	3.55	184	0.55
6	unknown	1079	3.23	3.24	133	0.40
7	unknown	1170	3.50	3.52	133	0.40
8	unknown	1692	5.07	5.09	153	0.46
9	unknown	1000	2.99	3.01	102	0.31
10	unknown	591	1.77	1.78	51	0.15
11	unknown	2170	6.50	6.52	143	0.43
12	unknown	1692	5.07	5.09	92	0.28
13	Emamectin	1306	3.91	3.93	72	0.22
14	unknown	14982	44.86	45.04	164	0.49
15	unknown	545	1.63	1.64	61	0.18
16	unknown	2306	6.90	6.93	164	0.49
17	unknown	625	1.87	1.88	164	0.49
18	unknown	239	0.71	0.72	123	0.37
19	unknown	409	1.22	1.23	102	0.31

Peak-No	Peak Name	LD	LQ	Bkg	LD	LQ	Bkg	LD	LQ
		% of applied		mg parent eq.			mg parent eq./l		
1	unknown	0.430	0.645	0.0001	0.0002	0.0003	0.0011	0.0023	0.0034
2	unknown	1.537	2.305	0.0004	0.0008	0.0012	0.0041	0.0082	0.0123
3	unknown	1.291	1.936	0.0003	0.0006	0.0010	0.0034	0.0069	0.0103
4	unknown	0.922	1.383	0.0002	0.0005	0.0007	0.0025	0.0049	0.0074
5	unknown	1.106	1.660	0.0003	0.0006	0.0008	0.0030	0.0059	0.0089
6	unknown	0.799	1.199	0.0002	0.0004	0.0006	0.0021	0.0043	0.0064
7	unknown	0.799	1.199	0.0002	0.0004	0.0006	0.0021	0.0043	0.0064
8	unknown	0.922	1.383	0.0002	0.0005	0.0007	0.0025	0.0049	0.0074
9	unknown	0.615	0.922	0.0002	0.0003	0.0005	0.0016	0.0033	0.0049
10	unknown	0.307	0.461	0.0001	0.0002	0.0002	0.0008	0.0016	0.0025
11	unknown	0.861	1.291	0.0002	0.0004	0.0006	0.0023	0.0046	0.0069
12	unknown	0.553	0.830	0.0001	0.0003	0.0004	0.0015	0.0030	0.0044
13	Emamectin	0.430	0.645	0.0001	0.0002	0.0003	0.0011	0.0023	0.0034
14	unknown	0.983	1.475	0.0002	0.0005	0.0007	0.0026	0.0053	0.0079
15	unknown	0.369	0.553	0.0001	0.0002	0.0003	0.0010	0.0020	0.0030
16	unknown	0.983	1.475	0.0002	0.0005	0.0007	0.0026	0.0053	0.0079
17	unknown	0.983	1.475	0.0002	0.0005	0.0007	0.0026	0.0053	0.0079
18	unknown	0.738	1.106	0.0002	0.0004	0.0006	0.0020	0.0039	0.0059
19	unknown	0.615	0.922	0.0002	0.0003	0.0005	0.0016	0.0033	0.0049

Validation of the HPLC Method

For validation of the HPLC method the selected sample (irradiated, 24 hours/ duplicate B) was re-analysed by HPLC. The eluate was collected in 1 minute intervals and measured by LSC.

Validation HPLC

Project	856664
Sample	Irradiated 19h / 2 / ACN
Date	March 4, 2005

Sample/Amount Injected

Sample	19h / 2 / ACN
Vol. injected (ml)	0.35
DPM injected	17945

Procedure

An aliquot of 350 µl of the selected samples was injected into the HPLC system. Radioactive fractions were collected and submitted to LSC.

Recovery

Sample		first fract.	Parent	third fract.	Total
	Retention time (min.)	0-37.5	37.5-39.0	39.0-80.0	
19h / 2 / ACN	Volume collected (ml)	38.00	3.00	25.00	
	Volume measured (ml)	38.00	3.00	25.00	
	DPM measured	6078	8661	3364	
	Recov. (DPM)	6078	8661	3364	18103
	Recov. (%)	33.57	47.84	18.58	100.00
	Recov. (%-injected)	**33.87**	**48.26**	**18.75**	**100.88**

Sample		Identity of Radioactivity		
		first fract.	Parent	third fract.
19h / 2 / ACN	ROI Data from HPLC radiomonitor	36.90	45.50	17.50
	Difference in % (%-ROI minus %-injected)	3.03	-2.76	-1.25

APPENDIX II

CALCULATION OF THE QUANTUM YIELD FOR ^{14}C-LABELLED EMAMECTIN

QUANTUM YIELD (Φ) OF PHOTOLYSIS

The quantum yield of disappearance is defined as the number of molecules reacted per number of photons absorbed. It is calculated from the experimentally determined rate of disappearance of the irradiated test item and from the total amount of light energy absorbed during the irradiation.

AII-1 ACTINOMETRY

AII-1.1 Actinometer Solution

A stock solution of p-Nitroanisole (PNA) in acetonitrile was prepared by dissolving 30.63 mg PNA in 20 ml acetonitrile in a volumetric flask. An aliquot of 1 ml of the stock solution was transferred to a 10 ml volumetric flask and the volume made up to the mark with acetonitrile to get dilution A. An aliquot of 2 ml of dilution A was transferred to a 2L volumetric flask. Thereafter, 1582 mg of pyridine were added and the volume was made up to the mark with purified water to obtain an actinometer solution (AS) containing 0.153 mg PNA/L ($1 \cdot 10^{-6}$ M), 791 µg pyridine/L (0.01 M), and 0.1% acetonitrile.

AII-1.2 Irradiation of the Actinometer Solution

The actinometer solution was irradiated in the same way as emamectin but for only 100 minutes (6000 seconds). Aliquots (200 µl) of the actinometer solution were taken every 20 minutes and analysed per HPLC (Section 2.6.2).

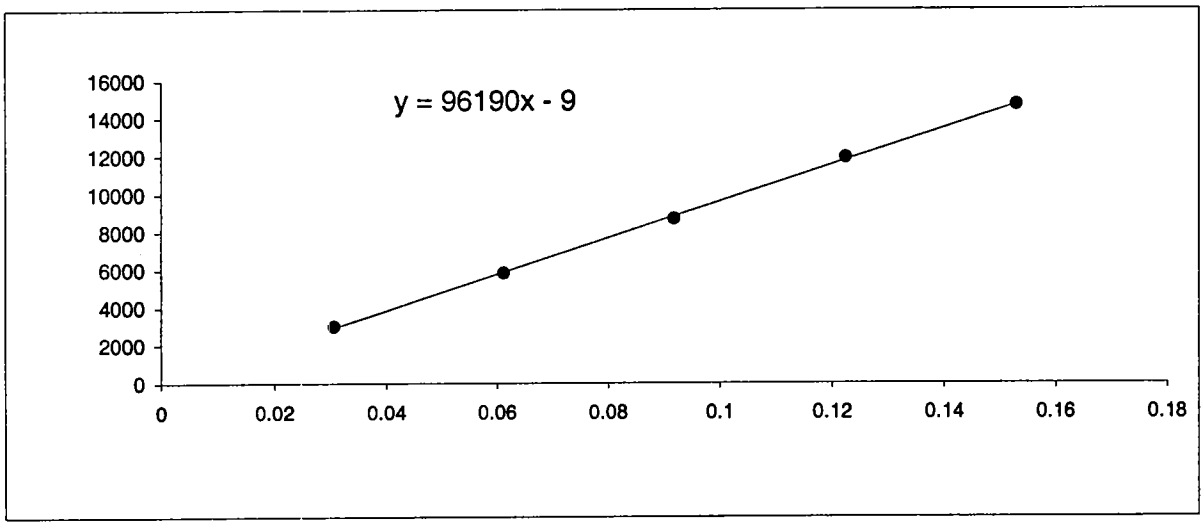
AII-1.3 Analysis of the Actinometer

The actinometer was analysed by HPLC. The HPLC was calibrated by standard solutions.

Aliquots from the initial non-irradiated actinometer solution (AS) were transferred to 10 ml measuring flasks and diluted with purified water to give the following calibration solutions:

Calibration solution	Volume solution (mL)	Final concentration (µg test item/mL)	Final concentration (mol test item/L)
0		0.1530	1.0E-6
1	8	0.1224	8.0E-7
2	6	0.0918	6.0E-7
3	4	0.0612	4.0E-7
4	2	0.0306	2.0E-7

Regression Model y = a + b*x



Correlation coeificient r^2 = 0.9995

AII-1.4 Calculation of the Quantum Yield Φ

The calculation of the quantum yield was performed according to the method described in the ECETOC Technical Report No. 12 [3]. The arithmetic operations were based on the equations and abbreviations summarised below. The photolysis quantum yield is defined as:

$$\Phi = \frac{\Delta M\,(t)}{P_{abs}\,(t)}$$

with $\Delta M\,(t)$ = Number of photo reacted molecules within time t
and $P_{abs}\,(t)$ = Number of absorbed photons in the same time.

The rate of disappearance of emamectin was calculated by applying linear first-order reaction kinetics.
The number of molecules degraded within time t was calculated by:

$$\Delta M\,(t) = M_0 - M(t)$$

M_0 is the number of test item molecules at time 0.

AII-1.5 Determination of P_{abs} (t)

When incident light of intensity P_{inc} (λ) (or photons P_{inc} (λ)) at a wavelength λ (nm) passes through a filter or other absorbing medium with absorbency E (λ) at a wavelength λ, the total number of absorbed photons P_{abs} per unit surface is calculated from:

$$\sum_{\lambda} P_{abs}(\lambda) = \sum_{\lambda} P_{inc}(\lambda) - \sum_{\lambda} P_{trans}(\lambda)$$

P_{abs} = Absorbed photons or light intensity
P_{inc} = Incident photons or light intensity
P_{trans} = Transmitted (or non-absorbed) photons or light intensity

If the absorbancy (extinction coefficient) is defined as $E = \log (I_{inc} / I_{trans})$ then the transmitted light intensity is calculated from:

$$I_{trans} = I_{inc} \cdot 10^{-E(\lambda)}$$

According to Lambert Beer's law, $\varepsilon(\lambda) = E(\lambda) \cdot d^{-1} \cdot C^{-1}$

$\varepsilon(\lambda)$ = Extinction coefficient
d = Optical path length, distance in cm
C = Concentration of the chemical in mol · litre^{-1}

Since the intensity is proportional to the number of photons, the above equation can be expressed as follows:

$$P_{trans} = P_{inc} \cdot 10^{-E(\lambda)}$$

and

$$\sum_{\lambda} P_{abs}(\lambda) = \sum_{\lambda} P_{inc}(\lambda) - \sum_{\lambda} (P_{inc} \cdot 10^{-E(\lambda)})$$

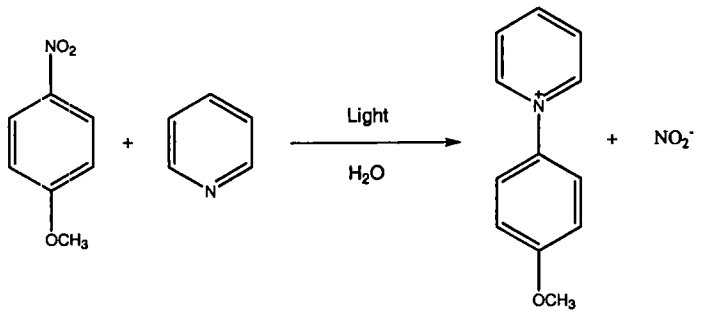
$$= \sum_{\lambda} (P_{inc}(\lambda) \cdot (1 - 10^{-E(\lambda)}))$$

Thus, to determine the number of absorbed photons $\sum P_{inc}(\lambda) \cdot (1\text{-}10^{-E(\lambda)})$, it is necessary to obtain the number of incident photons $P_{inc}(\lambda)$. P_{inc} is obtained from the actinometric measurement.

For the available incident light intensity, it is first necessary to know the "relative" distribution of energy across the spectral emission of the lamp at the wavelength range from 290 nm upwards, expressed in 5 nm waveband intervals. These data were measured by direct photometry using the LI-1800 spectrometer. With these data the relative energy absorbed by the actinometer can be calculated: $\sum P_{rel}(\lambda) \cdot 1\text{-}10^{-E(\lambda)})$. With the relative light absorbed by the actinometer and the incident light absorbed (known from the actinometer measurement) a correction factor is calculated by which the relative lamp spectrum is corrected yielding $P_{inc}(\lambda)$.

AII-1.6 Determination of the incident light intensity by actinometry

When p-nitroanisole (PNA) and pyridine (PYR) are irradiated in aqueous solution at $\lambda > 300$ nm, the following reaction occurs:



The quantum yield for this reaction is independent of the wavelength. The actinometer quantum yield depends on the pyridine concentration in the actinometer according to

$$\Phi_{act.} = 0.437 \cdot [PYR] + 0.000282$$

The photoreaction proceeds cleanly over 10 half-lives to give the products. Oxygen does not affect the reaction, nor are secondary reactions of either NO_2^- or p-pyridiniumanisole observed. The reaction has a small negative Arrhenius activation energy of –3 kcal/mol i.e., the reaction slows slightly with increasing temperature. The concentration of PNA was measured by HPLC. The reaction followed pseudo first order kinetics:

$$C = C_0 \exp(-k_1 \cdot t)$$

The rate constant k_1 is determined in the actinometer experiment. With the formula the number of molecules reacted within a certain time period $\Delta M_{Act.}$ (t) can be calculated and from this the absorbed incident light intensity is calculated by:

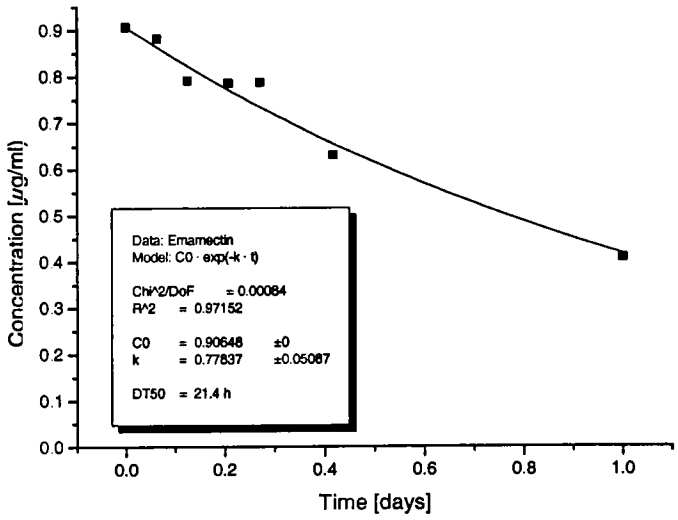
$$I_{Act,abs} = \Delta M_{Act.} (t) / \Phi_{act.}$$

AII-2 DETERMINATION OF PHOTOLYSIS RATE Of THE TEST ITEM

Molecular weight of the test item:	1008.2 g/mol
Volume of the test solution:	50 ml (V)
Irradiated surface:	26.42 cm^2 (IS)
Initial concentration:	0.906 mg/l
Apparatus:	Heraeus Suntest CPS, Original Hanau

For the determination of the quantum yield, the following values were used:

Irradiation time (days)	emamectin Concentration (mg/l)
0.000	0.906
0.063	0.882
0.125	0.791
0.208	0.785
0.271	0.787
0.417	0.630
1.000	0.407



AII-2.1 Kinetic Analysis

For the determination of the quantum yield, first-order kinetics was applied using the experimental points of the test item as shown above. The variation of the concentration with time is given by the following equation:

$$C = C_0 \exp(-k \cdot t)$$

The half-life (DT-50) can be expressed as:

$$DT\text{-}50 = \frac{\ln(2)}{k}$$

With k = 0.77837 $days^{-1}$
C_0 = 0.906 mg/l
DT-50 = 0.89 days (or 21.4 hours)

Therefore, the number of reacting molecules (ΔM) within the time t = DT-50 test item/100 (days):

$\Delta M(t) = M_0 - M(t)$
$\Delta M(t) = M_0 \cdot (1\text{-}\exp(-k \cdot t))$
$= C_0/\text{molecular weight} \cdot \text{Volume}/1000 \cdot N \cdot (1\text{-}\exp \cdot k\, t)$
$= 1.870 \cdot 10^{-14}$ test item molecules in 0.00891 days using the vessel surface (S) of 26.42 cm^2 and the volume of 50 ml.

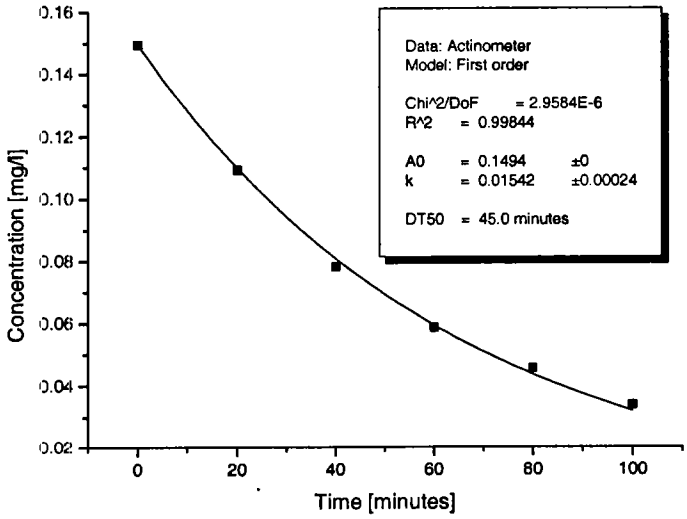
N = Avogadro's constant ($6.022 \cdot 10^{23}$ mol^{-1})

AII-3 ACTINOMETRIC MEASUREMENTS FOR DETERMINATION OF PHOTON FLUX

Molecular weight of p-nitroanisole:	153.14 g/mol
Volume of the test solution:	50 ml (V)
Irradiated surface:	26.42 cm^2 (IS)
Initial concentration:	0.1494 mg/l
Apparatus:	Heraeus Suntest CPS, Original Hanau

The following actinometer concentration was determined during the irradiation:

Irradiation time (min)	Actinometer Concentration (mg/l)
0	0.1494
20	0.1091
40	0.0780
60	0.0585
80	0.0454
100	0.0338



AII-3.1 Number of p-nitroanisole Molecules which Disappear during Irradiation

For the determination of the actinometer concentration, first-order kinetics was applied using the experimental points of the test item as shown above. The variation of the concentration with time is given by the following equation:

$$C = C_0 \exp(-k \cdot t)$$

The half-life (DT-50) can be expressed as:

$$DT\text{-}50 = \frac{\ln(2)}{k}$$

With k = 0.01542 minutes^{-1}
C_0 = 0.1494 mg/l
DT-50 = 44.95 minutes

Therefore, the number of reacting molecules (ΔM) within the time t = DT-50 actinometer/100 (minutes):

$\Delta M\ (t) = M_0 - M\ (t)$
$\Delta M\ (t) = M_0 \cdot (1\text{-}\exp\ (-k \cdot t))$
$= C_0$/molecular weight · Volume/1000 · N · (1-exp · k t)
$= 2.029 \cdot 10^{-14}$ test item molecules in 0.450 minutes using the vessel surface (S) of 26.42 cm^2 and the volume of 50 ml.

N = Avogadro's constant ($6.022 \cdot 10^{23}$ mol^{-1})

AII-3.2 Total Amount of Absorbed Photons by the Actinometer in the Range 290 nm to 450 nm

The quantum yield of the p-nitroanisole actinometer is

$\Phi_{Act.} = 0.437 \cdot [\text{pyridine}] + 0.000282$ [ref 7]

and is independent of the wavelength but depends on pyridine concentration.

With the pyridine concentration being 0.01 mol/l

$\Phi_{Act.}$ = 0.004652 photo-reactions per absorbed photon

Therefore, the number of photons absorbed (P_{abs}) by the actinometer:

P_{abs} = ΔM / 0.004652 photons = $4.362 \cdot 10^{16}$
in 0.450 minutes and 26.42 cm^2 surface

AII-3.3 Number of Photons (P_{inc}) Available for Photolysis in the Range 290 nm to 490 nm

P_{abs} is used to calculate the "absolute" value of available light for every 5 nm spectral band. Since p-nitroanisole is a weakly absorbing actinometer the following correction is necessary:

$P_{inc} = P_{abs} / \Sigma\ [Ri\ (\lambda) - (1 - 10^{-E(\lambda)})]$
$= 1.979 \cdot 10^{19}$ photons in 0.450 minutes and 26.42 cm^2 surface.

* $E(\lambda)$ = Absorbance of the actinometer (A) at equal path lengths used for photolysis. In this case, E was determined with 1 cm path length and corrected with factor V/S.

* $R(\lambda)$ = The ratio of the absorbed intensity in each spectral band, calculated from the relative values.

AII-3.4 Determination of the Total Light Intensity Absorbed by the Test Item

First, the number of photons emitted (ΣP_{inc}) by the "Suntest" light source in the time (DT-50; test item)/100 was calculated from the actinometer experiment.

ΣP_{inc} = $P_{inc} \cdot$ [time (test item) / time (Actinometer)]
[area (test item) / area (Actinometer)]
= $5.552 \cdot 10^{20}$ photons in 0.0089 days and at 26.42 cm^2 surface

The total absorbed photons (P_{abs}) by the test item is calculated as:

$$P_{abs} = (\sum_{\lambda} P_{inc}) \cdot \sum_{\lambda} [R(\lambda) \cdot (1 - 10^{-E(\lambda)})]$$

The total absorbed light intensity is equal to the sum of the absorbed photons over the spectral range in which the chemical absorbs.

* $E(\lambda)$ = Absorbance of the test item at equal path lengths as its photolysis. In this case, E was determined with 1 cm path length and corrected with factor V/S.

* $R(\lambda)$ = The ratio of the absorbed intensity in each spectral band, calculated from the relative values with the sum $R(\lambda) = 1$

Thus,

P_{abs} = $1.276 \cdot 10^{15}$ photons in 0.0089 days (= (DT-50, test item)/100) and at 26.42 cm^2 surface.

AII-3.5 Quantum Yield Φ

In the end the quantum yield of the test item can be calculated by dividing the number of reacted molecules by the number of absorbed incident photons:

$\Phi_{test\ item}$ = $\Delta M\ (t) / I_{test\ item,\ abs}$
$\Phi_{test\ item}$ = 1.44E-2

Table AII-1: Spectra: incident light intensity and absorption of the test item.

	1	2	3	4
	Incident light	Test item	Test item	factor
Wave length [nm] λ	Spectrum [measured] *	concentration absorbance [measured]	Ext. Coef. in irradiated system	irradiated concentration to absorbance . conc.
290	0.0235	0.974278	189	0.00017
295	0.0783	0.820326	159	0.00017
300	0.1956	0.679307	132	0.00017
305	0.3411	0.547395	106	0.00017
310	0.5074	0.433206	84	0.00017
315	0.7356	0.341644	66	0.00017
320	0.9482	0.276449	54	0.00017
325	1.1823	0.228097	44	0.00017
330	1.4208	0.193686	38	0.00017
335	1.6798	0.16789	33	0.00017
340	1.9312	0.148438	29	0.00017
345	2.1470	0.13455	26	0.00017
350	2.3512	0.123562	24	0.00017
355	2.5587	0.114234	22	0.00017
360	2.7338	0.106833	21	0.00017
365	2.9645	0.099721	19	0.00017
370	3.2993	0.092125	18	0.00017
375	3.3701	0.086069	17	0.00017
380	3.7154	0.08005	16	0.00017
385	3.8629	0.073625	14	0.00017
390	4.1738	0.066987	13	0.00017
395	5.1877	0.061386	12	0.00017
400	5.1986	0.057037	11	0.00017
405	5.1626	0.052069	10	0.00017
410	5.8946	0.04873	9	0.00017
415	6.0504	0.045947	9	0.00017
420	6.4970	0.042457	8	0.00017
425	6.5774	0.040754	8	0.00017
430	6.7565	0.038037	7	0.00017
435	7.1734	0.035593	7	0.00017
440	7.7599	0	0	0.00017
445	7.7203	0	0	0.00017
450	9.2218	0	0	0.00017

* Light measured: Measured by the Spectrophotometer LI-1800

Table AII-2: Spectrum of the test substance.

Wave length [nm] λ	2 Absorbance [measured] (optical path-length :1 cm)	4 factor irradiated concentration to absorbance . conc.	5 Corrected absorbance (2 * 4)	6 Sample Absorbance (E) (5 * G/IA)	7 Test Item (1-10^-E)
290	0.9743	0.00017	0.0002	0.0003	0.0007
295	0.8203	0.00017	0.0001	0.0003	0.0006
300	0.6793	0.00017	0.0001	0.0002	0.0005
305	0.5474	0.00017	0.0001	0.0002	0.0004
310	0.4332	0.00017	0.0001	0.0001	0.0003
315	0.3416	0.00017	0.0001	0.0001	0.0003
320	0.2764	0.00017	0.0000	0.0001	0.0002
325	0.2281	0.00017	0.0000	0.0001	0.0002
330	0.1937	0.00017	0.0000	0.0001	0.0001
335	0.1679	0.00017	0.0000	0.0001	0.0001
340	0.1484	0.00017	0.0000	0.0000	0.0001
345	0.1346	0.00017	0.0000	0.0000	0.0001
350	0.1236	0.00017	0.0000	0.0000	0.0001
355	0.1142	0.00017	0.0000	0.0000	0.0001
360	0.1068	0.00017	0.0000	0.0000	0.0001
365	0.0997	0.00017	0.0000	0.0000	0.0001
370	0.0921	0.00017	0.0000	0.0000	0.0001
375	0.0861	0.00017	0.0000	0.0000	0.0001
380	0.0801	0.00017	0.0000	0.0000	0.0001
385	0.0736	0.00017	0.0000	0.0000	0.0001
390	0.0670	0.00017	0.0000	0.0000	0.0001
395	0.0614	0.00017	0.0000	0.0000	0.0000
400	0.0570	0.00017	0.0000	0.0000	0.0000
405	0.0521	0.00017	0.0000	0.0000	0.0000
410	0.0487	0.00017	0.0000	0.0000	0.0000
415	0.0459	0.00017	0.0000	0.0000	0.0000
420	0.0425	0.00017	0.0000	0.0000	0.0000
425	0.0408	0.00017	0.0000	0.0000	0.0000
430	0.0380	0.00017	0.0000	0.0000	0.0000
435	0.0356	0.00017	0.0000	0.0000	0.0000
440	0.0000	0.00017	0.0000	0.0000	0.0000
445	0.0000	0.00017	0.0000	0.0000	0.0000
450	0.0000	0.00017	0.0000	0.0000	0.0000

G: Irradiated volume
IA: irradiated surface (area)

Table AII-3: Spectrum of the actinometer solution.

Wave length [± 2 nm] lambda	8 Absorbance [measured] (optical path-length :1 cm)	9 factor irradiated PNA concentration to absorbance . conc.	10 Corrected absorbance (8 * 9)	11 Absorbance (E) (10 * G/IA)	12 Actinometer (1-10^-E)
290	0.1299	0.0488	0.0063	0.0120	0.0272
295	0.1515	0.0488	0.0074	0.0140	0.0317
300	0.1715	0.0488	0.0084	0.0158	0.0358
305	0.1888	0.0488	0.0092	0.0174	0.0393
310	0.2009	0.0488	0.0098	0.0185	0.0418
315	0.2067	0.0488	0.0101	0.0191	0.0430
320	0.205	0.0488	0.0100	0.0189	0.0426
325	0.196	0.0488	0.0096	0.0181	0.0408
330	0.1812	0.0488	0.0088	0.0167	0.0378
335	0.1617	0.0488	0.0079	0.0149	0.0338
340	0.1397	0.0488	0.0068	0.0129	0.0293
345	0.1171	0.0488	0.0057	0.0108	0.0246
350	0.096	0.0488	0.0047	0.0089	0.0202
355	0.0759	0.0488	0.0037	0.0070	0.0160
360	0.0579	0.0488	0.0028	0.0053	0.0122
365	0.0432	0.0488	0.0021	0.0040	0.0091
370	0.031	0.0488	0.0015	0.0029	0.0066
375	0.0215	0.0488	0.0010	0.0020	0.0046
380	0.0146	0.0488	0.0007	0.0013	0.0031
385	0.0087	0.0488	0.0004	0.0008	0.0018
390	0.0054	0.0488	0.0003	0.0005	0.0011
395	0.0033	0.0488	0.0002	0.0003	0.0007
400	0.0018	0.0488	0.0001	0.0002	0.0004
405	0.0011	0.0488	0.0001	0.0001	0.0002
410	0.0007	0.0488	0.0000	0.0001	0.0001
415	0.0006	0.0488	0.0000	0.0001	0.0001
420	0.0003	0.0488	0.0000	0.0000	0.0001
425	0.0003	0.0488	0.0000	0.0000	0.0001
430	0.0003	0.0488	0.0000	0.0000	0.0001
435	1E-04	0.0488	0.0000	0.0000	0.0000
440	0	0.0488	0.0000	0.0000	0.0000
445	0	0.0488	0.0000	0.0000	0.0000
450	0	0.0488	0.0000	0.0000	0.0000

G: Irradiated volume
IA: irradiated surface (area)

Table AII-4: Spectrum of the absolute light incident intensity

Wave length [nm] λ	1 Relative incident light intensity	13 Conversion of light to photons **μ einstein * m-2** [1 * λ / (h * C)]	14 Relative photon values [13 / sum (13)]	15 Correction factor **actinometer** Ri[1-10^-E] [14*(1-10^-E)] **14 * 12**	16 Correction factor **test item** Ri[1-10^-E] [14*(1-10^-E)] **14 * 7**
290	0.0235	0.057	0.000073	0.000002	5.38615E-08
295	0.0783	0.193	0.000247	0.000008	1.53629E-07
300	0.1956	0.491	0.000627	0.000022	3.23456E-07
305	0.3411	0.870	0.001112	0.000044	4.62106E-07
310	0.5074	1.315	0.001680	0.000070	5.52911E-07
315	0.7356	1.937	0.002475	0.000106	6.42363E-07
320	0.9482	2.536	0.003241	0.000138	6.80616E-07
325	1.1823	3.212	0.004105	0.000168	7.11206E-07
330	1.4208	3.919	0.005009	0.000189	7.36863E-07
335	1.6798	4.703	0.006011	0.000203	7.66612E-07
340	1.9312	5.488	0.007014	0.000205	7.90869E-07
345	2.1470	6.191	0.007913	0.000195	8.08707E-07
350	2.3512	6.878	0.008791	0.000178	8.25113E-07
355	2.5587	7.592	0.009704	0.000155	8.42014E-07
360	2.7338	8.226	0.010513	0.000129	8.53178E-07
365	2.9645	9.044	0.011559	0.000106	8.75599E-07
370	3.2993	10.203	0.013041	0.000086	9.12581E-07
375	3.3701	10.563	0.013500	0.000062	8.82654E-07
380	3.7154	11.800	0.015082	0.000047	9.17123E-07
385	3.8629	12.430	0.015887	0.000029	8.88527E-07
390	4.1738	13.605	0.017389	0.000020	8.84848E-07
395	5.1877	17.127	0.021890	0.000015	1.02074E-06
400	5.1986	17.380	0.022214	0.000008	9.62464E-07
405	5.1626	17.475	0.022336	0.000005	8.83454E-07
410	5.8946	20.200	0.025818	0.000004	9.55684E-07
415	6.0504	20.986	0.026823	0.000003	9.36200E-07
420	6.4970	22.807	0.029150	0.000002	9.40142E-07
425	6.5774	23.364	0.029862	0.000002	9.24472E-07
430	6.7565	24.283	0.031036	0.000002	8.96766E-07
435	7.1734	26.081	0.033334	0.000001	9.01281E-07
440	7.7599	28.537	0.036474	0.000000	0.00000E+00
445	7.7203	28.714	0.036700	0.000000	0.00000E+00
450	9.2218	34.684	0.044331	0.000000	0.00000E+00
455	9.4709	36.017	0.046034	0.000000	0.00000E+00
460	10.9855	42.236	0.053983	0.000000	0.00000E+00
465	14.8574	57.743	0.073803	0.000000	0.00000E+00
470	14.7985	58.133	0.074301	0.000000	0.00000E+00
475	11.2461	44.648	0.057066	0.000000	0.00000E+00
480	11.5951	46.518	0.059456	0.000000	0.00000E+00
485	11.8883	48.191	0.061594	0.000000	0.00000E+00
490	11.2371	46.021	0.058821	0.000000	0.00000E+00
	Sum:	**782.4**	**1.000000**	**0.002204**	**0.000023**