Section A4.2(a) **Analytical Methods for Detection and Identification**

Annex Point IIA4.2

Method Validation for the Analysis of Sumithrin in Soil

Additional clean-up of the sample extract was performed using 15 g of activated Florisil contained in a glass chromatographic column. The residue in the flat bottom flask was dissolved in 3 mL of a mixed solvent (hexane/ethyl acetate, 20: 1 , v/v). This was quantitatively transferred to the Florisil column using three additional 3-mL washes of the mixed solvent. The sample solution and rinses were allowed to percolate through the column until the solvent reached the top of the packing. The eluate was discarded. The Sumithrin was eluted from the column using 45 mL of the mixed solvent. The first 5 mL were discarded. The remaining 40 mL were collected in a flat bottom flask, concentrated to 1-2 mL using rotary evaporation and then transferred to a glass screw-capped culture tube using hexane. The solution was taken to dryness using N_2. The residue was dissolved in a known volume of toluene and quantitated using gas chromatography (GC) and a mass selective detector (MSD).

5.2 Detection

Non-entry field

5.2.1 Separation method

Gas Chromatography

Instrumentation used for the chromatography of Sumithrin was a Hewlett-Packard (HP) 5890A Series II GC equipped with a HP 5970 MSD. Samples were injected with a HP 7673 autoinjector. Data were obtained with a Unix system. General chromatography and MSD parameters are as follows:

Column:

J & W Scientific DB-5, 30 m x 0.25 mm i.d. fused silica capillary with a film thickness of 0.25 µm. DB-5 is a 5 % phenyl and 95 % methyl silicone.

Temperatures:

Column:	95 °C for 0.75 mm, 15 °C/min from 95 to 250 °C, 10 °C/min from 250 to 275 °C, hold for 7 minutes at 275 °C
Injector:	225 °C
Transfer Line:	275 °C
Detector:	275 °C

Carrier: Helium at a flow of 30 cm/sec

Injection Volume: 2 µl

5.2.2 Detector

HP 5970 MSD

Acquisition Mode: Single ion monitoring (SIM), ions 123 and 183 monitored

Temperatures:

Detector:	275 °C

Carrier: Helium at a flow of 30 cm/sec

Electron Multiplier: 1600 to 2200

Purge: Purge turned on at 0.75 min

Elution Time: Isomers elute between 15.5 and 16.5 min

Data Acquisitions and Calculations

Peak heights and areas for ions with a mass of 123 and 183 for cis and trans Sumithrin were obtained with the Unix data system that controlled the operation of the mass spectrometer. The two ions were used at the elution time determined by the injection of reference standards.

For calculation purposes, peak areas were used. Initial calculations were performed using the sum of the peak areas for ions 123 and 183 for each isomer. An interference was noted with the trans isomer for ion 123 . Although this interference seemed insignificant, peak areas for ion 183 only for both ions were chosen to quantify the Sumithrin residues. Thus, the peak area along with the concentration of each isomer of Sumithrin (isomeric ratios of 19/81 of cis/trans) was entered into a quadratic formula to determine a regression curve. Peak areas of ion 183 found for each isomer for the unknown samples were then entered into the regression curve and the nanograms of each isomer were calculated. Sample weights and volumes were then entered into the following equation to determine the final ppm of each

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Section A4.2(a) **Analytical Methods for Detection and Identification**

Annex Point IIA4.2

Method Validation for the Analysis of Sumithrin in Soil

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isomer.

ppm residue = (ng/mL detected X final volume (mL)) ÷ (sample weight (g) X 1000)

Total Sumithrin was then obtained by adding together the total amount of cis and trans Sumithrin found in the unknown samples. Recoveries of fortified samples were determined by the formula:

% recovery = ((ppm residue found - average ppm residue in control) ÷ ppm residue added) x 100

Linear regression was not used to construct the standard reference curves because a slight curvature was noted when plotting concentration versus peak area.
Calculation of the regression curve was performed using a data software package obtained from Beckman Instruments entitled "Computerized Automated Laboratory Systems" (CALS). All other calculations were performed using the spread sheet program Quattro Pro.
The residue level in the treated samples was also corrected for moisture content as follows:

Corrected ppm, dry basis = (ppm wet basis ÷ (100 - % soil moisture)) x 100

5.2.3 Standard(s)

The Sumithrin standard was a mixture of cis and trans isomers. The isomer ratio for cis:trans was 19:81. The Sumithrin standard was stored in a freezer when not in use.
Solutions made from the standard were refrigerated when not in use.
Details of the preparation of stock solutions and dilution of working standards are contained in the study raw data and are not presented in the study report.

Refer to figure A4.2(a)-1 for a typical chromatogram of a Sumithrin calibration standard.

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5.2.4 Interfering substance(s)

No substances are expected to interfere.

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5.3 Linearity

Non-entry field

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5.3.1 Calibration range

A calibration curves were prepared containing the following concentrations of cis and trans Sumithrin:-

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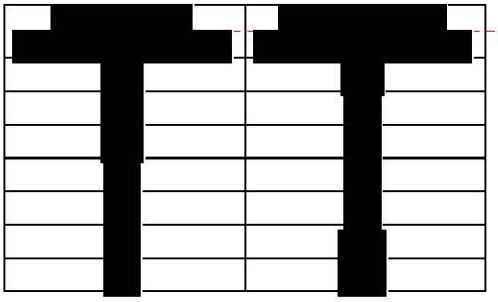

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Refer to Figures A4 2(a)-5 and 6 for typical calibration lines.

5.3.2 Number of

Each standard was injected once.

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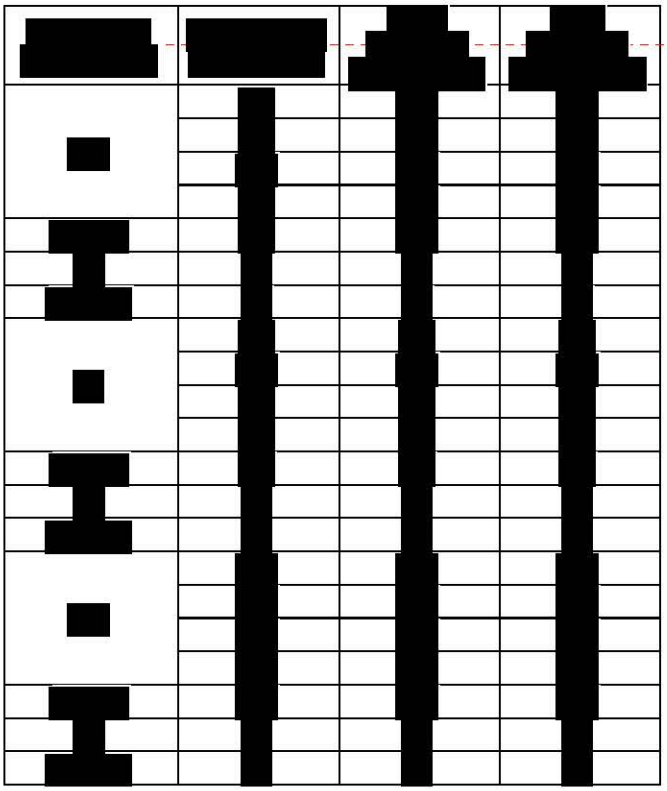
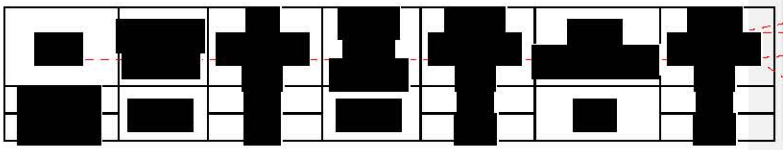
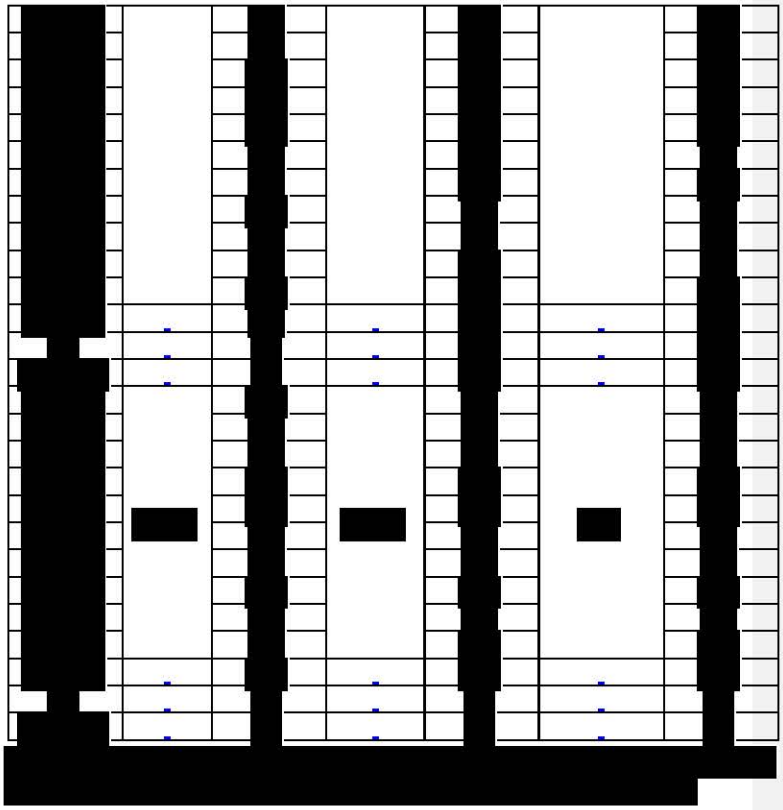
Section A4.2(a) **Annex Point IIA4.2**	**Analytical Methods for Detection and Identification** **Method Validation for the Analysis of Sumithrin in Soil**	Formatted Table
measurements		
5.3.3 Linearity	The detector response (area) for Sumithrin® was plotted against the standard concentration. The correlation coefficient was calculated to be 0.0.99904 for cis Sumithrin and 0.99922 for trans Sumithrin.	Formatted: Font: 10 pt
5.4 Specifity: interfering substances	No other substances were found to interfere. The controls were not found to contain any interfering peaks at the retention time of Sumithrin when quantified using the 183 amu ion.	Formatted: Font: 10 pt
5.5 Recovery rates at different levels	Refer to Figures A4.2(a)-2 and 3 for typical chromatograms of soil samples fortified with Sumithrin.	Formatted: Font: 10 pt
		Formatted: Font: 10 pt Formatted Table
5.5.1 Relative standard deviation	Refer to Section 3.5.	Formatted: Font: 10 pt
5.6 Limit of determination	The limit of determination or LOQ was established to be 0.010 mg/kg. The limit of detection was 100 ng/ml for total Sumithrin.	Formatted: Font: 10 pt
5.7 Precision	Non-entry field	Formatted: Font: 10 pt
5.7.1 Repeatability		Formatted: Font: 10 pt Formatted: Font: 10 pt Formatted Table

Section A4.2(a) **Annex Point IIA4.2**	**Analytical Methods for Detection and Identification** **Method Validation for the Analysis of Sumithrin in Soil**	Formatted Table



5.7.2 Independent laboratory validation	An independent laboratory validation has not been performed.	**Formatted:** Font: 10 pt

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6 APPLICANT'S SUMMARY AND CONCLUSION

6.1 Materials and methods	The analytical method consists of extraction with methanol, liquid-liquid clean-up using methylene chloride followed by clean-up by activated florisil column chromatography and determination with gas chromatograph with Mass Selective Detection (MSD).	**Formatted:** Font: 10 pt
6.2 Conclusion	The method is considered to be acceptable in terms of accuracy, precision, linearity and specificity.	**Formatted:** Font: 10 pt
6.2.1 Reliability	[redacted]	**Formatted:** Font: 10 pt
6.2.2 Deficiencies	The number of replicates for the assessment of accuracy was 4 instead of 5, however, as three concentration levels were analysed instead of the required two levels this deviation is considered not to be significant. Suitable means and RSDs have been determined based upon 4 replicates and demonstrate that the method is robust.	**Formatted:** Font: 10 pt

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Evaluation by Competent Authorities
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Section A4.2(a)	Analytical Methods for Detection and Identification
Annex Point IIA4.2	Method Validation for the Analysis of Sumithrin in Soil

Date	[redacted]
Materials and methods	
Conclusion	
Reliability	
Acceptability	
Remarks	
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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Section A4.2(b) Annex Point IIA4.2 IUCLID 6.2/1 Analytical Methods for Detection and IdentificationMethod Validation for the Analysis of Sumithrin in Air

Official use only

~~7~~ REFERENCE

10.3	**Reference**	[redacted]
10.4	**Data protection**	Yes
10.4.1	Data owner	Sumitomo Chemical Co., Ltd.
10.4.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

11 GUIDELINES AND QUALITY ASSURANCE

11.1	**Guideline study**	Biocidal Products Directive (98/8/EC). The study design was based on the guidelines described in the EU Guidance document on residue analytical methods: Working document SANCO/825/00 rev.7 (17/03/04).
11.2	**GLP**	Yes
11.3	**Deviations**	None

12 MATERIALS AND METHODS

12.1	**Preliminary treatment**	Non-entry field
12.1.1	Enrichment	**Preparation of Traps [1a/b, 4d]** SKC air-sampling tubes (Catalogue Number 226-30-06, 11 cm x 0.8 cm diameter) are used for sampling. The tubes consist of a glass wool plug, 400 mg of XAD-2 resin, a further glass wool plug then a back-up of 200 mg of XAD-2 resin. The packing is retained with another plug of glass wool. Glass air-sampling tubes should be covered with aluminium foil to protect from light during sampling. **Sampling Procedure [1a/b, 4a]** Air is sampled by drawing air through the traps using a vacuum pump at a rate of 1 L/minute. Flow rate can be controlled using aquarium valves or flow restrictors on the interconnecting tubing. Air is drawn through the traps for six hours to give a total sample of 360 L of air through each trap. Record the temperature and relative humidity of the laboratory air at regular intervals during sampling. **Analysis of the Traps [1a/b, 4d]** 1. Unpack the rear portions of each trap and separately transfer the contents (including rear glass wool) to suitable glass sample vials. 2. Add 1 mL toluene to the remaining air trap and leave to soak through packing. Note: This is required to reduce possible loss of adsorbent particles when unpacking, due to static on the glass trap.

3. Unpack the front portion of the trap (including front glass wool) separately into other vials. Note: The middle portion of glass wool should be included with the front portion of trap material.
4. Rinse the trap into the vial containing the front trap material with 9 mL toluene.
5. Sonicate for 10 minutes then filter the extract through glass wool into a glass tube.
6. Extract each portion of trap with a second 10 mL portion of toluene and sonicate for 10 minutes.
7. Combine extracts by filtering through glass wool.
8. Evaporate extracts to less than 10 mL volume under nitrogen in a TurboVap set at 45°C.
9. Transfer to a volumetric flask and dilute to the volume (minimum final volume of 10 mL) with toluene.
10. Analyse the extract by GC/MS using the instrumental conditions specified below.
11. Samples above an extract concentration of 0.5 µg/mL may not require evaporation. Instead, they may be diluted to a suitable volume with toluene, to obtain a concentration within the calibration range.

12.1.2 Cleanup

No clean-up was required.

12.2 Detection

Non-entry field

12.2.1 Separation method

Instrumentation:-Trace GC Ultra Gas Chromatograph (Thermo Finnigan)
Column#:-DB-17MS (15 m x 0.25 mm, 0.25 µm film thickness)
Injection volume:-2 µL, splitless
Injection temperature#:-250°C
Carrier gas:-helium 1.5 mL/min
Oven temperature program:-

Rate (°C/min)	Temperature (°C)	Hold (min)
-	100	2
6	220	5
60	300	3

Note:-Parameters marked # may not be modified. Minor adjustments to the remaining parameters may be required in order to fully optimise the system.

12.2.2 Detector

Detector:-Trace DSQ MS (Thermo Finnigan)
Detector temperature:-200°C
Ionisation mode#:-electron impact (EI+ SIM);
Ions monitored#:-see below

Compound	Retention times	Quantitation ion	Confirmation	Confirmation

	(min)	(*m/z*)	ion 1 (*m/z*)	ion 2 (*m/z*)
d-phenothrin	26.2	123	183	153

Note:-Parameters marked # may not be modified. Minor adjustments to the remaining parameters may be required in order to fully optimise the system.

12.2.3 Standard(s)

Preparation of Standard Solutions
Duplicate primary stock standard solutions of d-phenothrin are prepared in acetone.

Preparation of Stock Solutions
In duplicate, accurately weigh *ca.* 20 mg (corrected for purity) of d-phenothrin into separate 20 mL volumetric flasks and dilute each to the mark using acetone to give primary stock standards of concentration 1000 µg/mL d-phenothrin.

Fortification Solutions [1a/b, 4b]
Prepare a suitable fortification solution in acetone by serial dilution from the primary stock solution (1000 µg/mL in acetone).

Concentration (µg/mL)	Volume (mL)	Final volume (mL)	Concentration (µg/mL)
1000	1	10	100

Calibration Standards
Prepare appropriate calibration standards in toluene, from the primary stock solution (1000 µg/mL in acetone), to cover the range 0 to 0.5 µg/mL, with a lowest calibration level of 0.01 µg/mL.

Calculations

Residues of d-phenothrin are determined by the interpolation of the total peak areas of d-phenothrin from the standard regression equation, as follows:

The calibration line is determined by plotting the responses from the calibration solutions (R) against the of amount test substance injected (A) to generate a straight line graph.

$R = B0 + B1 \times A$

where B1 is the gradient and B0 is the intercept.

Concentrations of test substance (A) in sample extracts are calculated from their response using the equation:

Concentration of extract A (µg/mL) = (peak area – intercept)/slope

The residue of d-phenothrin in each test sample of air is calculated as follows:

Residue (mg/m^3) = extract concentration (µg/ml) x factor

Factor = final volume (10 ml) x dilution factor (if required) / volume of

Section A4.2(b) Annex Point IIA4.2 IUCLID 6.2/1 Analytical Methods for Detection and IdentificationMethod Validation for the Analysis of Sumithrin in Air

air (360L)

Procedural recovery data from fortified samples are calculated via the following equation:

Recovery (%) = ((A-C)/S) x 100

Where:-

A = concentration found in test sample (mg/m^3 air)
C = concentration (or interference) found in control sample (mg/m^3 air)
S = concentration added to fortified sample (mg/m^3 air)

12.2.4 Interfering substance(s): No substances are expected to interfere.

12.3 Linearity: Non-entry field

12.3.1 Calibration range: A calibration curve 0.01, 0.02, 0.05, 0.075, 0.100, 0.250, 0.400, 0.500 µg/ml was prepared.
Refer to Figure A4_1(1)-2 for a typical calibration line.

12.3.2 Number of measurements: Each standard was injected once.

12.3.3 Linearity: The detector response (area) for Sumithrin® was plotted against the standard concentration. The correlation coefficient was calculated to be 0.9933.

12.4 Specifity: interfering substances: No other substances were found to interfere. The controls were not found to contain any interfering peaks at the retention time of Sumithrin.

12.5 Recovery rates at different levels

Validation at ambient conditions

Fortification Level (mg/m^3)	Measured Concentration (mg/m^3)	% Recovery	Mean % Recovery	RSD (%)
Control A	0.00000	-	-	-
Control B	0.00000	-	-	-
0.00120	0.00119	99	[redacted]	6.8
	0.00112	93		
	0.00129	108		
	0.00109	91		
	0.00115	96		
0.0120	0.01043	87	[redacted]	5.4
	0.01007	84		
	0.01042	87		
	0.01009	84		
	0.00908	76		

Validation at elevated temperature and humidity

Fortification Level (mg/m^3)	Measured Concentration (mg/m^3)	% Recovery	Mean % Recovery	RSD (%)
Control A	0.00000	-	-	-

Section A4.2(b) Annex Point IIA4.2 IUCLID 6.2/1 Analytical Methods for Detection and IdentificationMethod Validation for the Analysis of Sumithrin in Air

Control B	0.00000	-	-	-
0.00120	0.00102	85		
	0.00113	94		
	0.00087	73	[redacted]	10.3
	0.00108	90		
	0.00114	95		
0.0120	0.01097	91		
	0.01081	90		
	0.01024	85	[redacted]	9.2
	0.01013	84		
	0.01264	105		

12.5.1 Relative standard deviation: The Overall RSD = 10.0% for ambient conditions and 9.4% for elevated conditions.

12.6 Limit of determination: The limit of determination was established to be 0.001200 mg/m^3

12.7 Precision: Non-entry field

12.7.1 Repeatability: Repeatability was not assessed within this report.

12.7.2 Independent laboratory validation: An independent laboratory validation has not been performed.

13 APPLICANT'S SUMMARY AND CONCLUSION

13.1 Materials and methods: Control traps (XAD-2 resin packed glass tubes; main front portion of 400 mg, rear 'breakthrough' portion of 200 mg) were fortified with known amounts of d phenothrin, at two levels in quintuplicate. The levels were equivalent to air concentrations of 0.0012 mg/m3 (LOQ) and 0.012 mg/m3 (10x LOQ). The traps were then flushed with air, under ambient or elevated conditions of temperature and humidity, for six hours before being analysed.

d-Phenothrin residues were extracted from XAD-2 resin packed glass tubes (200/400 mg; SKC Cat No. 226-30-06) with toluene. Detection, quantification and confirmation were by gas chromatography with mass spectrometric detection (GC/MS), in the electron impact (EI) mode.

13.2 Conclusion: The method is considered to be acceptable in terms of accuracy, precision, linearity and specificity.

13.2.1 Reliability: [redacted]

13.2.2 Deficiencies: [redacted]

Evaluation by Competent Authorities	
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Date	[redacted]
Materials and method	
Conclusion	
Reliability	
Acceptability	
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	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

[redacted]

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Section A4.2(c) **Analytical Methods for Detection and Identification**

Annex Point IIA4.1/4.2 & IIIA-IV.1 **Analytical Method for Drinking Water**

Official use only

48 REFERENCE

4.18.1 Reference	[redacted]
4.28.2 Data protection	Yes
4.2.18.2.1 Data owner	Sumitomo Chemical Co., Ltd.
4.2.28.2.2 Companies with a letter of access	Not applicable
4.2.38.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

59 GUIDELINES AND QUALITY ASSURANCE

5.19.1 Guideline study	[redacted] European Commission Directive 96/46/EC, July 16, 1996 European Commission, Guidance Document on residue Analytical Methods, SANCO/825/00 rev. 7, March 17, 2004 TNsG, Analytical Methods. In Support of the Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market, (Draft Revision Document CA-May08-Doc.6.9)
5.29.2 GLP	Yes
5.39.3 Deviations	None

610 MATERIALS AND METHODS

6.110.1 Preliminary treatment	
6.1.110.1.1 Enrichment	**Preparation of the SPE** - C18 (500 mg) SPE cartridge was washed with 10 mL toluol - the cartridge was then washed with 10 mL acetone - afterwards the cartridge was washed with 10 mL water - the column must not be allowed to run dry **Preparation of the samples** - 200 mL sample water were transferred into a 250-mL measuring flask - the fortification samples were spiked as required - the measuring flasks were filled up to the calibration mark using sample water - the resulting solution was mixed well by shaking **Extraction** - the sample was completely loaded onto the SPE column and passed through slowly - finally the column was sucked to dryness by strong vacuum - 8 mL acetone were used for elution into a 10 mL pyrex glass - the acetone was evaporated to dryness in a gentle nitrogen stream

- the residue was reconstituted in 1000 µl toluol
- the final solution was transferred into an amber GC vial

6.1.210.1.2 Cleanup: Not applicable

6.210.2 Detection

6.2.110.2.1 Separation method: **GC/MS:**

GC System	AGILENT 6890 SERIES SYSTEM WITH AGILENT 7683 SERIES AUTOSAMPLER
Carrier gas	Helium
INJECTION TECHNIQUE	Splitless injection with 2µL injection volume. Injection temperature was 225°C. Purge time was 0.60 min, purge flow was 30.0 mL/min. Gas saver was on. Saver flow was 20.0 mL/min and saver time was 2.00 min.
GC capillary column	AGILENT HP 5 MS COLUMN: 15 M LENGTH, 0.25 MM INNER DIAMETER, 0.25 µM FILM THICKNESS. Column flow 1.0 mL/min (constant flow).
Oven temperature program	95°C (0.75 min hold), heat rate 15°C/min to 250°C (hold for 0 min) and heat rate 10°C/min to 275°C (hold for 7 min)

6.2.210.2.2 Detector:

MS detection	Agilent 5973 MS operated in electron impact ionisation mode with selected ion monitoring (SIM)
Data Acquisition	*Acquisition and peak calculations were performed with Agilent chemstation software.*
Retention time:	~ 13.2 MIN FOR SUMITHRIN
QUANTIFICATION:	Quantification of the analytical reference item was performed using a Microsoft Excel template using the regression model $y=a*x^b$

Mass	m/z	Dwell Time	Method
Mass 1	123	50	2nd Confirmatory
Mass 2	183	50	Primary
Mass 3	350	200	1st Confirmatory

6.2.310.2.3 Standard(s): A 1000 µg/mL Sumithrin stock solution was prepared by dissolving 13.394 mg of a 96.7% analytical standard in 12.952 mL of toluol and placing in an ultrasonic bath for about 5 min.

Defined volumes of the stock solutions were diluted using acetone to obtain fortification solutions of 10.0, 1.00 and 0.10 µg/mL.

Toluol was used to dilute the stock solution to obtain intermediate and calibration solutions. The range of concentrations of the calibration solutions were 0.005 to 2.00 µg/mL.

Refer to Figure 4.2(c)-1 for a representative chromatogram (primary and confirmatory method) of a 0.005 µg/mL calibration standard and to Figure 4.2(c)-2 for a representative chromatogram (primary and confirmatory method) of a 0.02 µg/mL

calibration standard.

~~6.2.4~~10.2.4 Interfering substance(s)	No substances were expected to interfere. Refer to Figure 4.2(c)-3 for a typical chromatogram of an untreated drinking water sample.

~~6.3~~10.3 Linearity

~~6.3.1~~10.3.1 Calibration range	For quantification the range was 0.02 to 2.0 µg/mL. For qualification, including the detection of residues an extended range of 0.005 to 2.0 µg/mL was used. External calibration was performed based on peak areas.
~~6.3.2~~10.3.2 Number of measurements	Seven calibration standards in the range of 0.02 to 2.0 µg/mL were used for the quantification and 9 calibration standards in the range of 0.005 to 2.0 µg/mL were used for the qualification. Each standard was injected 5 times.
~~6.3.3~~10.3.3 Linearity	The correlation was calculated using a least square fit of a potential function ($y=a \cdot x^b$). Data from the calibration generated from the GC/MS analysis used for quantification (primary method (range 0.02 to 2.00 µg/mL)) and qualification (confirmatory method (range 0.005 to 2.0000 µg/mL)) are summarised below: Primary method $y=260801x^{1.3291}$ $r=0.9963$ 1st Confirmatory method $y=14697x^{1.3337}$ $r=0.9984$ 2nd Confirmatory method $y=352689x^{1.2427}$ $r=0.9915$
~~6.4~~10.4 Specifity: interfering substances	No interferences above 30% of the LOQ at the retention time of Sumithrin were detected in the untreated control samples.
~~6.5~~10.5 Recovery rates at different levels	The validation summary results of Sumithrin at 0.1 µg/L and 1.0 µg/L in the Primary method are shown below:

Fortification Level [µg/L]	Mean Recovery [%]	Range [%]	RSD [%]	Number of Analyses
0.1	99	78 - 108	11	5
1.0	87	70 - 100	15	5
overall	93	70 - 108	15	10

The average recovery rates ranged from 87% to 99% with a relative standard deviation of ≤15%.

The validation summary results of Sumithrin at 0.1 µg/L and 1.0 µg/L in the 1st confirmatory method are shown below:

Fortification Level [µg/L]	Mean Recovery [%]	Range [%]	RSD [%]	Number of Analyses
0.1	104	101- 109	3	3
1.0	99	96 - 103	3	3
overall	102	96 - 109	4	6

The average recovery rates ranged from 99% to 104% with a relative standard deviation of ≤4%.

The validation summary results of Sumithrin at 0.1 µg/L and 1.0 µg/L in the 2nd confirmatory method are shown below:

Fortification Level [µg/L]	Mean Recovery [%]	Range [%]	RSD [%]	Number of Analyses
0.1	118	115 - 120	2	3
1.0	96	92 - 99	3	3
overall	107	92 - 120	10	6

The average recovery rates ranged from 96% to 118% with a relative standard deviation of ≤10%. Although the mean recovery of the lower fortification level extends with 118% that is outside the required range of 70% - 110%, the result is acceptable because they are used only for confirmation and the deviation is only 8% with the low RSD of 2%.

~~6.5.1~~10.5.1 Relative standard deviation

Refer to Section 3.5 above.

~~6.6~~10.6 **Limit of determination**

The validated limit of quantification (LOQ) is defined as the lowest fortification level with mean recoveries ranging from 70% to 110% at a relative standard deviation (RSD) of ≤20%. The LOQ for Sumithrin in drinking water was 0.1 µg/L.

The limit of detection (LOD) was estimated from the lowest detectable calibration standard concentration used (0.005 µg/mL). The corresponding limit of detection for Sumithrin in drinking water was 0.02 µg/L.

~~6.7~~10.7 **Precision**

~~6.7.1~~10.7.1 Repeatability

Primary Method (See Table 4.2(c)-2

For drinking water the relative standard deviations were 11% and 15%. These values are within the limit of 20% of the given guidelines, showing the precision of the analytical method.

Confirmatory Method (See Tables 4.2(c)-3 and 4.2(c)-4

For drinking water (1st confirmatory method, m/z 350) the relative standard deviations were 3% and 3%. These values are within the limit of ≤20% of the given guidelines, showing the precision of the analytical method.

For drinking water (2nd confirmatory method) the relative standard deviations were 2% and 3%. These values are within the limit of ≤20% of the given guidelines, showing the precision of the analytical method.

Section A4.2(c)	**Analytical Methods for Detection and Identification**
Annex Point IIA4.1/4.2 & IIIA-IV.1	**Analytical Method for Drinking Water**

~~6.7.2~~10.7.2 Independent laboratory validation	Not applicable
	~~7~~11 APPLICANT'S SUMMARY AND CONCLUSION
~~7.1~~11.1 **Materials and methods**	The purpose of the study was to develop and validate a residue analytical method for the determination of Sumithrin in drinking water. Local tap water from Itingen, Switzerland was sampled and characterised prior to analysis. An analytical method for the determination of Sumithrin in drinking water was validated. 5 replicates at 0.1 and 1.0 µg/L and control samples were prepared and analysed by concentrating the sample on a C18 (500 mg) SPE cartridge, eluting using acetone, then evaporating the eluant and reconstituting in 1 ml of toluol. Detection was via GC-MS with SIM.
~~7.2~~11.2 **Conclusion**	A method with a limit of quantification of 0.1 µg/L and a limit of detection of 0.02 µg/L in drinking water was validated for the determination of Sumithrin in drinking water. The GC/MS method was found to be acceptable in terms of accuracy, precision, specificity and linearity.
~~7.2.1~~11.2.1 Reliability	[redacted]
~~7.2.2~~11.2.2 Deficiencies	[redacted]

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
[redacted]	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4.2(c)-1: Drinking Water Characteristics

Source of Drinking Water	Local Tap Water from Harlan Laboratories, 4452 Itingen, Switzerland. Collected June 04, 2009.
Dry Residue	0.5 g/L
Silt Content	0.3 mg/L
pH-Value	7.83
Dissolved Organic Carbon	3.320 mg C/L
Hardness	19°dH

Table 4.2(c)-2: Individual validation results of Sumithrin in Drinking Water (Primary Method)

Internal Sample ID	Fortification Level [µg/L]	primary method Sumithrin (Quantifier m/z 183) x [µg/mL]	R [µg/L]	Recovery
		Controls		
11	not applicable	< limit of detection		
23	not applicable	not detected		
		Lower Fortification Level: 0.10 µg/L		
13	0.10	0.0262	0.1048	105%
14	0.10	0.0270	0.1079	108%
15	0.10	0.0269	0.1078	108%
25	0.10	0.0244	0.0976	98%
26	0.10	0.0195	0.0779	78%
			Average (n=5)	99%
			RSD(n=5)	11%
		Higher Fortification Level: 1.00 µg/L		
20	1.00	0.2468	0.9871	99%
21	1.00	0.2330	0.9319	93%
22	1.00	0.2507	1.0029	100%
27	1.00	0.1745	0.6982	70%
28	1.00	0.1782	0.7127	71%
			Average (n=5)	87%
			RSD(n=5)	15%
			Overall Average (n=10)	93%
RSD: Relative standard deviation;			Overall RSD (n=10)	15%
n: Number of replicates used for calculation				

Table 4.2(c)-3: Individual Validation Results of Sumithrin in Drinking Water (1st Confirmatory Method)

Internal Sample ID	Fortification Level [µg/L]	1st confirmation method Sumithrin (Qualifier m/z 350) x [µg/mL]	R [µg/L]	Recovery
		Controls		
11	not applicable	< limit of detection		
		Lower Fortification Level: 0.10 µg/L		
13	0.10	0.0254	0.1014	101%
14	0.10	0.0273	0.1091	109%
15	0.10	0.0256	0.1025	103%
			Average (n=3)	104%
			RSD (n=3)	3%
		Higher Fortification Level: 1.00 µg/L		
20	1.00	0.2446	0.9784	98%
21	1.00	0.2406	0.9625	96%
22	1.00	0.2569	1.0277	103%
			Average (n=3)	99%
			RSD (n=3)	3%
			Overall Average (n=6)	102%
RSD: Relative standard deviation;			Overall RSD (n=6)	4%
n: Number of replicates used for calculation				

Table 4.2(c)-4: Individual Validation Results of Sumithrin in Drinking Water (2nd Confirmatory Method)

Internal Sample ID	Fortification Level [µg/L]	2nd confirmation method		
		Sumithrin (Qualifier m/z 123)		
		x [µg/mL]	R [µg/L]	Recovery
		Controls		
11	not applicable	< limit of detection		
		Lower Fortification Level: 0.10 µg/L		
13	0.10	0.0289	0.1154	115%
14	0.10	0.0299	0.1197	120%
15	0.10	0.0297	0.1187	119%
			Average (n=3)	118%
			RSD(n=3)	2%
		Higher Fortification Level: 1.00 µg/L		
20	1.00	0.2429	0.9717	97%
21	1.00	0.2309	0.9236	92%
22	1.00	0.2469	0.9878	99%
			Average (n=3)	96%
			RSD(n=3)	3%
			Overall Average (n=6)	107%
RSD: Relative standard deviation;			Overall RSD (n=6)	10%
n: Number of replicates used for calculation				

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Section A4.2(c) Annex Point IIA4.2 IUCLID 6.2/1 Analytical Methods for Detection and Identification Method Validation for the Analysis of Sumithrin in Water

Official use only

812 REFERENCE

13.3	**Reference**	[redacted]
13.4	**Data protection**	Yes
13.4.1	Data owner	Sumitomo Chemical Co., Ltd.
13.4.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

14 GUIDELINES AND QUALITY ASSURANCE

14.1	**Guideline study**	U.S. EPA-FIFRA 40 CFR Section 158.145
14.2	**GLP**	[redacted]
14.3	**Deviations**	

15 MATERIALS AND METHODS

15.1	**Preliminary treatment**	Non-entry field
15.1.1	Enrichment	500 ml aliquots, measured using a graduated cylinder, of control test water were transferred to 1L separatory funnels. The samples were fortified with Sumithrin at concentrations ranging from 0.320 to 104 µg/l. Duplicate samples were prepared for each concentration. Following fortification, approximately 2 g of NaC1 were added to each sample and the samples were shaken until the NaCl had completely dissolved.
15.1.2	Cleanup	Liquid-liquid clean-up was performed as follows:- 100 ml portions of dichloromethane were added to each sample and the samples were shaken for approximately 2 minutes. After the phases were allowed to separate, the dichloromethane phases were eluted over powder funnels containing sodium sulphate (which had been prewashed with dichloromethane) into 500 ml flasks. The extraction was repeated once again and the sodium sulphate was rinsed with an additional 10 ml portion of dichloromethane. The combined dichloromethane extracts were rotoevaporated to near dryness and reconstituted to appropriate volume using a 50% acetonitrile, 50% millipore® water solution. The samples were brought up in volumes such that the concentration of the diluted samples fell within the standard calibration range. During the analysis, it was determined that 2.50 µg/l was the lowest Sumithrin concentration that could be accurately measured using HPLC methods.
15.2	**Detection**	Non-entry field

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15.2.1 Separation method

The samples were analyzed for Sumithrin using a Waters Model 510 HPLC pump equipped with a Varian 2050 UV detector and a Shimadzu Sil—6A autosampler. The chromatographic data was collected and stored using a Hewlett Packard 1000 Minicomputer with Beckman CALS System® software. The operating parameters were as follows:

Column: Alltech Econosil C18 (RP), 25 cm x 4.6 mm ID, 10 micron, serial #022588—11.

Mobile Phase:

50% HPLC grade Acetonitrile; 50% Millipore Water

Flow Rate: 1.5 ml/mm

Injection Volume: 200 p1

15.2.2 Detector

Varian 2050 UV detector; λ 215 nrn.

15.2.3 Standard(s)

A primary stock standard of Sumithrin at a concentration of 1.04 mg/ml in acetone was prepared by dissolving ~0.05g of Sumithrin in 50 ml acetone and stored in a refrigerator. Subsequent dilutions of the 1.04 mg/ml stock solution were prepared in acetone as spiking solutions and in 50% HPLC grade acetonitrile, 50% Millipore water for HPLC chromatography standards.

Standard Reference (µg/ml)	Volume Taken (ml)	Final Volume (ml)	Concentration (µg/ml)
1040 (Stock)	1.000	50	20.8**
20.8	0.060	100	0.012*
20.8	0.125	100	0.026*
20.8	0.250	100	0.050*
20.8	0.500	100	0.104*
20.8	1.000	100	0.208*

*50% HPLC grade acetonitrile, 50% Millipore water

** Acetone

Calculations

Calculations of the Sumithrin concentrations were performed using the external standard analysis function of a Hewlett Packard 1000 minicomputer using Beckman CALS System® software.

Concentrations of Sumithrin in the samples were determined directly from the standard curve by the following equation:

(ng/ml Sumithrin equivalents from standard curve equation) x (Volume for analysis in ml)/ (sample volume extracted in ml) = ng/ml Sumithrin (ppb)

15.2.4 Interfering substance(s)

No substances are expected to interfere.

15.3 Linearity

Non-entry field

15.3.1 Calibration range

A calibration curve 0.012, 0.026, 0.050, 0.104, 0.208 µg/ml was prepared.

Refer to Figure A4_1(1)-2 for a typical calibration line.

15.3.2 Number of measurements

Each standard was injected once.

15.3.3 Linearity

The detector response (area) for Sumithrin® was plotted against the standard concentration. The correlation coefficient was calculated to be 0.99955.

15.4 Specifity: interfering substances

No other substances were found to interfere. The controls were not found to contain any interfering peaks at the retention time of Sumithrin.

15.5 Recovery rates at different levels

Fortification Level (µg/l)	Measured Concentration (µg/l)	% Recovery	Mean % Recovery	RSD (%)
Control A	<0.250	-	-	-
Control B	<0.250	-	-	-
0.52	<0.250	-	-	-
0.52	<0.250	-	-	-
1.04	0.438	42.1*	-	-
1.04	0.398	38.3*	-	-
2.5	2.04	81.6	82.7	8.1
2.5	1.99	79.6		
5.2	4.61	88.7		
5.2	4.39	84.4		
52	43.4	83.5		
52	49.1	94.4		
104	76.1	73.2		
104	79.5	76.4		

* Outside acceptance criteria. Not included in calculation of the mean and %RSD.

15.5.1 Relative standard deviation

The RSD = 8.1%

15.6 Limit of determination

The limit of determination was established to be 2.50 µg/l.

~~X7~~

15.7 Precision

Non-entry field

15.7.1 Repeatability

Repeatability was not assessed within this report.

15.7.2 Independent laboratory validation

An independent laboratory validation has not been performed.

16 APPLICANT'S SUMMARY AND CONCLUSION

16.1 Materials and methods

The method of analysis involves liquid-liquid extraction clean-up of water followed by determination of levels of Sumithrin by HPLC UV detection at 215 nm.

16.2 Conclusion

The method is considered to be acceptable in terms of accuracy, precision, linearity and specificity.

16.2.1 Reliability

[redacted]

16.2.2 Deficiencies

[redacted]

This method does not meet the requirements of the drinking water regulations i.e. the LOD is >0.1 µg/l. The method does however cover the range required in the LD_{50} aquatic toxicology tests and is therefore considered to be fit for purpose.

Evaluation by Competent Authorities

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Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A4

Reference list by section number

Section No./Reference No.	Author(s)	Year	Title, Source (where different from company) Company, Report No. GLP (where relevant) / (Un) Published	Data Protection Claimed (Yes/No)	Owner
A4_1/01	[redacted]	1997	Enforcement Analytical Method for Sumithrin® Technical Grade. [redacted]	Y	Sumitomo Chemical Co., Ltd.
A4_1/02	[redacted]	1997	Enforcement Analytical Method for Sumithrin® Technical Grade. [redacted]	Y	Sumitomo Chemical Co., Ltd.
A4_1/03	[redacted]	1997	Enforcement Analytical Method for Sumithrin® Technical Grade. [redacted]	Y	Sumitomo Chemical Co., Ltd.
A4_1/04	[redacted]	1997	Enforcement Analytical Method for Sumithrin® Technical Grade. [redacted]	Y	Sumitomo Chemical Co., Ltd.
A4_1/05	[redacted]	1997	Enforcement Analytical Method for Sumithrin® Technical Grade. [redacted]	Y	Sumitomo Chemical Co., Ltd.

Section No./Reference No.	Author(s)	Year	Title, Source (where different from company) Company, Report No. GLP (where relevant) / (Un) Published	Data Protection Claimed (Yes/No)	Owner
A4_1/6	CIPAC	2002	CIPAC Method 356 - d-Phenothrin Source: CIPAC Report No. none [redacted] Published: Y	N	CIPAC
A4_1/6	[redacted]	2002	CIPAC Method 356 - d-Phenothrin Small Scale Collaborative Study on the Determination of d-Phenothrin in d-Phenothrin Technical by Gas Chromatography [redacted]	N	CIPAC
A4_2/b	[redacted]	2006	Sumithrin (d-Phenothrin): Validation of an Analytical Method for the determination of Residues in Air [redacted]	Y	Sumitomo Chemical Co., Ltd.
A4 2/c	[redacted]	2009	Sumithrin: Validation of a Multi-Residue Method for the Determination of Sumithrin in Drinking Water	Y	[redacted]
A4_2/d	[redacted]	1988	Method Validation for the Analysis of Sumithrin in Aquatic Test Water [redacted]	Y	Sumitomo Chemical Co., Ltd.

Reference List by Author

Author(s)	Section No./ Reference No.	Year	Title, Source (where different from company) Company, Report No. GLP (where relevant) / (Un) Published	Data Protection Claimed (Yes/No)	Owner
CIPAC	A4_1/6	2002	CIPAC Method 356 - d-Phenothrin Source: CIPAC Report No. none	N	CIPAC
[redacted]	A4_ 2/c	2009	Sumithrin: Validation of a Multi-Residue Method for the Determination of Sumithrin in Drinking Water	Y	[redacted]
[redacted]	A4_1/6	2002	CIPAC Method 356 - d-Phenothrin Small Scale Collaborative Study on the Determination of d-Phenothrin in d-Phenothrin Technical by Gas Chromatography	N	CIPAC
[redacted]	A4_1/05	1997	Enforcement Analytical Method for Sumithrin® Technical Grade.	Y	Sumitomo Chemical Co., Ltd.
[redacted]	A4_2/c	1988	Method Validation for the Analysis of Sumithrin in Aquatic Test Water	Y	Sumitomo Chemical Co., Ltd.

Author(s)	Section No./ Reference No.	Year	Title, Source (where different from company) Company, Report No. GLP (where relevant) / (Un) Published	Data Protection Claimed (Yes/No)	Owner
[redacted]	A4_2/b	2006	Sumithrin (d-Phenothrin): Validation of an Analytical Method for the determination of Residues in Air [redacted]	Y	Sumitomo Chemical Co., Ltd.

Section A4.1 (1) Analytical Methods for Detection and Identification
Enforcement Analytical Method for Sumithrin® Technical Grade-Determination of Sumithrin Content

Official use only

		1. REFERENCE
1.1	**Reference**	[redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Sumitomo Chemical Co., Ltd.
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	U.S. EPA Product Properties Test Guidelines OPPTS 830.1800
2.2	**GLP**	[redacted]
2.3	**Deviations**	
		3 MATERIALS AND METHODS
3.1	**Preliminary treatment**	Non-entry field
3.1.1	Enrichment	Determination of Sumithrin® Content Weigh accurately about 0.1 g of each Sumithrin® T.G. and Sumithrin® standard, and add exactly 10 mL of the internal standard solution to prepare a sample solution and a standard solution. Perform the test with 1 µL each of the sample and the standard solutions by GC.
3.1.2	Cleanup	No clean-up is required as there are no potentially interfering materials. Standard solutions prepared in solvent are being quantified.
3.2	**Detection**	Non-entry field
3.2.1	Separation method	Gas Chromatography was used for the determination of Sumithrin®. Column: A glass column (3 mm id. x 1 m), packed with 2 % PEG 20M on Chromosorb W AW DMCS (60 to 80 mesh). Temperatures: Oven, 210 °C Injection port and detector, 240°C. Carrier gas: Nitrogen. Flow rate: Adjust the flow rate so that the retention time of Sumithrin® is about 12 minutes. Refer to Figure A4_1(1)-1 for a typical chromatogram.
3.2.2	Detector	Flame ionisation detection (FID) was employed.
3.2.3	Standard(s)	Approximately 80, 90, 100, 110 and 120 mg of Sumithrin® standard was accurately weighed and dissolved in exactly 10 mL of the internal standard solution (di-(2-ethylhexyl) phthalate) to make calibration solutions (80- 120mg/10 mL). The ratio of peak area of Sumithrin® to

Section A4.1 (1) Analytical Methods for Detection and Identification Enforcement Analytical Method for Sumithrin® Technical Grade- Determination of Sumithrin Content

that of the internal standard was plotted against the amount of Sumithrin® in the solution to make a calibration curve.

The concentration of Sumithrin® in the Sumithrin® T.G. was determined using the equation below:-

$$C = \frac{Ws \times Q_T \times P}{W_X \times Q_S}$$

where C: the content (%) of Sumithrin® in Sumithrin® T.G.

W_S: the amount (mg) of Sumithrin® standard.

W_T: the amount (mg) of Sumithrin® T.G.

Q_S: the ratio of the peak area of Sumithrin® against that of the internal standard in the standard solution.

Q_T: the ratio of the peak area of Sumithrin® against that of the internal standard in the sample solution.

P: the purity (%) of Sumithrin® standard.

3.2.4 Interfering substance(s)

No substances are expected to interfere as the standard is prepared in analytical reagent grade acetone. The method developed, adequately separates the active substance from its impurities.

3.3 Linearity

NON-ENTRY FIELD

3.3.1 Calibration range

A calibration curve (8, 9, 10, 11 and 12 mg/ml) was prepared containing 10 mg/ml internal standard (di-(2-ethylhexyl) phthalate).

Refer to Figure A4_1(1)-2 for a typical calibration line.

3.3.2 Number of measurements

Each standard was injected once.

3.3.3 Linearity

The ratio of Sumithrin® peak area to internal standard was calculated and plotted. The correlation coefficient was calculated to be 1.0000.

3.4 Specificity: interfering substances

No other substances were found to interfere. Refer to Figure A4.1(1)-3

3.5 Recovery rates at different levels

~~X1~~

Six separate sub-samples from a sample of Sumithrin® T.G. (10 mg/ml) were analysed and the results were as follows:-

Percentage Recovery	Mean Recovery (%)	RSD (%)
93.7, 93.9, 92.9 94.1, 93.8, 94.0	93.7	0.46

Different concentration levels were not evaluated, as this is not applicable to purity determinations.

3.5.1 Relative standard deviation

The RSD = 0.5%

3.6 Limit of determination

The limit of determination, based on the lowest calibration standard was 8 mg/ml.

Section A4.1 (1) Analytical Methods for Detection and Identification Enforcement Analytical Method for Sumithrin® Technical Grade- Determination of Sumithrin Content

3.7 Precision — Non-entry field

3.7.1 Repeatability — Two different analysts analysed the standards (10 mg/ml) and good precision between the results was found, as shown below:- ~~X2~~

Analyst	% Recovery	Mean Recovery (%)	RSD (%)
A	93.7, 93.9, 92.9 94.1, 93.8, 94.0	93.7	0.37
B	93.7, 93.9, 93.6	93.7	

3.7.2 Independent laboratory validation — An independent laboratory validation is not required for this type of method.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods — The method of analysis involves dissolving 0.1g of Sumithrin® T.G. in 10 ml of acetone containing internal standard and quantifying the solution using GC-FID.

4.2 Conclusion — The method is considered to be acceptable in terms of accuracy, precision, linearity and specificity. ~~X3~~

4.2.1 Reliability — [redacted]

4.2.2 Deficiencies — [redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
Date	
Materials and method	
Conclusion	
Reliability	
Acceptability	
Remarks	
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

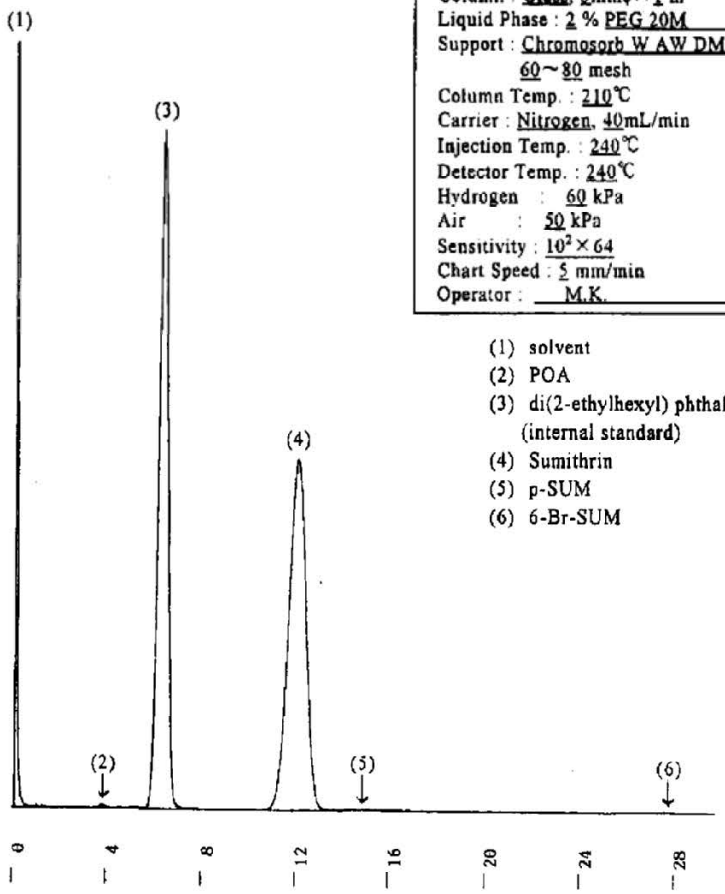
Figure A4_1(1)-1 Typical gas chromatogram for the determination of Sumithrin® content

Sumitomo Chemical Co., Ltd. Study No. 3256

Page 37 of 45

Gas Chromatogram (FID)

Date : 1997.5.24
Apparatus : Shimadzu GC-14B
Sample : Sumithrin T.G. 40817G
10 mg/mL of acetone , 1μL
Column : Glass, 3mmϕ × 1 m
Liquid Phase : 2 % PEG 20M
Support : Chromosorb W AW DMCS
60~80 mesh
Column Temp. : 210℃
Carrier : Nitrogen, 40mL/min
Injection Temp. : 240℃
Detector Temp. : 240℃
Hydrogen : 60 kPa
Air : 50 kPa
Sensitivity : $10^2 \times 64$
Chart Speed : 5 mm/min
Operator : M.K.



(1) solvent
(2) POA
(3) di(2-ethylhexyl) phthalate (internal standard)
(4) Sumithrin
(5) p-SUM
(6) 6-Br-SUM

Figure A4_1(1)-2 Calibration curve for the determination of Sumithrin in Sumithrin® TG.

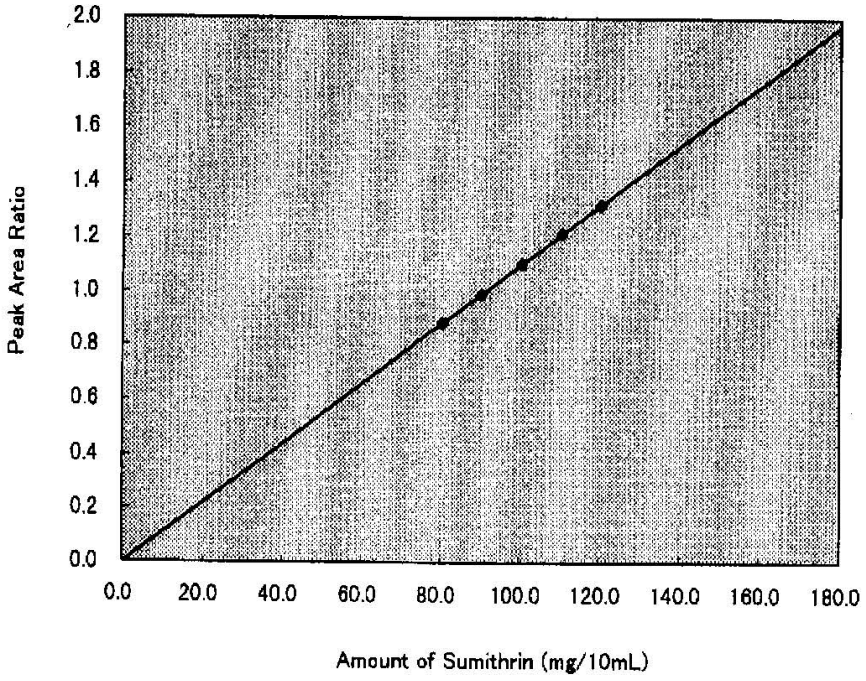
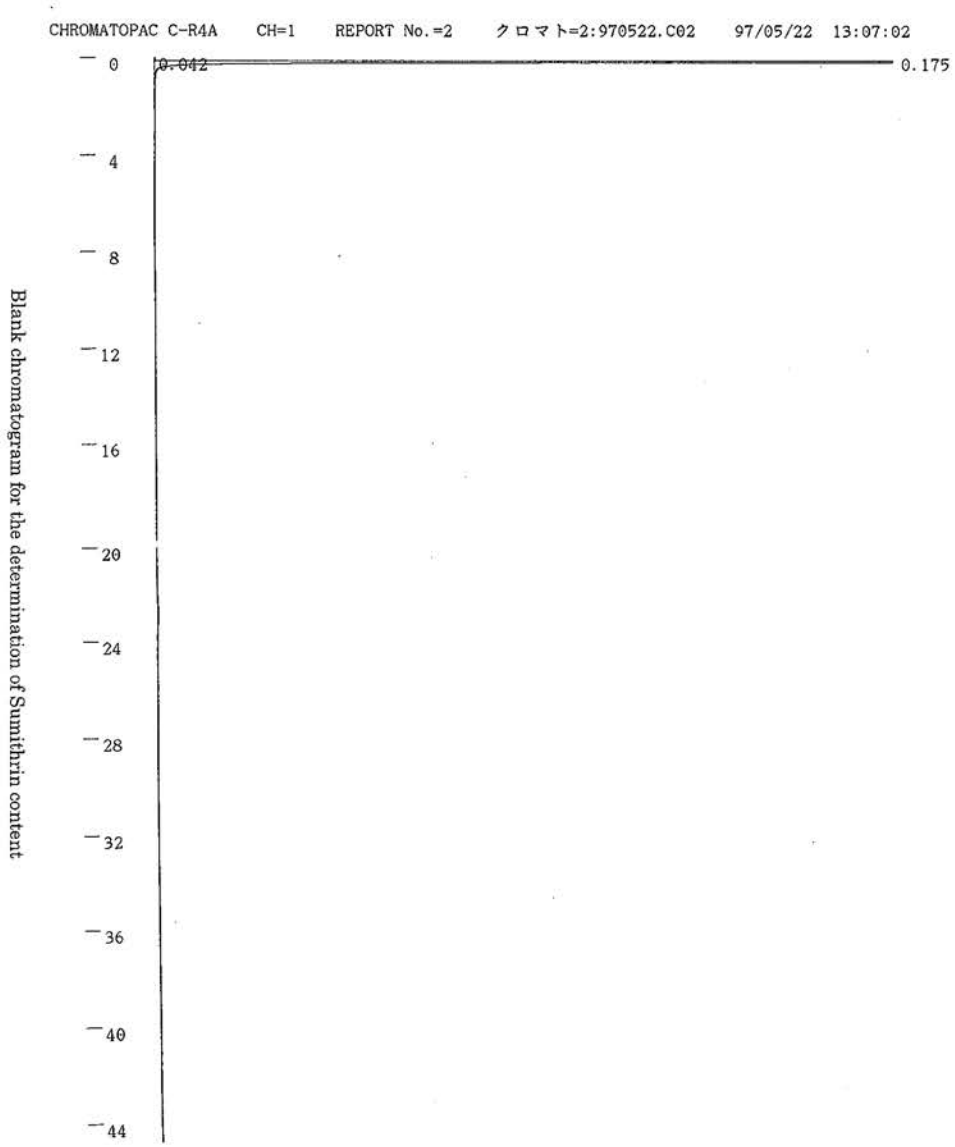


Figure A4_1(1)-3 Typical Blank gas chromatogram for the determination of Sumithrin® content



** 定量計算結果 **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	0.042	28	6			0.0008	
	2	0.175	3700189	1264311	VE		99.9992	
		TOTAL	3700218	1264318			100	

Section A4.1(2) Annex Point IIA4.1 Analytical Methods for Detection and Identification Enforcement Analytical Method for Sumithrin® Technical Grade - Determination of Geometric Isomer Ratios

Official use only

		5 REFERENCE
1	**REFERENCE**	[redacted]
1.1	**Data protection**	Yes
1.1.1	Data owner	Sumitomo Chemical Co., Ltd.
1.1.2		
1.1.3	Criteria for data protection	Data submitted to the ms after 13 may 2000 on existing a.s. for the purpose of its entry into annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	U.S. EPA Product Properties Test Guidelines OPPTS 830.1800
2.2	**GLP**	[redacted]
2.3	**Deviations**	
		3 MATERIALS AND METHODS
3.1	**Preliminary treatment**	*Non-entry field*
3.1.1	Enrichment	Determination of Geometric Isomer Ratios Dissolve 0.10 g of Sumithrin® T.G. in 10 mL of acetone to prepare a sample solution. Perform the test with 1 µL of the sample solution by GC.
3.1.2	Cleanup	No clean-up is required as there are no potentially interfering materials. The samples comprise of standard solutions prepared in solvent.
3.2	**Detection**	*Non-entry field*
3.2.1	Separation method	Gas Chromatography (GC) was used. Column: A glass column (3 mm id. x 2 m) packed with 5 % silicone DC QF-I on Chromosorb W AW DMCS (60 to 80 mesh). Temperatures: Oven, 170°C; Injection port and detector, 220°C. Carrier gas: Nitrogen. Flow rate: Adjust the flow rate so that the retention time of *trans*-isomer is about 40 minutes. Refer to Figure A4_1(2)-1 for a typical chromatogram.
3.2.2	Detector	Flame ionisation detection (FID) was employed.
3.2.3	Standard(s)	Approximately 80, 90, 100, 110 and 120 mg of Sumithrin® standard was accurately weighed and dissolved in exactly 10 mL of the internal standard solution (di-(2-ethylhexyl) phthalate) to make calibration solutions (80- 120 mg/10 mL). The ratio of peak area of Sumithrin® to that of the internal standard was plotted against the amount of Sumithrin® in the solution to make a calibration curve.

Section A4.1(2) Annex Point IIA4.1 Analytical Methods for Detection and Identification Enforcement Analytical Method for Sumithrin® Technical Grade - Determination of Geometric Isomer Ratios

The peak areas of *cis*- and *trans*- isomers in the sample solution were measured and calculated using the following equation:-

$$C = \frac{At \times 100}{Ac + At}$$

where, C: *trans*-isomer ratio (%).

Ac: peak area of *cis*-isomer,

At: peak area of *trans*-isomer.

3.2.4 Interfering substance(s)

No substances are expected to interfere as the standard is prepared in analytical reagent grade acetone. The method developed, adequately separates the active substance from its impurities.

3.3 Linearity

Non-entry field

3.3.1 Calibration range

A calibration curve (8, 9, 10, 11 and 12 mg/ml) was prepared containing 10 mg/ml internal standard (di-(2-ethylhexyl) phthalate).

3.3.2 Number of measurements

Each standard was injected once.

3.3.3 Linearity

The ratio of Sumithrin® peak area to internal standard was calculated and plotted. The correlation coefficient was calculated to be 1.0000.

3.4 Specifity: interfering substances

No other substances were found to interfere. Refer to Figure A4.1(2)-2

3.5 Recovery rates at different levels

Results for the determination of isomer ratio in the standard mixtures (10 mg/ml)

Sample No.	Calculated value (%)		Found value (%)	
	cis-SUM	*trans*-SUM	*cis*-SUM	*trans*-SUM
1	30.1	69.9	29.5	70.6
2	25.2	74.8	24.4	75.7
3	20.2	79.8	19.3	80.7
4	0.6	99.4	1.1	98.9

Results for the repeatability for the determination of *trans*-isomer ratio

Found Value (%)	Mean (%)	RSD (%)
80.4, 80.3, 80.2 80.3, 80.3, 80.2	80.3	0.1

Recovery rates for Accuracy and linearity data can be calculated using the accuracy data described in report. Refer to Table 4.1(2)-1

3.5.1 Relative standard deviation

RSD = 0.1%

3.6 Limit of determination

The limit of determination, based on the lowest calibration standard was 8 mg/ml.

3.7 Precision

Non-entry field

3.7.1 Repeatability

Two different analysts analysed the standards and good precision

Section A4.1(2) Annex Point IIA4.1 Analytical Methods for Detection and Identification Enforcement Analytical Method for Sumithrin® Technical Grade - Determination of Geometric Isomer Ratios

between the results was found, as shown below:-

Analyst	Found Value (%)	Mean (%)	Overall Mean (%)	RSD (%)
A	80.4, 80.3, 80.2 80.3, 80.3, 80.2	80.3	80.0	0.50
B	79.5, 79.5, 79.5	79.5		

3.7.2 Independent laboratory validation

An independent laboratory validation is not required for this type of method.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

The method of analysis involves dissolving 0.1g of Sumithrin® T.G. in 10 ml of acetone containing internal standard and quantifying the *cis* and *trans* isomers using GC-FID.

4.2 Conclusion

The method is considered to be acceptable in terms of accuracy, precision, linearity and specificity.

4.2.1 Reliability [redacted]

4.2.2 Deficiencies [redacted]

Figure A4_1(2)-1 Typical gas chromatogram for the determination of geometrical isomer ratio of Sumithrin® T.G.

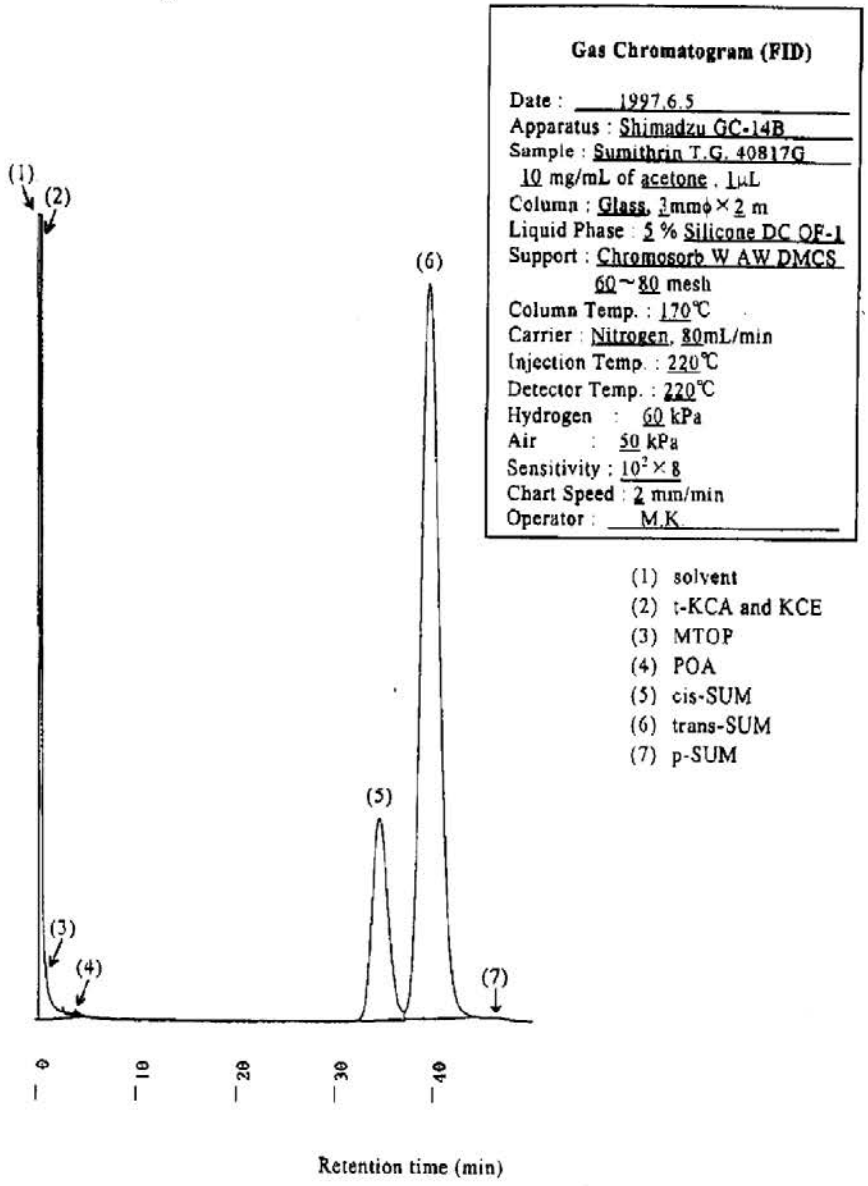
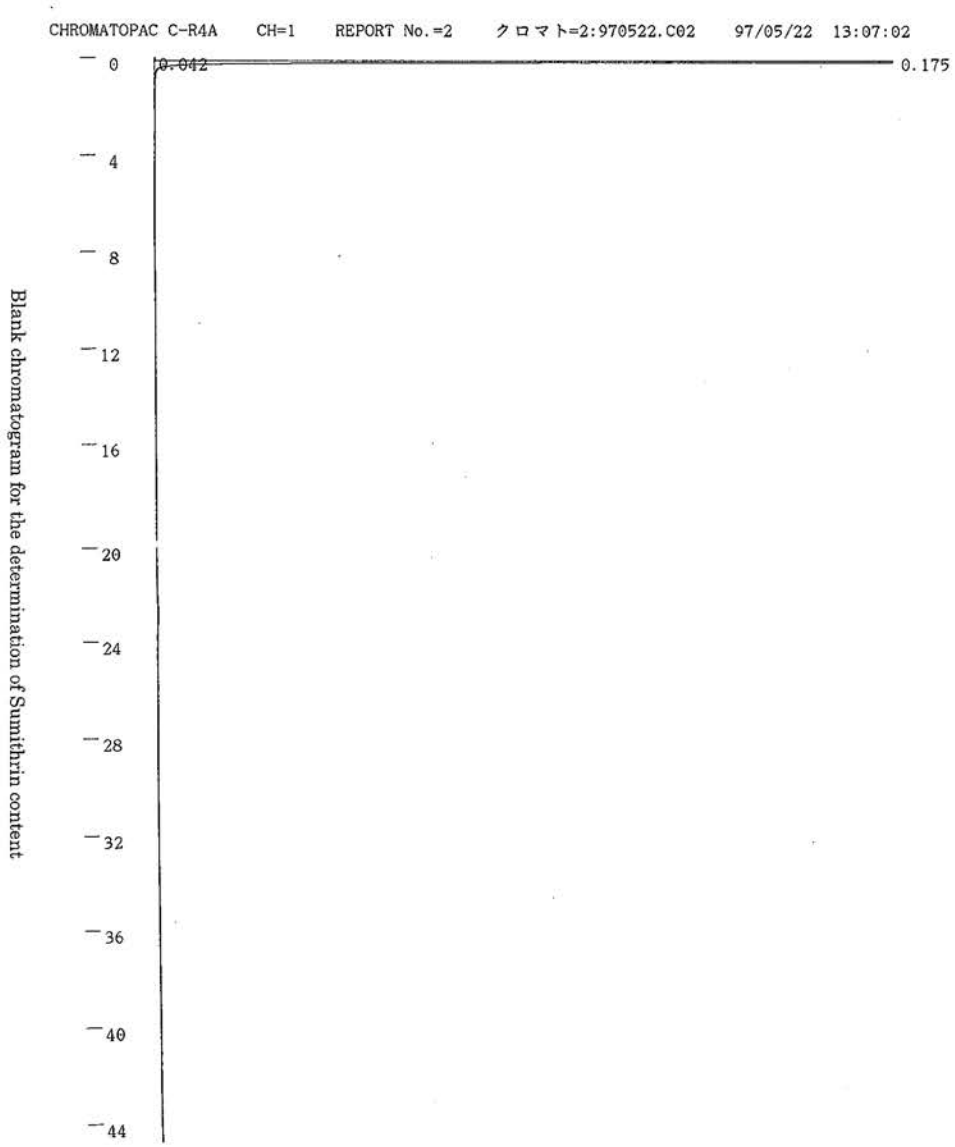


Figure A4_1(2)-2 Typical Blank gas chromatogram for the determination of Sumithrin® content



** 定量計算結果 **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	0.042	28	6			0.0008	
	2	0.175	3700189	1264311	VE		99.9992	
		TOTAL	3700218	1264318			100	