Section A3.14 Annex Point IIIA, (-)	Viscosity	
	Justification for non-submission of data	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [x]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	RH-287T is a solid with a melting point quite higher than room temperature (about 40-42°C). Therefore, its viscosity was not determined.	
Undertaking of intended data submission [ ]	No studies are planned.	
	Evaluation by Competent Authorities	
Date	18 September 2007	
Evaluation of applicant's justification	Agree with applicant's version	
Conclusion	Agree with applicant's version	
Remarks	-	

Section A3.15 Annex Point IIA, III. 3.11.	Explosive properties	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [x] Other justification [ ]	
Detailed justification:	Detailed justification is considered as confidential information	

Section A3.15 Annex Point IIA, III. 3.11.	Explosive properties
	[redacted]
Undertaking of intended data submission [ ]	No studies are planned.
	Evaluation by Competent Authorities
Date	14 January 2008
Evaluation of applicant's justification	Agree with applicant's version
Conclusion	Agree with applicant's version
Remarks	-

Section A3.16 Annex Point IIA, III 3.12.	Oxidising properties	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Detailed justification is considered as confidential information [redacted]	
Undertaking of intended data submission [ ]	No studies are planned.	
	Evaluation by Competent Authorities	
Date	28 January 2008	
Evaluation of applicant's justification	Agree with applicant's version	
Conclusion	Agree with applicant's version	
Remarks	-	

Directive 98/8/EC on the placing of biocidal products on the market.

Dossier for the inclusion of an active substance in the Annex 1

4,5-Dichloro-2-octyl-2H-isothiazol-3-one (DCOIT)

Product type 21: Antifouling products

Document III-A (A4)
Study summaries – Active substance

Section A4: Analytical Methods for the Active Substance

TABLE OF CONTENTS

Section A4.1.a
Annex Point IIA, IV.4.1.

Analytical Method for Detection and Identification of the pure active substance 4,5-dichloro-2-octyl-2H-isothiazol-3-one in RH-287T

[redacted]

1 REFERENCE

Official use only

Section A4.1.a
Annex Point IIA, IV.4.1.

Analytical Method for Detection and Identification of the pure active substance 4,5-dichloro-2-octyl-2H-isothiazol-3-one in RH-287T

1.1 Reference

[redacted]

Reference type: Study report

Year: 2002

Report date: 01 June 2002

1.2 Data protection Yes

1.2.1 Data owner: Rohm and Haas Company.

1.2.2

1.2.3 Criteria for data protection: [redacted]

[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study There are no official guidelines available for the analytical methods. The method was developed according to the principles described in the Technical Notes for Guidance on data requirements.

2.2 GLP Yes

Section A4.1.a Annex Point IIA, IV.4.1.	Analytical Method for Detection and Identification of the pure active substance 4,5-dichloro-2-octyl-2H-isothiazol-3-one in RH-287T

2.3	**Deviations**	N/A

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Dissolve 50-65 mg of (molten) RH-287T sample in 25 ml acetonitrile containing internal standard and inject 5 µl into HPLC.
3.1.2	Cleanup	No clean-up required.
3.2	**Detection**	
3.2.1	Separation method	Reverse phase HPLC using a 5 µm particles, 15 cm x 4.6 mm i.d. C18 column with 70 % acetonitrile/30 % water isocratic mobile phase at a flow of 2.3 ml/minutes. In these conditions, the retention time of 4,5-dichloro-2-octyl-2H-isothiazol-3-one is approximately 4.8 min.
3.2.2	Detector	Variable wavelength UV detector set at 254 nanometers
3.2.3	Standard(s)	Dimethyl phthalate as an internal standard
3.2.4	Interfering substance(s)	None.
3.3	**Linearity**	
3.3.1	Calibration range	Concentrations are estimated by an internal standard, single point calibration method. The standard is prepared by dissolving 50-65 mg RH-287T (purity > 99 %) in 25 ml internal standard solution (500 ppm w/v dimethyl phthalate in acetonitrile).
3.3.2	Number of measurements	Not applicable.
3.3.3	Linearity	Linearity of the detector response at 254 nm was conducted at 5 levels ranging from 7.78 mg to 77.8 mg RH-287T (purity > 99 %) dissolved in 25 ml internal standard solution. The method is linear with correlation coefficient of 0.999964.
3.4	**Specifity: interfering substances**	Potential low level impurities in RH-287T do not interfere with 4,5-dichloro-2-octyl-2H-isothiazol-3-one or the internal standard peaks. Both the dimethyl phthalate internal standard and 4,5-dichloro-2-octyl-2H-isothiazol-3-one peaks in sample preparation were identified by LC/MS.
3.5	**Recovery rates at different levels**	Not applicable.
3.5.1	Relative standard deviation	

Section A4.1.a
Annex Point IIA, IV.4.1.

Analytical Method for Detection and Identification of the pure active substance 4,5-dichloro-2-octyl-2H-isothiazol-3-one in RH-287T

3.6	**Limit of determination**	Not applicable as the analysis is for RH-287T containing > 99 % 4,5-dichloro-2-octyl-2H-isothiazol-3-one .
3.7	**Precision**	
3.7.1	Repeatability	In order to determine repeatability of the analytical procedure, one technical sample was prepared five times and each preparation was injected in duplicate. Standard deviation of the measurement is ± 0.882 %.
3.7.2	Independent laboratory validation	Technical sample was analyzed in two different labs, by different analysts on different instruments. The standard deviation is ± 0.785 %.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	50-65 mg of molten RH-287T are dissolved in 25 ml internal standard solution (dimethyl phthalate in acetonitrile) and 5 µl are injected into HPLC for analysis. The analytical procedure involves Reverse Phase HPLC using a 5 µm particles, 15 cm x 4.6 mm i.d. C18 column with a 70 % acetonitrile/30 % water isocratic mobile phase at a flow of 2.3 ml/min and UV detection at 254 nanometers. Concentrations are estimated by an internal standard, single point calibration method.
4.2	**Conclusion**	The test method meets all the necessary method validation criteria for the determination of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in RH-287T.
4.2.1	Reliability	[redacted]
4.2.2	Deficiencies	[redacted]

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	25 September 2007, revised 6 January 2009
Materials and methods	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A4.2.a
Annex Point IIA, IV4.2

Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in Sediment and Soil

[redacted]

Official use only

1 REFERENCE

1.1	**Reference**	Reference type: Study report Year: 2004 Report date: 18 November 2004
		[redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2		
1.2.3	Criteria for data protection	[redacted]
		[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	There are no official guidelines available for the analytical methods. The method was developed according to the principles described in the Technical Notes for Guidance on data requirements.
2.2	**GLP**	Yes
2.3	**Deviations**	N/A

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	**Soil**: Twenty grams of soil was extracted with 50 ml of acetonitrile by shaking (by hand for 2-3 minutes). The extraction solvent was separated from the soil by filtration. The filtration cake was rinsed with an additional 10-15 ml of acetonitrile. **Sediment**: Twenty grams of sediment was extracted with 50 ml of acetonitrile by shaking (by hand for 2-3 minutes). The extraction solvent was separated from the sediment by filtration. The filtration cake was

Section A4.2.a
Annex Point IIA, IV4.2

Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in Sediment and Soil

		rinsed with an additional 10-15 ml of acetonitrile.
3.1.2	Cleanup	**Soil**: The acetonitrile extract was diluted with 100 ml of water and the resulting solution partitioned with 100 ml of dichloromethane. The dichloromethane fraction was concentrated on a rotary evaporator and made up to 5 ml with acetonitrile. **Sediment**: The acetonitrile extract was diluted with 100 ml of water and the resulting solution partitioned with 100 ml of dichloromethane. The dichloromethane fraction was concentrated on a rotary evaporator and then applied to an ENVI-Carb (1 gram) solid phase extraction cartridge that had been conditioned previously with acetonitrile and water. The extract was pulled through the cartridge under vacuum at a flow rate of about 2 ml/min. The organic eluant was collected and transferred to a round bottom flask where it was concentrated on a rotary evaporator and made up to 5 ml with acetonitrile.
3.2	**Detection**	
3.2.1	Separation method	**Soil**: The separation method for soils employed reversed phase HPLC and the conditions are outlined in Table 4.2(a)-1 (see at the end of section 4.2.a). Separation was on a C-8 column using a solvent gradient of water and acetonitrile with a flow rate of 0.4 ml/min. In these conditions, the retention time of 4,5-dichloro-2-octyl-2H-isothiazol-3-one is about 13 min. **Sediment**: The separation method for soils sediments employed reversed phase HPLC and the conditions are outlined in Table 4.2(a)-2 (see at the end of section 4.2.a). Separation was on an extra densely bonded and double endcapped C-8 column using a solvent gradient of water and methanol with a flow rate of 0.4 ml/min. In these conditions, the retention time of 4,5-dichloro-2-octyl-2H-isothiazol-3-one is about 17.5 min.
3.2.2	Detector	**Soil**: The detector used was a mass spectrometer and conditions are outlined in Table 4.2(a)-3. *(see at the end of section 4.2.a)* **Sediment**: The detector used was a mass spectrometer and conditions are outlined in Table 4.2(a)-3. *(see at the end of section 4.2.a)*
3.2.3	Standard(s)	^{12}C-RH-287T (Lot : 14-SS-18F, purity : 99.86 %) was used as an external standard. Known concentrations were spiked into the soil or sediment and then quantified. Lot No.: 14-SS-18F Purity: 99.86% ^{14}C-RH-287T was used to verify the recovery of 4,5-dichloro-2-octyl-2H-isothiazol-3-one during the individual extraction/clean-up steps.
3.2.4	Interfering substance(s)	No substances interfering with the detection of *4*,5-dichloro-2-octyl-*2H*-isothiazol-3-one in soil or sediment were observed under the conditions employed.
3.3	**Linearity**	

Section A4.2.a
Annex Point IIA, IV4.2

Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in Sediment and Soil

3.3.1	Calibration range	The calibration standards are prepared in blank soil/sediment extracts. **Soil**: The concentrations of 4,5-dichloro-2-octyl-2H-isothiazol-3-one RH-5287 that were used for preparing the calibration curve were 0.2 µg/ml, 0.5 µg/ml, 1.0 µg/ml and 2.0 µg/ml. Each concentration was injected in triplicate. **Sediment**: The concentrations of 4,5-dichloro-2-octyl-2H-isothiazol-3-one (RH-287) that were used for preparing the calibration curve were 0.1 µg/ml, 0.2 µg/ml, 0.5 µg/ml, 1.0 µg/ml and 2.0 µg/ml. Each concentration was injected in triplicate.
3.3.2	Number of measurements	The calibration curves were prepared with triplicate injections at each calibration level. The two test soils and the sediment were fortified at two levels, 0.05 µg/g and 0.5 µg/g of soil/sediment. Generally, triplicate injections were made for each matrix and each concentration; however, there were a few replicates of the Spring House soil dosed at 0.05µg/g soil where only duplicate injections were employed.
3.3.3	Linearity	**Soil**: R^2 equals 0.9907 for the calibration curve in Spring House soil extracts and 0.9955 for the calibration curve in Bristol soil extracts. **Sediment**: R^2 equals 0.9965.

Section A4.2.a
Annex Point IIA, IV4.2

Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in Sediment and Soil

3.4 Specifity: interfering substances

In the test system employed, there were no interfering substances in either the sediment or soil.

3.5 Recovery rates at different levels

[redacted]

3.5.1 Relative standard deviation

[redacted]

Section A4.2.a
Annex Point IIA, IV4.2

Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in Sediment and Soil

3.6	**Limit of determination**	**Soil**: Limit of quantification (LOQ) is 0.05 µg/g of soil. The limit of detection, LOD, is one-third the LOQ or 0.02 µg/g. **Sediment**: Limit of quantification (LOQ) is 0.05 µg/g of sediment. The limit of detection, LOD, is one-third the LOQ or 0.02 µg/g.
3.7	**Precision**	
3.7.1	Repeatability	[redacted]
3.7.2	Independent laboratory validation	Not required.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	**Soil**: Twenty gram aliquots of 2 test soils were fortified with either 0.05µg/g of soil or 0.5 µg/g of soil. The soils were extracted with acetonitrile, the extracted diluted with water, the mixture partitioned with dichloromethane, and the organic fraction concentrated. The concentrate was separated and detected/quantified by LC-MS. **Sediment**: Twenty gram aliquots of fresh water sediment were fortified with either 0.05µg/g of sediment or 0.5 µg/g of sediment. The soils sediments were extracted with acetonitrile, the extracts diluted with water, and the mixture partitioned with dichloromethane. The dichloromethane phase was applied to an ENVI-Carb solid phase extraction cartridge and the eluant concentrated. The concentrate was separated and detected/quantified by LC-MS.
4.2	**Conclusion**	The results demonstrate that the method successfully quantifies 4,5-dichloro-2-octyl-2H-isothiazol-3-one (RH-287) in soil and sediment. The limit of detection quantification is 0.05 µg 4,5-dichloro-2-octyl-2H-isothiazol-3-one (RH-287)/g of soil/sediment. This method should be applicable to a wide variety of soils and sediments.
4.2.1	Reliability	[redacted]
4.2.2	Deficiencies	[redacted]

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	25 September 2007
Materials and methods	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-

Table 4.2(a)-1: HPLC Conditions for Soil Samples

Solvent Gradient:			
	Time	**Percent Water**	**Percent Acetonitrile**
	0.00	30	70
	1.00	30	70
	4.00	5	95
	15.00	5	95
	17.00	30	70
	20.00	30	70
Guard Column:	Pinnacle II C-8, 10 x 2 mm		
Analytical Column:	Pinnacle II C-8, 150 x 4.6 mm		
Flow Rate:	0.4 ml/min		
Injection Volume:	5 µl		
Typical 4,5-dichloro-2-octyl-2H-isothiazol-3-one RH-287 Retention time:	~13 min		

Table 4.2(a)-2: HPLC Conditions for Sediment Samples

Solvent Gradient:			
	Time	**Percent Water**	**Percent Acetonitrile/Methanol**
	0.00	60	40
	2.00	60	40
	14.00	10	90
	24.00	10	90
	28.00	60	40
	30.00	60	40
Analytical Column:	Zorbax Eclipse XBD-C-8, 150 x 3.0 mm, 5µm particle size		
Flow Rate:	0.4 ml/min		
Injection Volume:	5 µl or 10µl		
Typical 4,5-dichloro-2-octyl-2H-isothiazol-3-one RH-287 Retention time:	~17.5 min		

Table 4.2(a)-3: Mass Spectrometer Conditions used for Detecting 4,5-dichloro-2-octyl-2H-isothiazol-3-one (RH-287) in Soil and Sediment

Parameter	Soil	Sediment
Instrument	ThermoFinnigan LCQ^{DECA} Ion Trap	ThermoFinnigan LCQ^{DECA} Ion Trap
Aquire Time	30.00 min	30.00 min
Scan Mode	MS	MS
Scan Type	Selected Ion Monitoring	Selected Ion Monitoring
Selected Ions Monitored	282 and 284	282 and 284
Polarity	Positive ion	Positive ion

[redacted]

[redacted]

[redacted]	[redacted]			[redacted]		
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]			[redacted]			[redacted]
[redacted]			[redacted]			[redacted]
[redacted]			[redacted]			[redacted]

[redacted]

[redacted]

[redacted]	[redacted]			[redacted]		
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]			[redacted]			[redacted]
[redacted]			[redacted]			[redacted]
[redacted]			[redacted]			[redacted]

[redacted]

[redacted]	[redacted]					
	[redacted]			[redacted]		
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Section A4.2.b
Annex Point IIA, IV.4.2.

Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in Air

Official use only

1 REFERENCE

1.1 Reference

Reference Type: Study report

Year: 2003

Report date: 21 October 2003

[redacted]

1.2 Data protection Yes

1.2.1 Data owner: Rohm and Haas Company.

1.2.2

1.2.3 Criteria for data protection: [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study: There are no official guidelines available for the analytical methods. The method was developed according to the principles described in the Technical Notes for Guidance on data requirements.

2.2 GLP: Yes

2.3 Deviations: N/A

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment: Silica gel adsorption tubes are used for sampling airborne 4,5-dichloro-2-octyl-2H-isothiazol-3-one. Silica gel is extracted with methanol using an ultrasonic bath, filtered and diluted with water for HPLC separation with mass spectrometric (MS / MS) analysis detection.

3.1.2 Cleanup: No clean-up is required.

3.2 Detection

3.2.1 Separation method: Reversed phase HPLC using a 3 µm particles, procedure using 100 mm x 4.6 mm i.d., ODS (C18 or octadecylsilaneAgilent Hypersil BDS C18) column, with 80 % methanol/20 % water containing 0.1 % formic acid

Section A4.2.b
Annex Point IIA, IV.4.2.

Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in Air

		isocratic mobile phase. In these conditions, the retention time of 4,5-dichloro-2-octyl-2H-isothiazol-3-one is approximately 4 min.water containing 0.1% formic acid and methanol as solvents, isocratic at 80% methanol / 20% water containing formic acid.
3.2.2	Detector	Mass selective detector using APCI (Atmospheric pressure chemical ionization) mode and SRM (single reaction monitoring) (SRM) mode.
3.2.3	Standard(s)	DCOIT/RH-287T Analytical standards used with external standard calibration method.
3.2.4	Interfering substance(s)	None.
3.3	**Linearity**	
3.3.1	Calibration range	0.005, 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.0 µg/ml
3.3.2	Number of measurements	Not applicable.
3.3.3	Linearity	Correlation coefficient $r^2 = 0.999$
3.4	**Specifity: interfering substances**	The analytical method is highly specific using Highly specific technique by MS/MS detection using with Single Reaction Monitoring technique specific for 4,5-dichloro-2-octyl-2H-isothiazol-3-one/DCOIT parent ion molecular weight 282 and a fragment ion at 170 mass.

3.5 Recovery rates at different levels

[redacted]

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
	[redacted]	[redacted]

[redacted]

3.5.1 Relative standard deviation

[redacted]

3.6 Limit of determination

The limit of quantification is 0.2 µg/m^3.

3.7 Precision

3.7.1 Repeatability

[redacted]

3.7.2 Independent laboratory validation

[redacted]

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	A silica gel adsorption tubes is used for trapping airborne 4,5-dichloro-2-octyl-2H-isothiazol-3-one (DCOIT). DCOIT is extracted from the tubes using methanol and ultrasonic bath. The extract is then injected into an HPLC connected to a mass selective detector. The extraction procedure is reproducible and recovery of DCOIT from silica gel tubes is > 92% for 0.2, 2.0 and 20 µg/m^3 fortified samples. The HPLC MS / MS method is linear with a correlation coefficient of 0.999. The limit of quantification of DCOIT in the air is 0.2 µg/m^3 .
4.2	**Conclusion**	The test method meets all the necessary method validation criteria for the determination of low levels of 4,5-dichloro-2-octyl-2H-isothiazol-3-one (DCOIT) in air. Silica gel adsorption tubes traps airborne DCOIT efficiently without any breakthrough. Recovery of DCOIT from silica gel into methanol is >90%. Use of mass selective detector and single reaction monitoring (SRM) method improved accuracy and provided lower limit of quantification.
4.2.1	Reliability	1
4.2.2	Deficiencies	No

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	25 September 2007
Materials and methods	Agree with applicant's version
Conclusion	Agree with applicant´s version
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A4.2.c
Annex Point IIA, IV.4.2.

Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in water

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] [redacted] Reference type: Study report Year: 2003 Report date: 26 June 2003
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company.
1.2.2		
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	There are no official guidelines available for the analytical methods. The method was developed according to the principles described in the Technical Notes for Guidance on data requirements.
2.2	**GLP**	Yes
2.3	**Deviations**	N/A

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	4,5-dichloro-2-octyl-2H-isothiazol-3-one (DCOIT) is extracted from 1000 ml of water samples by liquid / liquid partition into 100 mL dichloromethane (MDC) twice. After filtering and drying the combined MDC dichloromethane layers over sodium sulphate, the extract is evaporated to dryness followed by dissolution of the residue in toluene for GC analysis.
3.1.2	Cleanup	Liquid /liquid extraction as explained above.
3.2	**Detection**	Electron Capture Detector (ECD)
3.2.1	Separation method	1. For Drinking, and surface waters and sea waters fortified with 0.02 ug µg/l: Capillary Gas Chromatography (GC) using Helium as carrier gas

Section A4.2.c **Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in water**

Annex Point IIA, IV.4.2.

		at a flow of 1.5 ml/min, a 30 meters x 0.25 mm i.d. RTX 1 (methyl silicone) column with 0.25 micron µm film and 1 microliter µl splitless injection. In these conditions, the retention time of 4,5-dichloro-2-octyl-2H-isothiazol-3-one is 8.3 - 9.0 minutes. 2. A slightly different chromatographic method was used for sea water fortified with 10 µg DCOIT/l : Capillary Gas Chromatography (GC) using Helium as carrier gas at a flow of 3 ml/min, a 25 meters x 0.53 mm i.d. DB-1 (methyl silicone) column with 5 micron µm film and 1 microliter µl splitless injection. In these conditions, the, retention time of 4,5-dichloro-2-octyl-2H-isothiazol-3-oneDCOIT ~ is approximately 15 minutes.
3.2.2	Detector	Electron Capture Detector (ECD) at 320 °C and make-up gas of nitrogen at 60 ml/min.
3.2.3	Standard(s)	DCOIT/RH-287T Analytical standards used with external standard calibration method.
3.2.4	Interfering substance(s)	None.
3.3	**Linearity**	
3.3.1	Calibration range	0.002, 0.005, 0.02, 0.05, 0.10, 0.20, 0.50, 1.0 µg/ml.
3.3.2	Number of measurements	Not applicable.
3.3.3	Linearity	Correlation coefficient $r^2 = 0.9974$.

Section A4.2.c **Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in water**

Annex Point IIA, IV.4.2.

3.4 Specificity: interfering substances

[redacted]

3.5 Recovery rates at different levels

[redacted]

[redacted]		
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]

[redacted]

3.5.1 Relative standard deviation

[redacted]

3.6 Limit of determination

The limit of quantification is 0.02 µg/L (0.02 ppb)

3.7 Precision

3.7.1 Repeatability

[redacted]

Section A4.2.c
Annex Point IIA, IV.4.2.

Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in water

		[redacted]
3.7.2	Independent laboratory validation	[redacted]

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

A liquid / liquid extraction followed by capillary GC Gas Chromatography (GC) analysis with Electron Capture Detection (ECD) method is developed for determination of 4,5-dichloro-2-octyl-2H-isothiazol-3-one (DCOIT) in three types of water: drinking, surface and sea. Water samples are analyzed by extraction with dichloromethane followed by filtering and drying of the extract. extract is evaporated to dryness, the residue after is then diluted dissolution in toluene is and injected into the GC.

The extraction procedure is reproducible and recovery of DCOIT is > 86.78 % for 0.02 and 10 ppb µg/l fortified samples.

The GC method is linear with a correlation coefficient of 0.9974.

The limit of quantification of (DCOIT) in the three types of water tested is 0.02 µg/L.

4.2 Conclusion

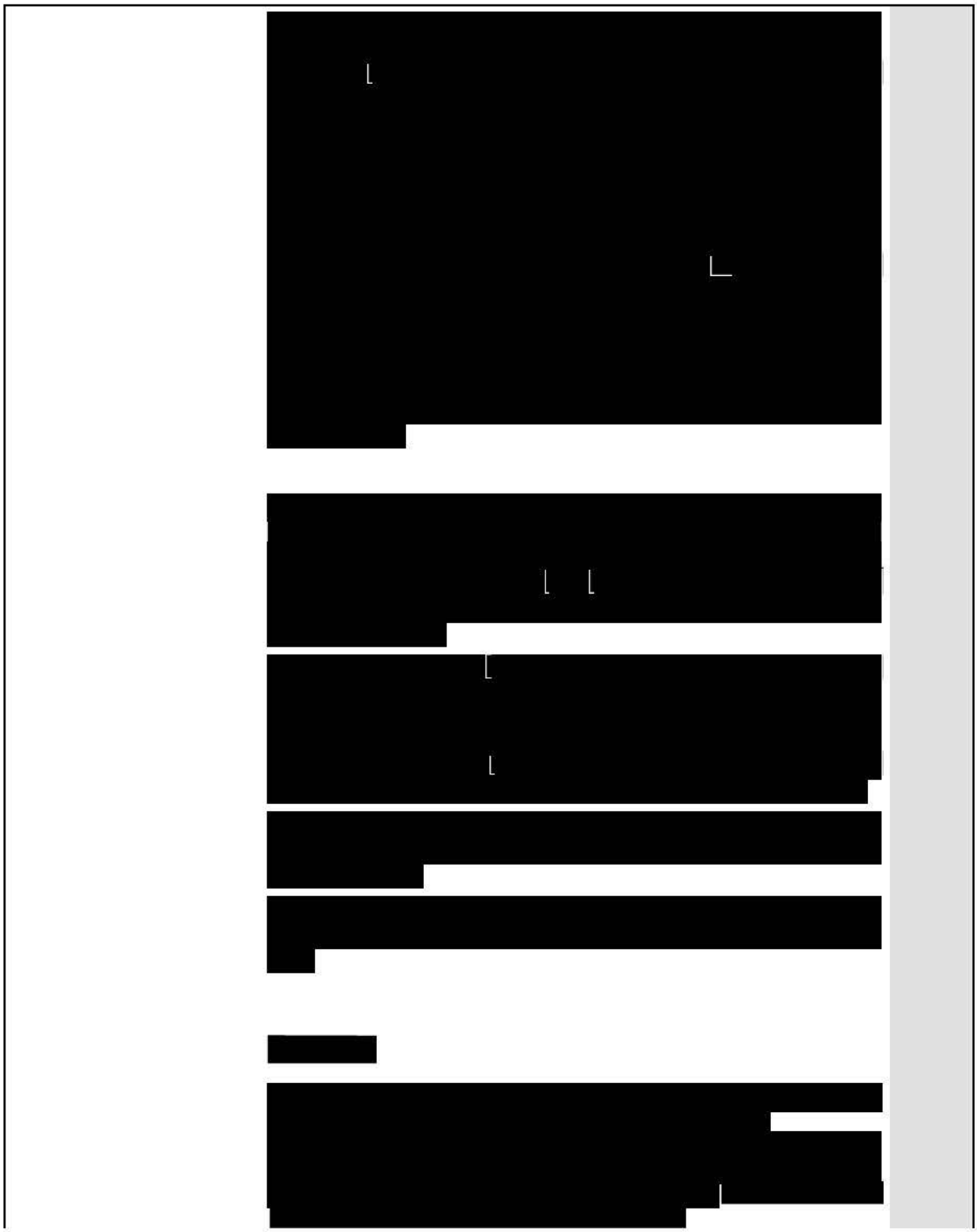
The test method meets all the necessary method validation criteria for the determination of low levels of DCOIT in drinking, surface and sea water. Use of halogen specific detector (ECD) provided improved accuracy and better limit of quantification.

4.2.1 Reliability: 1

4.2.2 Deficiencies: No

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	25 September 2007
Materials and methods	Agree with applicant's version
Conclusion	Agree with applicant´s version
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A4.2.d Annex Point IIA, IV.4.2.	Analytical method for the detection and identification of 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one in animal and human body fluids and tissues	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Detailed justification is considered as confidential information. [redacted]	



[redacted]

	[redacted]	
Undertaking of intended data submission [ ]	[redacted]	

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	10 October 2007
Evaluation of applicant's justification	Irritative damage to the respiratory tract is likely the main toxic effect seen in the referred acute toxicity study. Although a local effect, it is still the determinant of acute toxicity and the mechanism of toxicity is relevant for humans. In the CAs opinion the LC50 value obtained in the study warrants classification of DCOIT as toxic (T) or very toxic (T+) by inhalation (the result being on the borderline for classifications with toxic and very toxic). According to the TNsG on data requirements analytical methods for the analysis of active substances and for residues thereof in animal tissue must be submitted *where an active substance is classified as toxic or highly toxic*. Waiving is according to this not possible for the substance. In this case non-submission of an analytical method for the substance and its metabolites could nevertheless be scientific justifiable. As the observed effects after acute or repeated dosing of the chemical seem to reflect (mainly) a local tissue response, the appropriate analytical methods as suggested by the applicant are (principally) those pertaining to the substance in air and formulated products. Furthermore, there are indications that DCOIT does not bioaccumulate after oral exposure.
Conclusion	Non-submission of an analytical method for the detection and identification of DCOIT in animal and human body fluids and tissues is acceptable
Remarks	-

Section A4.2.e

Annex Point IIA, IV.4.2.

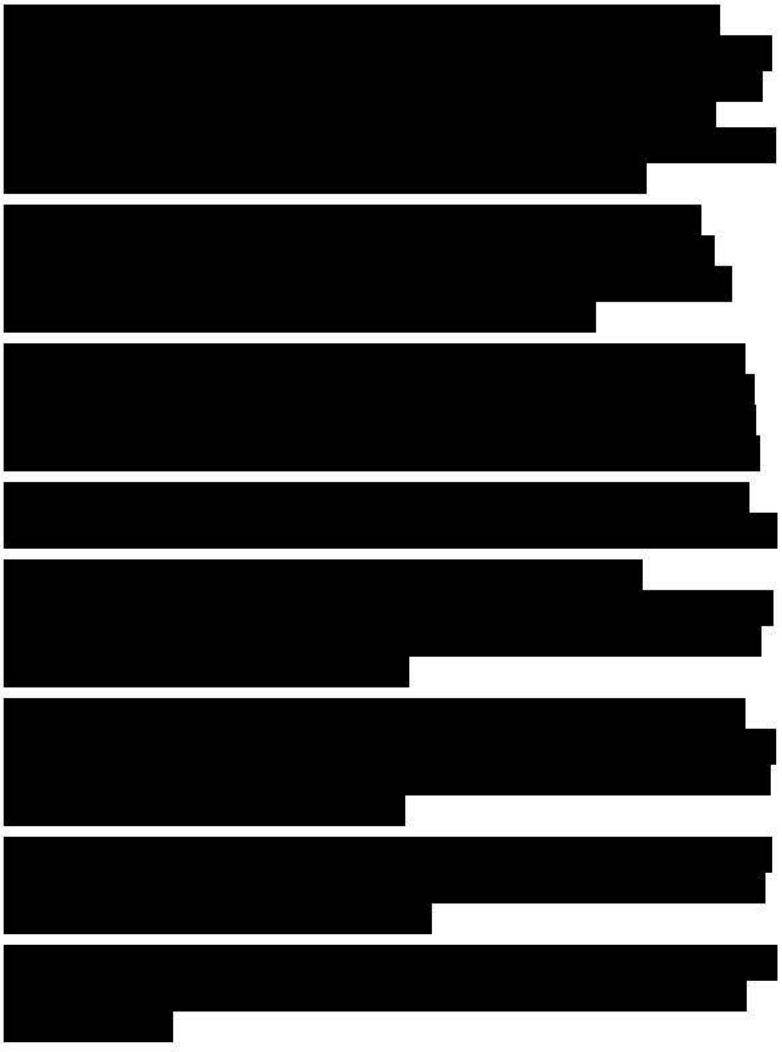
Analytical method for the detection and identification of degradation products of 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one in environmental compartments.

Justification for non-submission of data

Other existing data [ ] **Technically not feasible [ ]** **Scientifically unjustified [X]**

Limited exposure [ ] **Other justification [ ]**

Detailed justification: Detailed justification is considered as confidential information



[redacted]

Undertaking of intended data submission [ ] [redacted]

Evaluation by Competent Authorities

Evaluation by Rapporteur Member State

Date

8 January 2008

Evaluation of applicant's justification

DCOIT rapidly degrades primarily in the terrestrial and aquatic environment.

The following metabolites have to be considered:

- N-(n-octyl) malonamic acid (NNOMA): Formed in the seawater-sediment studies (max. 16%)
- N-(n-octyl) oxamic acid (NNOOA): Formed in the estuarine water degradation study (24% at all test concentrations)
- N-(n-octyl) acetamide (NNOA): Formed in the seawater-sediment studies (max. 12%)
- 2-chloro-2-(n-octyl) carbamoyl-1-ethene sulfonic acid: Formed in the estuarine water degradation study (ca 12% at 100 ppb)
- (1-chloro-2-(n-octyl) carbamoyl-1-ethene sulfonic acid: Formed in the estuarine water degradation study (ca. 9% at 100 ppb))*

*1-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid was not formed in amounts > 10%; however, concentrations of this metabolite were increasing during the course of the test, not reaching a plateau at the end of the study. It is possible that this metabolite might pass the 10% threshold if the study had been prolonged and therefore this metabolite may also have to be considered.

Regarding the fate and ecotoxicity of these metabolites, the following can be concluded:

- NNOMA is readily biodegradable, is less toxic than DCOIT, but is nevertheless acutely toxic to marine algae based on the ErC_{50} of 0.47 mg/L from a test with *Skeletonema costatum,* however, the test has some deficiencies.
- NNOOA is structurally similar to NNOMA. No experimental data are available. NNOOA is probably readily biodegradable as well. Regarding ecotoxicity, QSAR calculations were performed in ECOSAR v 0.99h. The program predicts $L(E)C_{50}$ > 100 mg/L. However, this prediction seems not to be robust. Moreover, the toxicity of NNOMA towards marine algae might be < 1 mg/L, while its toxicity towards freshwater algae is 11 mg/L. Due to the structural similarities of NNOMA and NNOOA, the possibility can not be excluded that also NNOOA might be similarly toxic to marine algae, even if its acute toxicity to freshwater algae might be > 100 mg/L.
- NNOA is readily biodegradable. The lowest acute endpoint from aquatic ecotoxicity tests with NNOA was a 72 hours ErC_{50} of 11 mg/L (freshwater algae).
- 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid is not readily biodegradable, and QSAR predicts 1-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid to be not readily biodegradable. No ecotoxicity data are available.

	According to the TNsG on data requirement, chapter 2, point 4.1 and 4.2 "Information on analytical methods is required concerning degradation products […] which are of toxicological or ecotoxicological concern (i.e. which are relevant for risk assessment) […]." (point 4.1) and "Methods for the analysis for parent compounds and/or metabolites of concern must be submitted" (point 4.2). The term "toxicological or ecotoxicological concern" is not further defined. No toxicological data are available on these metabolites; however, two of them are readily biodegradable and NNOOA is predicted to be readily biodegradable as well. Moreover, concentrations formed in the environment are expected to be very low and they become even lower due to ready biodegradation. It is therefore unlikely that humans would be exposed to them. Concerning the environment, the metabolites are 3-5 orders of magnitude less toxic to aquatic organisms than DCOIT itself. NNOMA and NNOA are readily biodegradable and it can be assumed that also NNOOA is readily biodegradable. Even if NNOMA and NNOA, and probably NNOOA as well, can still be considered as toxic to aquatic organisms, it is not assumed that they are of ecotoxicological concern as they are readily biodegradable, or predicted to be in case of NNOOA. Regarding the isomers 2-chloro-2-(n-octyl) carbamoyl-1-ethene sulfonic acid and 1-chloro-2-(n-octyl) carbamoyl-1-ethene sulfonic acid, QSAR predicts an LC_{50} for daphnids of 9.3 mg/L and 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid is not readily biodegradable. Experimental data on ecotoxicity is missing for both this metabolite and 1-chloro-2-(n-octyl) carbamoyl-1-ethene sulfonic acid and no final conclusion can be drawn on these substances at the moment.
Conclusion	There is still an ongoing discussion on whether DCOIT should be classified as N R50 only or as N R50/53 based on the properties of its breakdown products (for more details see Document III A8-9). As long as this issue is not finally concluded no decision can be drawn on the need for analytical method for metabolites of DCOIT.
Remarks	-

Section A4.3

Annex Point IIIA, IV.1

Analytical Method for Detection and Identification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in fish and shellfish

[redacted]

Official use only

1 REFERENCE

1.1 Reference

[redacted]

Reference type: Study report

Year: 2003

Report date: 8 December 2003

1.2 Data protection Yes

1.2.1 Data owner [redacted]

1.2.2

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study There are no official guidelines available for the analytical methods. The method was developed according to the principles described in the Technical Notes for Guidance on data requirements.

2.2	**GLP**	Yes
2.3	**Deviations**	N/A
	3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**	
3.1.1	Enrichment	10 g fish or shellfish samples are homogenized and extracted in acetonitrile followed by partitioning in dichloromethane/salt water (5 % NaCl). The dicholoromethane extract is evaporated to dryness and the residue is dissolved in 5 ml hexane.
3.1.2	Cleanup	The hexane solution is cleaned-up by adsorption chromatography using a Florisil column. 4,5-dichloro-2-octyl-2H-isothiazol-3-one is eluted using a hexane/ethyl acetate (7:3) mixture. The 4,5-dichloro-2-octyl-2H-isothiazol-3-one collected fraction is evaporated to 1 ml and extracted with acetonitrile. The acetonitrile extract is then evaporated to 0.5 ml, made up to 2.0 ml with methanol and filtered through a 0.45 µm PTFE filter before HPLC MS/MS analysis.
3.2	**Detection**	
3.2.1	Separation method	High Performance Liquid Chromatography (HPLC) using a 100 mm x 4.6 mm i.d. Hypersil BDS C-18 column with a gradient solvent program (water containing 0.1 % formic acid and methanol as mobile phase) at a flow of 1.0 ml/min. In these conditions, the retention time of the 4,5-dichloro-2-octyl-2H-isothiazol-3-one is approximately 4 min.
3.2.2	Detector	Mass Selective detector (MS), Ionization mode: Atmospheric Pressure Chemical Ionization (APCI), Scan Mode: Selective Reaction Monitoring at 282 from the first mass spectrometer and 170 from the second mass spectrometer.
3.2.3	Standard(s)	RH-287T analytical standards used. Calibration using external standard method.
3.2.4	Interfering substance(s)	None.
3.3	**Linearity**	
3.3.1	Calibration range	0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.0 µg/ml.
3.3.2	Number of measurements	Not applicable.
3.3.3	Linearity	Correlation coefficient $r^2 = 0.9999$.

3.4 Specifity: interfering substances

[redacted]

3.5 Recovery rates at different levels

[redacted]

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]

3.5.1 Relative standard deviation

[redacted]

3.6 Limit of determination

The limit of quantification is 0.01 mg/kg (0.01 ppm or 10 ppb) for the three types of fish tested.

3.7 Precision

3.7.1 Repeatability

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]

3.7.2 Independent laboratory validation

[redacted]

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	An analytical method, which includes extraction procedure and HPLC MS/MS analysis, is developed for determination of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in three types of fish: rainbow trout (non oily fish), mackerel (oily fish) and mussel (shellfish). The fish samples are solvent extracted, cleaned-up using a Florisil column followed by solvent extraction and HPLC MS/MS analysis of the final extract. The extraction procedure is reproducible and recovery of 4,5-dichloro-2-octyl-2H-isothiazol-3-one is > 86 % at 0.01mg/kg and 1.0 mg/kg fortification levels. The HPLC MS/MS method is linear with a correlation coefficient of 0.9999. The limit of quantification of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in the three types of fish tested is 0.01 mg/kg.
4.2	**Conclusion**	The test method meets all the necessary method validation criteria for the determination of residue levels of 4,5-dichloro-2-octyl-2H-isothiazol-3-one in rainbow trout, mackerel and mussel.
4.2.1	Reliability	1
4.2.2	Deficiencies	No

Evaluation by Competent Authorities

Evaluation by Rapporteur Member State

Date	6. August 2008
Materials and methods	Agree with applicant's version
Conclusion	Agree with applicant´s version
Reliability	1
Acceptability	Acceptable
Remarks	-

Directive 98/8/EC on the placing of biocidal products on the market.

Dossier for the inclusion of an active substance in the Annex 1

4,5-Dichloro-2-octyl-2H-isothiazol-3-one (DCOIT)

Product type 21: Antifouling products

Document III-A (A5)

Study summaries – Active substance

Section A5: Effectiveness Against Target Organisms and Intended Uses

TABLE OF CONTENT

Section A5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)		Official use only
5.1 Function (IIA5.1)	Broad spectrum antifouling agent. 4,5-dichloro-2-octyl-2H-isothiazol-3-one (DCOIT) is a broad spectrum antimicrobial biocidal active substance which exhibits rapid inhibition of growth at very low levels and cidal effects at higher levels or for longer contact periods. DCOIT may function as a broad spectrum antifouling agent for preventing the growth and settlement of soft fouling (ex., bacteria, fungi, algae) and hard fouling (ex., barnacles) organisms on submerged surfaces. In antifouling coatings, DCOIT may function as a bactericide, bacteristat, fungicide, fungistat, algicide, and algistat depending on the dose level applied, system conditions, and the level of microbial control desired. The basic antimicrobial efficacy of DCOIT, as determined from Minimum Inhibitory Concentration (MIC) values versus a range of fouling organisms are summarized in the following sections. Additional studies defining the Lethal Dose to provide 50% kill (LD_{50}) of marine algae and barnacle larvae demonstrated DCOIT as effective versus soft and hard fouling marine organisms.	
5.2 Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)		
5.2.1 Organism(s) to be controlled (IIA5.2)	DCOIT is active against a wide variety of organisms over a broad range of marine environmental conditions. A variety of organisms are involved in marine fouling and need to be controlled to prevent growth on surfaces. These include soft fouling microorganisms (bacteria, fungi, algae and cyanobacteria) and invertebrate organisms (hydroids, sponges and tunicates). Hard fouling organisms, such as barnacles, annelids, bryozoans, and mollusks, are also important fouling organisms. The organisms are of special interest in marine anti-fouling coatings used for surfaces on boats, various water craft, and submerged structures. The above list of microorganisms is representative of, but not limited to those which are common contaminants in marine fouling.	
5.2.2 Products, organisms or objects to be protected (IIA5.2)	DCOIT is dosed into anti-fouling paints and coatings to protect the surfaces of boats, various water craft, buoys and submerged structures (sluice doors, off-shore structures, …) from fouling (attached growth) by aquatic organisms.	
5.3 Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)		

Section A5 Effectiveness against target organisms and intended uses

5.3.1 Effects on target organisms (IIA5.3)

References

Reference Type: Study report

Year: 2005

Report date: 31 March 2005

[redacted]

[redacted] Willingham G.L. and Jacobson A.H. (1993), Efficacy and Environmental Fate of a New Isothiazolone Antifoulant, Proceedings of the Paint Research Association, Third Asia-Pacific Conference on Advances in Coatings, Inks, and Adhesive Technology, Singapore, Paper N° 14, p 1-13, published, May, 1993.

The antimicrobial efficacy of DCOIT relative to Product Type 21 (Antifouling Products) has been demonstrated in a variety of standard laboratory studies.
DCOIT was shown to be a highly effective antimicrobial agent when tested in Minimum Inhibitory Concentration (MIC) studies versus soft fouling (or slime-forming) microorganisms ([redacted]). The MIC values defines the lowest level of biocide which inhibits completely the growth of microorganisms. Thirty-two strains of bacteria, 23 strains of algae, and 30 strains of fungi were tested with DCOIT active ingredient under controlled laboratory conditions. The results showed that DCOIT was effective to inhibit microbial growth at concentrations ranging from <0.01 to 8.0 parts per million (ppm) for the 85 samples tested. DCOIT was most effective versus yeast and Gram positive bacteria (average MIC = 0.15 and 0.11 ppm, respectively), and least effective versus Gram negative bacteria (average MIC = 2.57 ppm). MIC values for DCOIT versus mold, blue-green algae, and green algae were similar (average MIC = 0.45, 0.51, and 0.61 ppm, respectively). The average MIC value for all 85 organisms tested was 1.1 ppm, indicating a highly active antimicrobial substance. All of these microorganisms are considered soft fouling organisms which attach to and grow on various submerged surfaces in the environment. Details on the specific MIC values and test conditions for each of the three major group of microorganisms are summarized in Table 5.3.

Additional studies with the green marine alga, *Enteromorpha*, and the marine diatom, *Amphora*, showed DCOIT as highly effective with LD_{50} values of 2.0 and 3.4 parts per billion (ppb), respectively ([redacted] Willingham, GL and Jacobson, AH, 1993). These are common soft fouling organisms which are known to readily attach to and grow on submerged marine surfaces.
Lethality studies with marine barnacles showed DCOIT as highly effective at 0.34 ppm (LD_{50}) for control of *Balanus nauplius* larvae [redacted] Willingham, GL and Jacobson, AH, 1993). These barnacles are common hard fouling organisms which readily attach to and colonize submerged marine surfaces.
The concentration of DCOIT required to inhibit and subsequently kill microorganisms in a product matrix is dependent upon the contact time as well as the amount of contamination in the system.

Reliability: 1, valid without restrictions

Section A5 Effectiveness against target organisms and intended uses

5.3.2 Likely concentrations at which the A.S. will be used (IIA5.3)

PT 21 Antifouling Products

For Product Type 21, RH-287T is to be formulated into marine antifoulant paints at 1% to 3% DCOIT as active substance. It is dispersed throughout the binder of the coating which is applied to the hull of the vessel by standard coating application techniques. For large vessels, the antifouling system is typically applied by airless spray. For small vessels, buoys, spot application, brush or roller application can be used. DCOIT antifouling agent is highly effective against a wide range of fouling organisms and provides outstanding long-term protection when used in combination with copper compounds or other biocides.

5.4 Mode of action (including time delay) (IIA5.4)

5.4.1 Mode of action

Reference

Reference Type: Study report

Year: 2006

Report date: 16 January 2006

[redacted]

Anti-fouling systems containing DCOIT protect treated surfaces against colonization of a broad range of organisms that foul marine vessels and submerged surfaces. DCOIT reacts with the proteins of organisms that come in contact with the coating surface (for example, algae, seaweed, barnacles). This results in interruption of the metabolic processes that utilize these proteins. Fouling organisms initiate specific physiological activities involved in attaching to solid surfaces that are disrupted by DCOIT. As a result the organisms do not successfully colonize the treated surfaces and biofouling is minimized.

DCOIT utilizes a two-step mechanism involving rapid growth inhibition leading to a loss of viability ([redacted]). Growth inhibition is the result of rapid disruption of the central metabolic pathways of the cell by inhibition of dehydrogenase enzymes. Key physiological activities that are rapidly inhibited in microbial cells are growth (reproduction) and respiration (oxygen consumption). These processes are critical in bacteria, algae, fungi, and invertebrates, which explains why DCOIT is such a broad spectrum biocide.

Inhibition of cellular activity is rapid (within minutes), whereas, cell

Section A5 Effectiveness against target organisms and intended uses

death (cidal activity) is observed after several hours contact. Cell death from DCOIT results from the progressive loss of protein thiols in the cell from one of multiple pathways. In general, the higher the concentration of DCOIT, the shorter the contact time required for inhibition and kill. Other isothiazolone biocides have been shown to generate free radicals within cells as metabolism is disrupted. The production of these radicals is considered a critical factor contributing to cell death and DCOIT is suspected to exhibit this same response in microbial cells.

The two step mechanism of action of DCOIT results in its broad spectrum of activity, low use levels for microbial control, and difficulty in attaining resistance.

[redacted]

5.4.2 Time delay

There is no specific time delay for use of antifouling biocides. See above description for mode of activity.

5.5 Field of use envisaged (IIA5.5)

This dossier has been prepared for the inclusion of DCOIT in Annex 1 for :

MG04: Other biocidal products

Product type PT21: Antifouling products.

In the frame of the biocidal products directive, DCOIT will also be supported by Rohm and Haas in the following product types :

MG02 : Preservatives

Product type 07 : Film preservatives

Product type 08 : Wood preservatives

Product type 09 : Fibre, leather, rubber and polymerised materials preservatives

Product type 10 : Masonry preservatives

Product type 11 : Preservatives for liquid-cooling and processing systems

Further specification (PT21)

DCOIT is recommended to be used in the formulation of antifouling paints to control the growth and settlement of fouling organisms on commercial or industrial boats and vessels, buoys, and submerged structures in water. DCOIT is used at 1-3 % in the antifouling paint. The antifouling paint is applied by brush, roller, or spray by professionals using appropriate safety procedures. The biocide-treated paint is applied after the surface has been cleaned and primer coated.. The antifouling paint is then allowed to air dry at least 48 hours prior to entry into water.

Section A5 Effectiveness against target organisms and intended uses

5.6	**User (IIA5.6)**	DCOIT antifouling agent is sold for manufacturing use only. Formulators using this product develop the marine antifoulant paint containing DCOIT alone or in combination with other biocides. The final formulated marine antifoulant paints are applied by professionals who are specialized in industrial and commercial applications.
	Industrial	Antifouling paint formulators dose the biocide into the paint to be applied to the desired surfaces.
	Professional	Professional users are recommended for application of the antifouling paint, containing the biocide, to the article to be treated.
	General public	DCOIT is not intended for use by the general public.
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)**	
5.7.1 Development of resistance		**Reference** Reference Type: Study report Year: 2006 Report date: 16 January 2006 [redacted] **General Comments on Antimicrobial Resistance** Resistance can be defined as a reduction in the susceptibility of an organism to an antimicrobial agent as a result of physiological or genetic changes. Thus, previously effective levels of biocides no longer have the same extent of control. Microorganisms do have the potential to develop resistance to any biocide, although this is largely a function of the use rate, environmental conditions, and microbial population density. Resistance is most likely to occur as a result of dosing biocides at levels below the minimum effective dose, poor stability of the biocide, extensive biofilm development, or efficacy gaps against certain groups of microorganisms. Resistance is a major problem in the medical field due to the growing number of microorganism showing resistance to antibiotics. In general, the mechanism of action of antibiotics is very different from industrial biocides. Industrial biocides typically have multiple targets or general modes of action. Antibiotics, however, have very specific targets, with often one enzyme or one pathway as the sole site of antimicrobial activity. For example, penicillin affects the peptide cross-linkage in the peptidoglycan layer of the bacterial cell wall.

Section A5 Effectiveness against target organisms and intended uses

Thus, antimicrobial agents which function by a single target – single mechanism are more likely to result in resistance.

A variety of mechanisms of developing resistance are known to occur in microorganisms. These include altering in the permeability of the cell wall or membrane to prevent the biocide from entering the cell, deactivation or degradation of the antimicrobial agent by biochemical reactions, and transport of the agent outside the cell, via efflux pumps.

Occurrence of Resistance to DCOIT

DCOIT has been used as a commercial antifouling agent since 1986. During this period of use, we have not observed any problems of resistance with DCOIT as an marine antifouling agent. There are also no published reports of microbial resistance to DCOIT. This is likely due to its unique mechanism of action, broad spectrum of activity, use concentrations, and pattern of use ([redacted]). These factors are described in more detail below.

The mechanism of action of DCOIT involves reaction with protein-thiol targets, including specific dehydrogenase enzymes, affecting a variety of metabolic processes within the cell. Developing resistance to multiple targets simultaneously by microorganisms is very difficult. Cells would essentially have to expend significant amounts of energy to repair and modify the various DCOIT targets and repair the damage while their overall metabolic processes and energy systems are shut down. Alteration of membrane components or transport of DCOIT outside the cell are also possible resistance strategies; however, this again requires the cell to synthesize cellular macromolecules while their energy generation and cell synthesis systems are disrupted.

Although it is known that biocides may exhibit cross-resistance to other biocides, the observation of cross-resistance of DCOIT to other biocides has not been demonstrated to be a problem. We have no experimental or commercial experience to indicate that cross-resistance is a problem of significant concern. The same factors mentioned above, which reduce the likelihood of developing resistance to DCOIT, would also be factors in reducing the likelihood of cross-resistance, since DCOIT and the other biocides/adjuvants have differing modes of activity.

The use of DCOIT in combination with cuprous oxide or other co-biocides in antifouling coatings further reduces the risk of the development of resistance.

5.7.2 Management strategies

Response to Potential Resistance Development

The appropriate response to potential resistance problems with DCOIT in use requires knowledge of the identity and nature of the contaminant, the source of contamination, and characteristics and history of the system in use. This knowledge dictates the correct response to be taken.

Section A5 Effectiveness against target organisms and intended uses

In the unlikely event that true resistance is observed with DCOIT in use, several potential remedies are available. These include applying only the maximum approved dose level, changing the active ingredient of the co-biocide and adding an additional biocide to broaden the spectrum of efficacy. Each of these approaches can be successful and selection of a remedial program must take into account a variety of factors.

5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	The estimation of total amounts of DCOIT placed on the EU (25 countries) and on the Norwegian market is given in the **confidential** part of the Dossier, section A5.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	8 April 2009
Materials and methods	Acceptable for evaluation of efficacy of the active substance for possible Annex I inclusion for PT21.
Conclusion	Acceptable
Reliability	-
Acceptability	Acceptable for the evaluation of DCOIT as an active substance for antifouling products.
Remarks	-