Document III-A / Section A7.4.1 and A7.4.2

Section A7.4.1.3.b/01 **Growth inhibition test of DCOIT on algae-Marine water, *Skeletonema costatum***

Annex Point IIA VII.7.3

4.3	**Results of controls**	control results performed as expected	x
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Yes, US EPA FIFRA 123-2, static 120 h algal study with analytical confirmation of test solution concentrations	
5.2	**Results and discussion**	No insoluble material was observed during the test. All measured concentrations in test media samples collected at 120 h were < the limit of quantitation (<0.050 µg ai/L)	x
5.2.1	NOE_rC	96 h = 1.44 µg DCOIT/L; 120 h = 0.193 µg DCOIT/L *(96-h NOE_bC=0.479 µg/L; 120-h NOE_bC = 0.479 µg/L)*	
5.2.2	E_rC_{50}	96 h and 120 h > 3.58 µg DCOIT/L	
5.2.3	E_bC_{50}	96 h = 1.49 µg DCOIT/L; 120 h = 1.84 µg DCOIT/L	
5.3	**Conclusion**	see validity criteria in table, below	x
5.3.1	Reliability	(1), reliable without restriction	x
5.3.2	Deficiencies	No	x

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	18 December 2007
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (4.2.3 and 4.3):** The growth rate in the controls was not exponential beyond 72 hours exposure. **Comment (4.2.6 and 5.2)**: Endpoints are based on measured concentrations at time 0. **Comment (5.2):** The 96 hours results based on initial measured concentrations can not be used as endpoints, as the removal of DCOIT from the test system in algae studies is rapid (see analytical measurements of test substance concentrations in the test on *Navicula Pelliculosa* IIIA 7.4.1.3a/03). Moreover, the growth rate in the controls was not exponential beyond 72 hours exposure. Because of the rapid decline of the test substance concentrations, the use of geometric mean concentrations over 96/120 hours would also not be meaningful. Therefore, results should be based on the initial phase of the test: 24 h NOErC = 0.479 µg/l 24 h ErC50 = 0.480 µg/l Unfortunately, variations of the cell density measurements at 24 hours are large and therefore the statistical power in the calculation of the NOEC is low. As a result, the apparent growth rate reduction at 0.479 µg/l is statistically not significant. Moreover, the dose-response curve is very steep and therefore there is practically no difference between the NOEC and the EC50.
Conclusion	**Comment (5.3):** Lack of analytical monitoring of the test substance concentration and large variations of cell density measurements at 24 hours make the establishment of a reliable NOEC difficult. However, the 24 h NOErC of 0.479 µg/l can be used as a first approach.
Reliability	**Comment (5.3.1 and 5.3.2):** Due to the deficiencies described, the reliability is changed from 1 to 2, reliable with restrictions.
Acceptability	Acceptable with the restrictions noted above.
Remarks	-

Section A7.4.1.3.b/01 Growth inhibition test of DCOIT on algae-Marine water, *Skeletonema costatum* – **TABLES AND FIGURES**

[redacted]

[redacted]

[redacted]

Table A7.4.1.3.b/01-4: Test conditions

Criteria	Details
Test temperature	19.5 to 20.1 °C
pH	8.0 ± 0.1
Aeration of dilution water	Not described
Light intensity	4020-4180 lux
Photoperiod	24 h light

Table A7.4.1.3.b/01-5: Cell concentration data

Test-Substance Concentration (initial measured)[1] [µg DCOIT/l]	Cell concentrations (mean values) [cells x 10^3/ml]											
	measured						Percent of control					
	0 h	24 h	48 h	72 h	96 h	120 h	0 h	24 h	48 h	72 h	96 h	120 h
0 (control)	10	24	102	647	870	1767	--	--	--	--	--	--
0 (solvent control)	10	21	94	664	924	1753	100	88	92	103	106	99
0.122	10	24	63	644	949	1758	100	100	62	100	109	99
0.193	10	28	103	715	1051	1790	100	117	101	111	121	101
0.479	10	15	110	653	895	1432	100	63	108	101	103	81
0.696	10	<10	52	519	879	1226	100	<42	51	80	101	69
1.44	10	<11	52	249	763	1083	100	<46	51	38	88	61
3.58	10	<10	<10	36	212	855	100	<42	<10	6	24	48
Temperature [°C]	19.5 to 20.1 °C											
pH	8.0 ± 0.1											

[1] specify, if TS concentrations were nominal or measured

Table A7.4.1.3.b/01-6: Validity criteria for algal growth inhibition test

	fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	yes	
Concentration of test substance ≥80% of initial concentration during test		yes

Figure A7.4.1.3.b/01-1: Growth of the marine alga, *Skeletonema costatum*, during the toxicity test with DCOIT

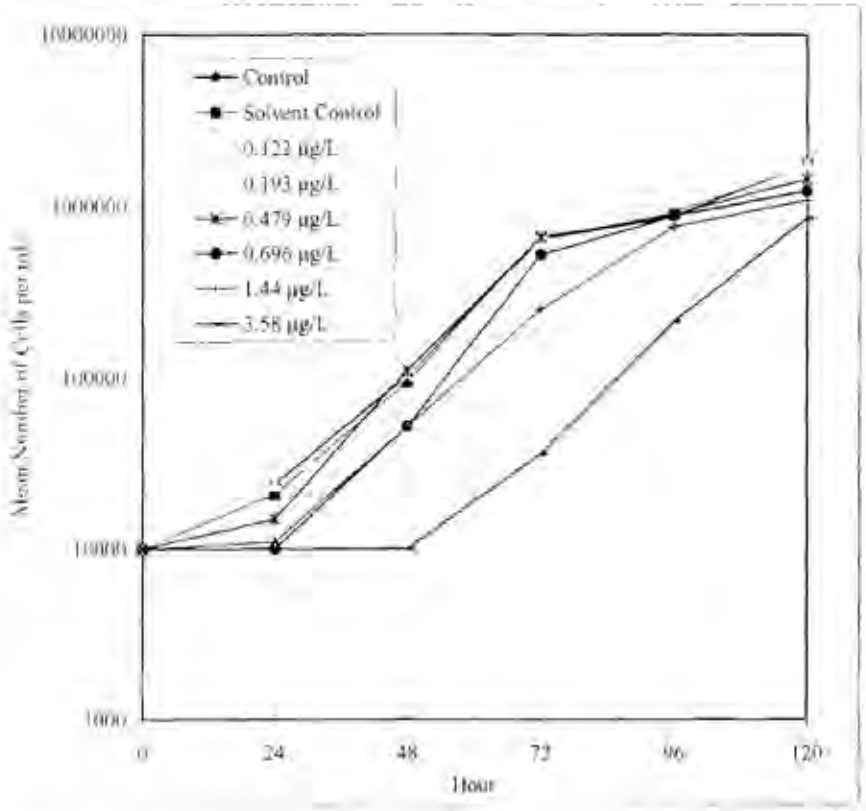

Figure 1. Growth of the marine alga, *Skeletonema costatum*, during the toxicity test with RH-287 Technical.

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Section A7.4.1.3.c/01 **Growth inhibition test of N-(n-octyl) malonamic acid on algae-Fresh water, *Selenastrum capricornutum***

Annex Point IIA VII.7.3

Official [redacted]

		1 REFERENCE
1.1	**Reference**	[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**	Yes, OECD 201, US EPA OPPTS 850.5400
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	N-(n-octyl) malonamic acid (NNOMA)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	The test substance is a metabolite of DCOIT.
3.1.3	Purity	99.72%
3.1.4	Composition of Product	[redacted]
3.1.5	Further relevant properties	
3.1.6	Method of analysis	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	[redacted]
3.3	**Reference substance**	
3.4	**Testing procedure**	
3.4.1	Culture medium	

Document III-A / Section A7.4.1 and A7.4.2

Section A7.4.1.3.c/01 **Growth inhibition test of N-(n-octyl) malonamic acid on algae-Fresh water, *Selenastrum capricornutum***

Annex Point IIA VII.7.3

3.4.2	Test organisms	[redacted]
3.4.3	Test system	
3.4.4	Test conditions	see table A7.4.1.3.c/01-4
3.4.5	Duration of the test	96 h
3.4.6	Test parameter	[redacted]
3.4.7	Sampling	
3.4.8	Monitoring of TS concentration	
3.4.9	Statistics	

4 RESULTS

4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal 0.031, 0.31, 0.63, 1.3, 2.5, 5.0, 10, 20 mg NNOMA/L
4.2.2	Actual concentrations of test substance	measured (mg NNOMA/L)

0 h	72 h	96 h
0.0254	NA	NA
0.229	0.169	0.155
0.595	0.451	0.211
0.925	0.817	0.274
1.82	2.09	0.904
5.06	4.67	2.20
8.52	9.86	3.64
15.4	18.7	16.2

minimum quantifiable limit (0.0600 mg NNOMA/L)

NA = not applicable

4.2.3	Growth curves	see Figure A7.4.1.3.c/01-1
4.2.4	Concentration / response curve	not in final report

Section A7.4.1.3.c/01 **Growth inhibition test of N-(n-octyl) malonamic acid on algae-Fresh water, *Selenastrum capricornutum***

Annex Point IIA VII.7.3

4.2.5	Cell concentration data	see table A7.4.1.3.c/01-5	
4.2.6	Effect data (cell multiplication inhibition)	Based on T0 measured concentrations: 0 - 72 h EC_{50} = 5.44 mg NNOMA/L; E_bC_{50} = 5.95 mg NNOMA/L; E_rC_{50} = 8.66 mg NNOMA/L; NOEC = 1.82 mg NNOMA/L; NOE_rC = 1.82 mg NNOMA/L Based on 0-72 h mean measured concentrations (geometric mean): NOE_rC = 1.95 mg NNOMA/L Based on 0-96 h mean measured concentrations (geometric mean): NOE_rC = 1.51 mg NNOMA/L	
4.2.7	Other observed effects	All test solutions appeared clear throughout the test with no visible precipitate.	
4.3	**Results of controls**	control results performed as expected	
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 201, US EPA OPPTS 850.5400, Acute static 96h algal study with analytical confirmation of test solution concentrations.	
5.2	**Results and discussion**	All test solutions appeared clear throughout the test with no visible precipitate.	
5.2.1	NOE_rC	Based on T0 measured concentration: 72/96 h NOE_rC = 1.82 mg NNOMA/L Based on mean measured concentration (geometric mean): 72 h NOE_rC = 1.95 mg NNOMA/L (geometric mean of 0h and 72h values) 96 h NOE_rC = 1.51 mg NNOMA/L (geometric mean of 0h, 72h and 96h values).	x
5.2.2	E_rC_{50}	Based on T0 measured concentration: 96 h = 10.8 mg NNOMA/L Based on geometric mean: 0-72 hr ErC50 = 9.32 mg a.i./L (95% confidence limits: 8.67 to 9.97 mg a.i./L) 0-96 hr ErC50 = 9.70 mg a.i./L (95% confidence limits: 9.08 to 10.3 mg a.i./L)	x
5.2.3	E_bC_{50}	96 h = 6.23 mg NNOMA/L	
5.3	**Conclusion**	see validity criteria in table A7.4.1.3.c/01-6	
5.3.1	Reliability	(1), reliable without restriction	

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Section A7.4.1.3.c/01 **Growth inhibition test of N-(n-octyl) malonamic acid on algae-Fresh water, *Selenastrum capricornutum***

Annex Point IIA VII.7.3

5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	07 April 2008
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (5.2.1 and 5.2.2):** The 96 hours results based on mean measured concentrations are considered the most reliable endpoints from this study.
Conclusion	Agree with applicant's version
Reliability	1, reliable without restrictions
Acceptability	Acceptable
Remarks	Test concentrations could not be maintained within 80% of nominal. But as results are based on mean measured concentrations, this is not considered a deficiency.

Section A7.4.1.3.c/01	Growth inhibition test of N-(n-octyl) malonamic acid on algae-Fresh water, *Selenastrum capricornutum* – **TABLES AND FIGURES**

[redacted]

[redacted]

[redacted]

Document III-A / Section A7.4.1 and A7.4.2

Table A7.4.1.3.c/01-4: Test conditions

Criteria	Details
Test temperature	24.0 – 25.3 degrees C
pH	7.3 to 8.5
Aeration of dilution water	Yes
Light intensity	4241 to 4721 lux
Photoperiod	24 h photoperiod daily

Table A7.4.1.3.c/01-5: Cell concentration data

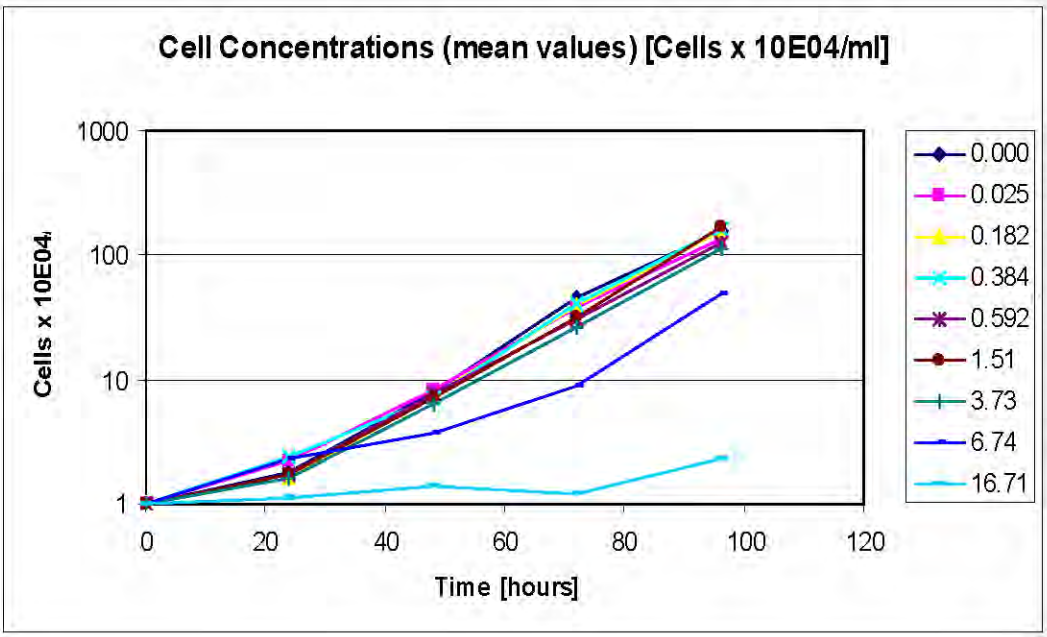
Test-Substance Concentration (mean measured 0-96h)[1] [mg NNOMA/l]	Cell concentrations (mean values) [cells x 10^4/ml]									
	measured					Percent of control				
	0 h	24 h	48 h	72 h	96 h	0 h	24 h	48 h	72 h	96 h
0 (control)	1.0	1.8	8.0	46	159	--	--	--	--	--
0.03	1.0	2.2	8.1	38	135	--	122	101	83	85
0.18	1.0	1.6	7.5	39	156	--	89	94	85	98
0.38	1.0	2.4	7.1	41	161	--	133	89	89	101
0.59	1.0	1.7	7.6	31	128	--	94	95	67	81
1.51	1.0	1.7	7.0	32	166	--	94	88	70	104
3.73	1.0	1.6	6.5	26	111	--	89	81	57	70
6.74	1.0	2.3	3.7	8.7	49	--	128	46	19	31
16.71	1.0	1.1	1.4	1.2	2.3	--	61	18	3	1
Temperature [°C]	24.0 - 24.8	--	--	24.1-25.0	24.0-25.3					
pH	7.4-7.6	--	--	7.3-7.8	7.4-8.5					

[1] specify, if TS concentrations were nominal or measured

Table A7.4.1.3.c/01-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	yes	
Concentration of test substance ≥80% of initial concentration during test		yes

Figure A7.4.1.3.c/01-1: Growth curves for *Selenastrum capricornutum* during a 96-hour exposure to N-(n-octyl) Malonamic acid (based on mean measured concentrations)



Document III-A / Section A7.4.1 and A7.4.2

Section A7.4.1.3.c/02 **Growth inhibition test of N-(n-octyl) malonamic acid on algae-Marine water, *Skeletonema costatum***

Annex Point IIA VII.7.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[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**	Yes, OECD guideline 201 and US EPA OPPTS 850.5400	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	N-(n-octyl) malonamic acid (NNOMA)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	The test substance is a metabolite of DCOIT	
3.1.3	Purity	99.72%	
3.1.4	Composition of Product	[redacted]	
3.1.5	Further relevant properties		
3.1.6	Method of analysis		
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**		
3.3	**Reference substance**		
3.4	**Testing procedure**		
3.4.1	Culture medium		

Document III-A / Section A7.4.1 and A7.4.2

Section A7.4.1.3.c/02 **Growth inhibition test of N-(n-octyl) malonamic acid on algae-Marine water, *Skeletonema costatum***

Annex Point IIA VII.7.3

		[redacted]
3.4.2	Test organisms	[redacted]
3.4.3	Test system	
3.4.4	Test conditions	see table A7.4.1.3.c/02-4
3.4.5	Duration of the test	96 h
3.4.6	Test parameter	[redacted]
3.4.7	Sampling	
3.4.8	Monitoring of TS concentration	
3.4.9	Statistics	

4 RESULTS

4.1	**Limit Test**	Not Performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal (mg NNOMA/L) 0.063, 0.13, 0.25, 0.50, 1.0
4.2.2	Actual concentrations of test substance	measured (mg NNOMA/L)

0 h	72 h	96 h
0.064	0.070	0.070
0.13	0.14	0.14
0.25	0.28	0.28
0.51	0.56	0.56
1.0	1.1	1.1

4.2.3	Growth curves	see Figure A7.4.1.3.c/02-1
4.2.4	Concentration / response curve	not in final report
4.2.5	Cell concentration data	see table A7.4.1.3.c/02-5
4.2.6	Effect data (cell multiplication inhibition)	0 - 72 h : EC_{50} = 0.24 mg NNOMA/L; E_rC_{50} = 0.44 mg NNOMA/L; NOEC = 0.064 mg NNOMA/L; NOE_rC = 0.064 mg NNOMA/L; NOE_bC = 0.064 mg NNOMA/L

Section A7.4.1.3.c/02 Growth inhibition test of N-(n-octyl) malonamic acid on algae-Marine water, *Skeletonema costatum*

Annex Point IIA VII.7.3

4.2.7	Other observed effects	All test solutions appeared clear with no visible precipitate	
4.3	**Results of controls**	control results performed as expected	x
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Yes, OECD guideline 201 and US EPA OPPTS 850.5400, Acute static 96h algal study with analytical confirmation of test solution concentrations.	
5.2	**Results and discussion**	All test solutions appeared clear with no visible precipitate	x
5.2.1	NOE_rC	96 h = 0.13 mg NNOMA/L	
5.2.2	E_rC_{50}	96 h = 0.47 mg NNOMA/L	
5.2.3	E_bC_{50}	96 h = 0.16 mg NNOMA/L	
5.3	**Conclusion**	see validity criteria in table A7.4.1.3.c/02-6	x
5.3.1	Reliability	(1), reliable without restriction	x
5.3.2	Deficiencies	No	x

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	18 December 2007
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (4.3 and 5.2):** The contol cultures showed almost no growth during the first 48 hours, indicating that the inoculum culture was not in a good condition. The number of cells was multiplied by a factor of 19 during 96 hours. This is within the criterion of OECD 201 (multiplication factor 16), but lower than the acceptance criterion in the EPA standard protocol (multiplication factor 24). Results from this test should be treated with caution.
Conclusion	**Comment (5.3):** The results should be treated with caution, as the control cultures showed almost no growth during the first 48 hours.
Reliability	**Comment (5.3.1 and 5.3.2):** Due to the deficiencies described, the reliability is changed from 1 to 2, reliable with restrictions.
Acceptability	Acceptable with the restrictions noted above.
Remarks	-

Section A7.4.1.3.c/02	Growth inhibition test of N-(n-octyl) malonamic acid on algae-Marine water, *Skeletonema costatum* – **TABLES AND FIGURES**

[redacted]

[redacted]

[redacted]

Table A7.4.1.3.c/02-4: Test conditions

Criteria	Details
Test temperature	20.6 to 22.0 °C
pH	7.8 to 8.1
Aeration of dilution water	Yes
Light intensity	4091 to 4400 lux
Photoperiod	16hr light : 8hr dark photoperiod

Table A7.4.1.3.c/02-5: Cell concentration data

Test-Substance Concentration (measured)[1] [mg NNOMA/l]	Cell concentrations (mean values) [x 10^4 cells/ml]									
	measured					Percent of control				
	0 h	24 h	48 h	72 h	96 h	0 h	24 h	48 h	72 h	96 h
0 (control)	1.0	1.2	1.1	3.7	19	--	--	--	--	--
0.064	1.0	0.81	1.1	3.1	22	--	68	100	84	116
0.13	1.0	0.73	0.82	2.1	15	--	61	75	57	79
0.25	1.0	1.2	0.71	2.0	4.9	--	100	65	54	26
0.51	1.0	0.94	0.78	1.4	2.6	--	78	71	38	14
1.0	1.0	0.18	0.33	0.30	0.77	--	9	30	8	4
Temperature [°C]	0 h = 20.6 – 21.0			96 h = 21.8 – 22.0						
pH	0 h = 7.9-8.1			96 h = 7.8-8.0						

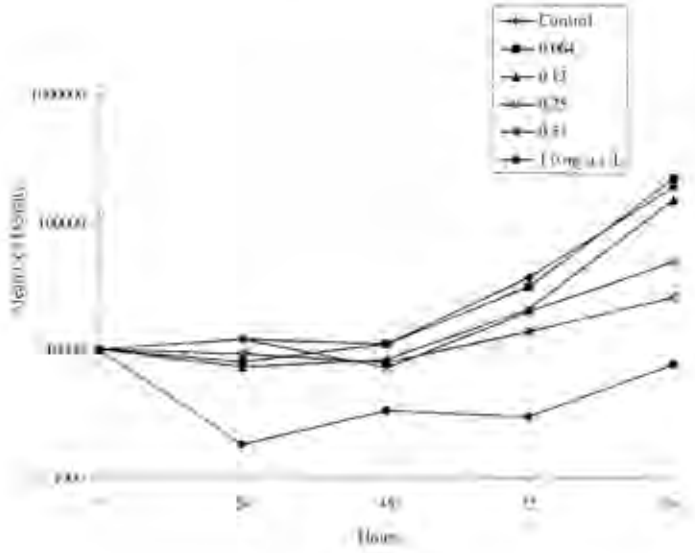
[1] specify, if TS concentrations were nominal or measured

Table A7.4.1.3.c/02-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	yes	
Concentration of test substance ≥80% of initial concentration during test	yes	

Figure A7.4.1.3.c/02-1: Growth curves for *Skeletonema costatum* during a 96-hour exposure to N-(n-octyl) Malonamic acid

Figure 1. Growth Curves for *Skeletonema costatum* During a 96-Hour Exposure to N-(n-octyl) Malonamic Acid, Technical, PMN



Document III-A / Section A7.4.1 and A7.4.2

Section A7.4.1.3.c/03 **Growth inhibition test of N-(n-octyl) acetamide on algae-Fresh water, *Selenastrum capricornutum***

Annex Point IIA VII.7.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[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**	Yes, OECD 201, US EPA OPPTS 850.5400	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	N-(n-octyl) acetamide (NNOA)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	The test substance is a metabolite of DCOIT	
3.1.3	Purity	97.06%	
3.1.4	Composition of Product	[redacted]	
3.1.5	Further relevant properties		
3.1.6	Method of analysis		
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**		
3.3	**Reference substance**		
3.4	**Testing procedure**		
3.4.1	Culture medium		

Document III-A / Section A7.4.1 and A7.4.2

Section A7.4.1.3.c/03 **Growth inhibition test of N-(n-octyl) acetamide on algae-Fresh water, *Selenastrum capricornutum***

Annex Point IIA VII.7.3

3.4.2	Test organisms	[redacted]
3.4.3	Test system	
3.4.4	Test conditions	see table A7.4.1.3.c/03-4
3.4.5	Duration of the test	96 h
3.4.6	Test parameter	[redacted]
3.4.7	Sampling	
3.4.8	Monitoring of TS concentration	
3.4.9	Statistics	

4 RESULTS

4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal 0.63, 1.3, 2.5, 5.0, 10, 20 mg NNOA/L	
4.2.2	Actual concentrations of test substance	0 h measured / 72 h measured / 96 h measured (mg NNOA/L)	x
4.2.3	Growth curves	see Figure A7.4.1.3.c/03-1	x
4.2.4	Concentration / response curve	not in final report	
4.2.5	Cell concentration data	see table A7.4.1.3.c/03-5	x
4.2.6	Effect data (cell multiplication inhibition)	0 - 72 h EC_{50} = 5.8 mg NNOA/L; E_bC_{50} = 6.0 mg NNOA/L; E_rC_{50} = 11 mg NNOA/L; 0-72 h NOEC = 2.7 mg NNOA/L; NOE_rC = 2.7 mg NNOA/L	

0 h	72 h	96 h
0.522	0.407	MQL
1.28	1.04	0.302
2.68	1.47	0.271
5.88	1.69	0.548
11.2	MQL	MQL
18.7	MQL	MQL

MQL = less than minimum quantifiable limit (0.0600 mg NNOA/L)

Section A7.4.1.3.c/03 **Growth inhibition test of N-(n-octyl) acetamide on algae-Fresh water, *Selenastrum capricornutum***

Annex Point IIA VII.7.3

4.2.7	Other observed effects	Through the first 72 h, all test solutions appeared clear with no visible precipitate or surface films. At 96 h, all treatments through 11 mg NNOA/L appeared clear, while the 19 mg NNOA/L treatment appeared cloudy.	
4.3	**Results of controls**	control results performed as expected	
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 201, US EPA OPPTS 850.5400, Acute static 96h algal study with analytical confirmation of test solution concentrations.	
5.2	**Results and discussion**	Through the first 72 h, all test solutions appeared clear with no visible precipitate or surface films. At 96 h, all treatments through 11 mg NNOA/L appeared clear, while the 19 mg NNOA/L treatment appeared cloudy.	x
5.2.1	NOE_rC	Based on initial measured concentrations: 72 h = 2.7 mg NNOA/L	
		Based on mean measured concentrations: 72 h = 1.99 mg NNOA/L	
5.2.2	E_rC_{50}	72 h = 11 mg NNOA/L based on initial measured concentrations	
5.2.3	E_bC_{50}	72 h = 6.0 mg NNOA/L based on initial measured concentrations	
5.3	**Conclusion**	see validity criteria in table, below	x
5.3.1	Reliability	(1), reliable without restriction	x
5.3.2	Deficiencies	No	x

Document III-A / Section A7.4.1 and A7.4.2

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	18 December 2007
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (4.2.2):** A review of the raw data showed that there were no problems with the analytical method used. It seems rather that the degradation of NNOA did not follow the typical degradative trend that residues in lower concentrations are typically reduced beyond MQL before those at the higher dose levels. The cell density in the higher dose concentrations were significantly lower than in those at the lower concentrations (at 72 and 96 hours, respectively), something that might have had an influence on the biodegradation of the test substance during the test. **Comment (4.2.3, 4.2.5 and 5.2)**: The growth in the control is not logarithmic during the latter 24 hours, and partly inhibited cultures tend to catch up with the control between 72 and 96 hours. Therefore, 72 hours results are used. **Comment (5.2)**: The statistical determination of the ErC_{50} value based on geometric mean concentrations was not feasable because at the two highest test concentrations the analytical results were < MQL. Using this approach would have lead to an ErC_{50}, which would be lower than the NOEC. Therefore the ErC_{50} based on initial measured concentrations is used as an endpoint from this study. This ErC_{50} should be used with caution as the test substance is not stable under the test conditions.
Conclusion	Agree with applicant's version. However, the EC_{50} should be used with caution (see comment 5.2).
Reliability	Comment **(5.3.1 and 5.3.2)**: Due to the restrictions described, the reliability is changed from 1 to 2, reliable with restrictions.
Acceptability	Acceptable with the restrictions noted above.
Remarks	-

Section A7.4.1.3.c/03	Growth inhibition test of N-(n-octyl) acetamide on algae-Fresh water, *Selenastrum capricornutum* – TABLES AND FIGURES

[redacted]

[redacted]

[redacted]

Table A7.4.1.3.c/03-4: Test conditions

Criteria	Details
Test temperature	22.1 to 24.9 °C
pH	7.3 to 8.1
Aeration of dilution water	Yes
Light intensity	4009 to 4690 lux
Photoperiod	continuous lighting

Table A7.4.1.3.c/03-5: Cell concentration data

Test-Substance Concentration (mean measured 0-72)[1] [mg NNOA/l]	Cell concentrations (mean values) [cells x 10^4/ml]									
	measured					Percent of control				
	0 h	24 h	48 h	72 h	96 h	0 h	24 h	48 h	72 h	96 h
0 (control)	1.0	2.6	12	63	117	--	--	--	--	--
0 (solvent control)	1.0	3.5	9.8	66	136	--	135	82	105	116
0.46	1.0	2.4	9.4	47	151	--	92	78	75	129
1.16	1.0	2.1	8.1	39	125	--	81	68	62	107
1.99	1.0	2.4	8.0	52	143	--	92	67	83	122
3.16	1.0	1.5	7.5	32	132	--	58	63	51	113
0.57	1.0	0.70	1.2	6.8	26	--	27	10	11	22
0.75	1.0	0.55	0.56	0.88	0.70	--	21	5	1	1
Temperature [°C]	22.1-24.0	--	--	23.9-24.9	23.2-23.5					
pH	7.5-7.6	--	--	7.3-7.5	7.4-8.1					

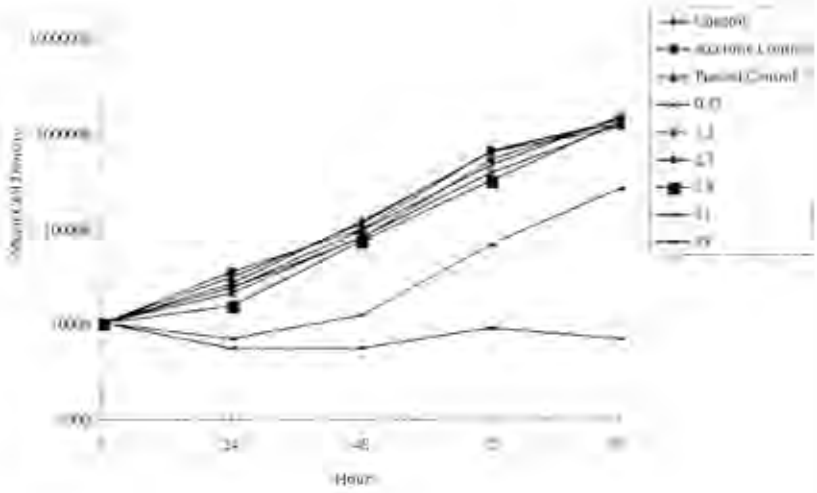
[1] specify, if TS concentrations were nominal or measured

Table A7.4.1.3.c/03-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	yes	
Concentration of test substance ≥80% of initial concentration during test		yes

Figure A7.4.1.3.c/03-1: Growth curves for *Selenastrum capricornutum* during a 96-hour exposure to N-(n-octyl) Acetamide

Figure 1 Growth Curves for *Selenastrum capricornutum* During a 96-Hour Exposure to N-(n-Octyl) Acetamide



Document III-A / Section A7.4.1 and A7.4.2

Section A7.4.1.4 Inhibition to microbial activity (aquatic, activated sludge)

Annex Point IIA VII.7.4 and IIIA VII.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[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**	Yes, OECD 209	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	RH-287 Technical	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	98.1% DCOIT	
3.1.4	Composition of Product	[redacted]	
3.1.5	Further relevant properties		x
3.1.6	Method of analysis		
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**		x

Document III-A / Section A7.4.1 and A7.4.2

Section A7.4.1.4 — Inhibition to microbial activity (aquatic, activated sludge)

Annex Point IIA VII.7.4 and IIIA VII.3

3.3	**Reference substance**	[redacted]
3.3.1	Method of analysis for reference substance	
3.4	**Testing procedure**	
3.4.1	Culture medium	
3.4.2	Inoculum / test organism	
3.4.3	Test system	
3.4.4	Test conditions	see table A7.4.1.4/01-4
3.4.5	Duration of the test	3 h
3.4.6	Test parameter	respiration inhibition
3.4.7	Analytical parameter	[redacted]
3.4.8	Sampling	
3.4.9	Monitoring of TS concentration	
3.4.10	Controls	
3.4.11	Statistics	

4 RESULTS

4.1	**Preliminary test**	Performed
4.1.1	Concentration	0, 0.57, 5.7
4.1.2	Effect data	respiration rate at 0.57 mg/L = 91% control rate; respiration rate at 5.7 mg/L = 78% control rate

Document III-A / Section A7.4.1 and A7.4.2

Section A7.4.1.4 Inhibition to microbial activity (aquatic, activated sludge)

Annex Point IIA VII.7.4 and IIIA VII.3

4.2 Results test substance

4.2.1 Initial concentrations of test substance: Nominal (mg/L) 5.7 x

4.2.2 Actual concentrations of test substance: Measured (mg DCOIT/L) x

Replicate	0.5 h	1 h	2 h	3 h
1	0.542	0.195	<0.100	<0.100
2	0.143	<0.100	<0.100	0.172

4.2.3 Growth curves: Not applicable

4.2.4 Cell concentration data: Not applicable

4.2.5 Concentration/ response curve: Not applicable

4.2.6 Effect data

Nominal concent. DCOIT tech (mg/L)	Rep	Oxygen concentration (mg/L)		Time to 6.5 mg/L O2 (min.)	Time to 2.5 mg/L O2 (min.)	Respiration rate
		Initial	Final			
0 (control)	1	8.5	0.3	3.0	7.25	56
	2	8.7	0.9	4.0	8.0	60
0.07	1	8.4	0.4	2.5	7.25	51
	2	8.1	0.6	2.5	7.25	51
0.22	1	8.2	0.5	3.0	7.5	53
	2	8.0	0.5	2.5	7.25	51
0.64	1	7.8	0.6	2.0	7.0	48
	2	8.3	0.5	2.75	7.5	51
1.9	1	8.3	0.6	3.0	8.0	48
	2	8.3	0.4	1.25	7.25	40
5.7	1	9.1	1.3	4.25	9.25	48
	2	8.7	1.7	4.25	9.25	48

3 h EC_{50} > 5.7 mg/L x

4.2.7 Other observed effects: TS degraded rapidly x

4.3 Results of controls: control without test substance: respiration rate = 58 mg/L/h

4.4 Test with reference substance: Performed

Section A7.4.1.4 Inhibition to microbial activity (aquatic, activated sludge)

Annex Point IIA VII.7.4 and IIIA VII.3

4.4.1	Concentrations	3,5-dichlorophenol: 5.0, 12, 30 mg/L	
4.4.2	Results	EC_{50} = 12 mg/L	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 209, Activated sludge, respiration inhibition test	
5.2	**Results and discussion**	Because of the cloudiness caused by the activated sludge, insoluble material, if present, could not be observed in test vessels. Rapid degradation of the test substance was observed during the definitive activated sludge respiration inhibition test, where measured concentrations decreased from a nominal concentration of 5.7 mg/L to less than the quantitation limit of 0.100 mg/L within 2 hours. T ½ = less than 30 minutes.	x
5.2.1	EC_{20}	Not described	
5.2.2	EC_{50}	3 h > 5.7 mg/L, the approximate water solubility limit	x
5.2.3	EC_{80}	Not described	
5.3	**Conclusion**		x
5.3.1	Reliability	(1), reliable without restriction	x
5.3.2	Deficiencies	No	x

Document III-A / Section A7.4.1 and A7.4.2

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	22 August 2007, revised 22 August 2009
Materials and Methods	**Comment (3.1.5):** The water solubility of DCOIT at pH7 and 20°C is 3.47 mg/l.
	Comment (3.2): According to the study report, beads of insoluble test material were observed on the bottom of the stock solution vessel.
	Comment (3.4.9): No measurement of test substance concentration has been conducted at time 0. Due to this and because of the cloudiness caused by the activated sludge, the actual concentration dissolved in the test medium at the start of the test is unknown.
Results and discussion	**Comment (4.2.1):** The definitive respiration inhibition test was conducted with 5 nominal concentrations: 0.07, 0.22, 0.64, 1.9, 5.7 mg/l. The highest test concentration is above the water solubility.
	Comment (4.2.2): The measured test concentration was taken from the 5.7 mg/l test vessel, which is above the water solubility of DCOIT of 3.47 mg/l.
	Comment (4.2.6): A NOEC of 0.64 mg/L (15% inhibition; NOEC < EC20) can also been derived from this test.
	Comment (4.2.7): Out from the physical-chemical properties of DCOIT, it seems unlikely that the disappearance of the test substance is mainly due to biodegradation. The measured test concentration was taken from the 5.7 mg/l test vessel, which is above the water solubility of DCOIT. The test substance concentration was not measured at the beginning of the test. Moreover, study III A7.1.3a (Adsorption/desorption test with activated sludge) shows that DCOIT adsorbs to activated sludge to various degrees, dependant on the sludge concentration. At a sludge concentration of about 6 g dry weight sludge per liter $CaCl_2$ ca 90% of DCOIT adsorbed to the activated sludge. Therefore, it is likely that adsorption might have played a major role as an elimination mechanism in this test.
Conclusion	**Comment (5.2)**: It seems unlikely that the disappearance of the test substance is only due to biodegradation (see comment 4.2.7).
	Comment (5.2.2): The water solubility of DCOIT at pH7 and 20°C is 3.47 mg/l.
	Comment (5.3): As an endpoint a NOEC of 0.64 mg/L has been derived from this test.
Reliability	**Comment (5.3.1 and 5.3.2)**: Due to the restrictions described, the reliability is changed from 1 to 2, reliable with restrictions.
Acceptability	Acceptable with the restrictions noted above.
Remarks	-

Section A7.4.1.4	Inhibition to microbial activity (aquatic, activated sludge)
	TABLES AND FIGURES

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

Table A7.4.1.4/01-4: Test conditions

Criteria	Details
Test temperature	20 ± 2 °C
pH	7.5 at test initiation
Aeration of dilution water	Not described
Suspended solids concentration	4.4 g/L at test initiation

Section A7.4.2 Annex Point IIA7.5	Estimation of bioconcentration	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Several bioaccumulation studies have been conducted in fish and invertebrates. They are fully summarized in section A7.4.3.3.	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	18 October 2007	
Evaluation of applicant's justification	Agree with applicant's justification.	
Conclusion	Applicant's justification is 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 8: Wood preservatives

Document III-A (A7)
Study summaries – Active substance
Ecotoxicological profile including environmental fate and behaviour

Part V

Fate and behaviour in the environment

Section A7.4.3: Effects on aquatic organisms, further studies

Document III-A / Section A7.4.3

TABLE OF CONTENTS

Document III-A / Section A7.4.3

Section 7.4.3.1 **Annex Point IIIA XIII.2.1**	**Prolonged toxicity to an appropriate species of fish**	
	Justification for non-submission of data	Official use only
Other existing data [x] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	As outlined in the "Technical guidance document in support of the directive 98/8/EC concerning the placing of biocidal products on the market", this test is not required as it does not add information as needed in the risk assessment. The existing guidelines are not sufficient. Other studies are available under section A7.4.3.2.	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	17 December 2007	
Evaluation of applicant's justification	Agree with applicant's justification	
Conclusion	Test not considered necessary as long-term tests with 2 different fish species have been conducted (see section A7.4.3.2).	
Remarks	-	

Document III-A / Section A7.4.3

Section A7.4.3.2.a/01 **Effects on reproduction and growth rate of fish- Early life stage test, Fresh water fish, Rainbow trout**
Annex Point IIIA XIII.2.2

Official use only

	1 REFERENCE	
1.1	**Reference**	[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**	Yes, OECD 210, US EPA OPPTS 850.1400, US EPA FIFRA 72-4, US EPA TSCA 797.1600, EC Council Directive 91/414EEC.
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 METHOD	
3.1	**Test material**	Kathon 287T, ^{14}C-RH-287
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	99.3% [redacted]
3.1.4	Composition of Product	[redacted]
3.1.5	Further relevant properties	
3.1.6	Method of analysis	

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	[redacted]
3.3	**Reference substance**	[redacted]
3.4	**Testing procedure**	[redacted]
3.4.1	Dilution water	
3.4.2	Test organisms	
3.4.3	Handling of embryos and larvae (OECD 210/212)	
3.4.4	Test system	
3.4.5	Test conditions	see table A7.4.3.2.a/01-5
3.4.6	Duration of the test	97 d (61 d post-hatch)
3.4.7	Test parameter(s)	[redacted]
3.4.8	Examination / Sampling	
3.4.9	Monitoring of TS concentration	
3.4.10	Statistics	

4 RESULTS

4.1	**Range finding test**	Performed
4.1.1	Concentrations	0.19, 0.38, 0.75, 1.5, 3.0 µg DCOIT/L

4.1.2 Number/ percentage of animals showing adverse effects

Egg hatch: day 32 to day 37

Results after 37 days of treatment:

Dose (µg DCOIT/L)	%mortality	length (mm)	weight (g)
control	0	28	0.242
Acetone control	0	28	0.253
0.19	0	28	0.260
0.38	0	27	0.235
0.75	7	28	0.250
1.5	0	26	0.204
3.0	10	21	0.109

4.1.3 Nature of adverse effects

Sublethal effects: fish resting on bottom of test chamber in 3.0 µg DCOIT/L treatment

4.2 Results test substance

4.2.1 Initial concentrations of test substance

Nominal (µg DCOIT/L): 0.19, 0.38, 0.75, 1.5, 3.0

4.2.2 Actual concentrations of test substance

Mean measured concentrations: 0.15, 0.30, 0.56, 1.2, 2.6 µg DCOIT/L (total radioactivity)

Mean measured concentrations: 2.5 and 251,000 µg DCOIT/L (high TS treatment and diluter stock solution) (HPLC)

Analytical results are presented in Tables A7.4.3.2.a/01-7 and -8.

4.2.3 Effect data

Egg hatch began on day 32 and was 100% complete by day 37.

Dose (µg DCOIT/L)	%hatching success	%post-hatch survival	length (mm)	weight (g)
control	100	100	45.6	1.399
Acetone control	100	98	45.4	1.367
0.15	100	98	44.2	1.269
0.30	100	98	45.3	1.346
0.56	100	100	44.6	1.290
1.2	100	97	44.2	1.243
2.6	83	45	31.6	0.436

Normal swim-up behavior began on day 48 and was complete on day 58. Primary behavior abnormality was fish resting on the bottom of the test chamber at 2.6 µg DCOIT/L which began when swim-up was complete.

0.15 µg DCOIT/L: 1 dead, 1 resting on bottom, 1 spinal curvature
0.30 µg DCOIT/L: 1 dead, 1 resting on bottom
0.56 µg DCOIT/L: 1 resting on bottom, 1 fish with 2 heads
1.2 µg DCOIT/L: 2 dead

		2.6 µg DCOIT/L: 33 dead, up to 22 resting on bottom	
4.2.4	Concentration / response curve	Not described in report	
4.2.5	Other effects	Not applicable	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	100% hatch success; egg fertilization 98%	
4.3.2	Nature of adverse effects	control: no deaths, no abnormal behavior acetone control: 1 death, no abnormal behavior	
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 210, US EPA OPPTS 850.1400, US EPA FIFRA 72-4, US EPA TSCA 797.1600, EC Council Directive 91/414EEC, Early life stage toxicity study to fish under flow-through conditions with analytical confirmation of TS concentrations.	
5.2	**Results and discussion**	All treatments were clear and colorless with no visible precipitates or surface films. MATC, based on growth = 0.82 µg ai/L	x
5.2.1	NOEC	egg hatchability and survival: 1.2 µg DCOIT/L ; growth: 0.56 µg DCOIT/L	
5.2.2	LOEC	egg hatchability and survival: 2.6 µg DCOIT/L ; growth: 1.2 µg DCOIT/L	
5.3	**Conclusion**	see tables A7.4.3.2.A/01-6	
5.3.1	Other Conclusions		
5.3.2	Reliability	(1), reliable without restriction	
5.3.3	Deficiencies	No	

Document III-A / Section A7.4.3

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	19 December 2007
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (5.2):** The NOEC is based on mean measured concentrations of total radioactivity. No measurement of parent with HPLC has been conducted at this concentration level. Therefore, it is not quite clear if the test result reflects the toxicity of DCOIT towards fish or if it refers to a mixture of parent and metabolites. However, HPLC measurements at the 3.0 µg/L level (nominal) showed that test concentrations were constant at about 84% of nominal. It can therefore be assumed that the NOEC reflects the toxicity of DCOIT towards fish.
Conclusion	Agree with applicant's version
Reliability	1, reliable without restrictions
Acceptability	Acceptable
Remarks	-

Section A7.4.3.2.a/01	Effects on reproduction and growth rate of fish- Early life stage test, Fresh water fish, Rainbow trout – **TABLES AND FIGURES**

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Document III-A / Section A7.4.3

Table A7.4.3.2.a/01-5: Test conditions

Criteria	Details
Test temperature	10 ± 2 °C
Dissolved oxygen	7.4 – 11.0 mg/L
pH	7.8 – 8.9
Adjustment of pH	Not described
Aeration of dilution water	Yes
Intensity of irradiation	387±88 lux
Photoperiod	Developing embryos were incubated in semi-darkness. 16 h daylight: 8 h dark, starting one-week post-hatch

Table A7.4.3.2.a/01-6: Validity criteria for fish tests according to OECD Guidelines 210

	fulfilled	Not fullfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	**yes**	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	**yes**	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	**yes**	
Test substance concentrations maintained within ± 20% of mean measured values	**yes**	
No effect on survival nor any other adverse effect found in solvent control	**yes**	
Further criteria for poorly soluble test substances	**yes**	

Document III-A / Section A7.4.3

Table A7.4.3.2.a/01-7: Measured total radioactivity

Measured Total Radioactivity (TRR) as RH-287 Technical (ug a.i./L)
Based on LSC Analysis

Day	Control	Acetone Control	Level 1 (0.19)	Level 2 (0.38)	Level 3 (0.75)	Level 4 (1.5)	Level 5a (3.0)	Level 5b (3.0)
-2	---	<MQL	0.170	0.309	0.653	1.24	2.56	2.42
0	---	<MQL	0.163	0.284	0.622	1.28	2.54	2.54
7	---	<MQL	0.155	0.310	0.635	1.23	2.62	
14	---	<MQL	0.161	0.303	0.290	0.57	1.42	
15	---	<MQL	0.190	0.337	0.696	1.50	3.07	
21	---	<MQL	0.229	0.370	0.767	1.49	2.99	
28	---	<MQL	0.186	0.326	0.718	1.46	2.70	2.59
35	---	<MQL	0.190	0.347	0.716	1.44	2.90	
42	---	<MQL	0.160	0.279	0.648	1.33	2.62	
49	---	<MQL	0.175	0.323	0.678	1.31	2.67	
56	---	<MQL	0.128	0.247	0.558	1.30	2.70	
63	---	<MQL	0.149	0.288	0.538	1.13	2.45	2.40
70	---	<MQL	0.116	0.257	0.423	1.03	3.26	
72	---	<MQL	0.129	0.189	0.515	1.06	2.86	
77	---	<MQL	0.133	0.512	0.469	1.02	2.79	
85	---	<MQL	0.103	0.200	0.443	1.32	2.79	
91	---	<MQL	0.138	0.284	0.437	0.97	2.75	
97	---	<MQL	0.118	0.224	0.438	1.03	2.30	2.55
Mean	---	<MQL	0.150	0.300	0.560	1.20	2.60	
Mean % of Nominal	---	---	79	79	75	80	87	

Table A7.4.3.2.a/01-8: Measured DCOIT concentration

Measured Concentrations of RH-287 Technical (ug a.i./L)
Based on HPLC Analysis

Day	Level 5 (3.0)	Diluter Stock (232,000)	High Spike (3.96)
-2	2.25	251000	1.740
	2.36		
0	2.58	241000	3.370
	2.44		
28	2.65	230000	3.300
	2.81		
63	2.53	275000	3.690
	2.62		
97	2.22	257000	3.260
	2.29		
Mean	2.52	251000	3.410
Mean % of Nominal	84	108	86

Document III-A / Section A7.4.3

Section A7.4.3.2.b/01
Annex Point IIIA XIII.2.2

Effects on reproduction and growth rate of fish-Early life stage test, Marine water fish, Sheepshead minnow

Official use only

1 REFERENCE

1.1	**Reference**	[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**	Yes, US EPA FIFRA 72-4
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 METHOD

3.1	**Test material**	RH-287 Technical
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	96.9%
3.1.4	Composition of Product	[redacted]
3.1.5	Further relevant properties	[redacted]
3.1.6	Method of analysis	[redacted]
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	[redacted]
3.3	**Reference substance**	[redacted]

Section A7.4.3.2.b/01 **Effects on reproduction and growth rate of fish-Early life stage test, Marine water fish, Sheepshead minnow**
Annex Point IIIA XIII.2.2

3.4	**Testing procedure**	
3.4.1	Dilution water	[redacted]
3.4.2	Test organisms	
3.4.3	Handling of embryos and larvae	
3.4.4	Test system	
3.4.5	Test conditions	see table A7.4.3.2.b/01-5
3.4.6	Duration of the test	[redacted]
3.4.7	Test parameter(s)	
3.4.8	Examination / Sampling	
3.4.9	Monitoring of TS concentration	
3.4.10	Statistics	

4 RESULTS

4.1	**Range finding test**	Performed
4.1.1	Concentrations	7.6, 11.5, 21.2, 34.5, 70.0 µg DCOIT/L
4.1.2	Number/ percentage of animals showing adverse effects	Data not available. 96 h NOEC = 11.5 µg DCOIT/L based on survival
4.1.3	Nature of adverse effects	lethargy and loss of equilibrium
4.2	**Results test substance**	
4.2.1	Initial concentrations of	Nominal (µg DCOIT/L)

Section A7.4.3.2.b/01 **Effects on reproduction and growth rate of fish-Early life stage test, Marine water fish, Sheepshead minnow**
Annex Point IIIA XIII.2.2

test substance 0.96, 1.9, 4.0, 8.0, 16.0

4.2.2 Actual concentrations of test substance

Nominal (µg DCOIT/L)	0.96	1.9	4.0	8.0	16.0
0 h measured	0.55	1.1	2.9	5.6	14.0
7 d measured	0.61	1.1	2.7	6.3	13.0
14 d measured	0.42	0.87	2.2	5.1	12.0
21 d measured	0.54	1.0	2.3	5.3	13.0
28 d measured	0.58	1.8	3.3	7.8	17.0
35 d measured	0.54	1.5	3.8	5.6	14.0

4.2.3 Effect data

Mean measured concentration (µg DCOIT/L)	%survival hatch 4 d	%embryos hatched 4 d	%survival juveniles 32 d post hatch
Control	81.2	81.2	95.0
solvent control	85.0	85.0	82.5
0.54	82.5	85.0	92.5
1.2	83.8	85.0	95.0
2.9	80.0	80.0	65.0
6.0	71.2	71.2	70.0
14.0	26.2	65.0	10.0

Mean measured concentration (µg DCOIT/L)	Total length (mm)	weight (mg) at conclusion of the 35 days
Control	17.1	127.4
solvent control	20.0	166.7
0.54	19.0	145.7
1.2	19.7	148.9
2.9	19.8	172.7
6.0	20.1	168.9
14.0	18.6	151.6

Effects data	NOEC (µg DCOIT/L)	LOEC (µg DCOIT/L)	MATC (µg DCOIT/L)
% embryos hatched	6.0	14.0	9.2

Section A7.4.3.2.b/01 **Effects on reproduction and growth rate of fish-Early life stage test, Marine water fish, Sheepshead minnow**
Annex Point IIIA XIII.2.2

		time to hatch	14.0	>14.0	>14.0
		% survival to hatch (day 4)	6.0	14.0	9.2
		Sublethal effects	14.0	>14.0	>14.0
		Total length	14.0	>14.0	>14.0
		Weight	14.0	>14.0	>14.0

4.2.4 Concentration / response curve: Not described

4.2.5 Other effects: loss of equilibrium, lethargy, mortality

4.3 Results of controls

4.3.1 Number/ percentage of animals showing adverse effects:

100% survival at hatch = 40 fish per replicate

100% survival after hatch = 20 fish per replicate

Group	%survival hatch 4d	%embryos hatched 4d	%survival juveniles 32d post hatch
Control	81.2	81.2	95.0
Solvent Control	85.0	85.0	82.5

4.3.2 Nature of adverse effects: mortality

4.4 Test with reference substance: Not Performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods: US EPA FIFRA 72-4, Early life stage toxicity study to fish under flow-through conditions with analytical confirmation of TS concentrations.

5.2 Results and discussion

5.2.1 NOEC: egg hatchability: 6 µg DCOIT/L; survival and growth: 14 µg DCOIT/L

5.2.2 LOEC: egg hatchability: 14 µg DCOIT/L; survival: 14 µg DCOIT/L ; growth: > 14 µg DCOIT/L

5.3 Conclusion: see table A7.4.3.2.b/01-6

5.3.1 Other Conclusions: Not applicable

5.3.2 Reliability: (1), reliable without restriction

5.3.3 Deficiencies: No

Document III-A / Section A7.4.3

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	19 December 2007
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-

Document III-A / Section A7.4.3

Section A7.4.3.2.b/01	Effects on reproduction and growth rate of fish-Early life stage test, Marine water fish, Sheepshead minnow - **TABLES AND FIGURES**

[redacted]

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

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Table A7.4.3.2.b/01-5: Test conditions

Criteria	Details
Test temperature	mean = 29.8 °C; range: 29.0 – 30.5 °C
Dissolved oxygen	mean = 7.2; range: 5.8 - 8.5
pH	mean = 7.8; range: 7.3 – 8.3
Adjustment of pH	Not described
Aeration of dilution water	No
Intensity of irradiation	10 $uEs^{-1}m^{-2}$
Photoperiod	16 h light, 8 h dark

Table A7.4.3.2.b/01-6: Validity criteria for fish tests according to OECD Guidelines 210/212

	fulfilled	Not fullfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	**yes**	
Difference of water temperature < 1.5 °C between test chambers or successive days at any time during test; temperature within range for specific test species	**yes**	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ 75 %, specified for the specific test species	**yes**	
Test substance concentrations maintained within ± 20% of mean measured values	**yes**	
No effect on survival nor any other adverse effect found in solvent control Survival>80 %	**yes**	
Further criteria for poorly soluble test substances	**yes**	

Section A7.4.3.3.1.a Bioconcentration in fish-Freshwater, Bluegill Sunfish

Annex Point IIIA XIII.2.3

Official use only

1 REFERENCE

1.1	**Reference**	[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**	Yes. U.S. EPA 40 CFR § 158, Pesticide Assessment Guidelines Subdivision N § 165-4
2.2	**GLP**	No (References 1-3) Yes (Reference 4)

[redacted]

Section A7.4.3.3.1.a Bioconcentration in fish-Freshwater, Bluegill Sunfish

Annex Point IIIA XIII.2.3

[redacted]

2.3 Deviations No noted deviations.

3 MATERIALS AND METHODS

3.1 Test material ^{14}C-DCOIT (RH287). [redacted]

3.1.1 Lot/Batch number [redacted]

3.1.2 Specification

3.1.3 Purity

3.1.4 Further relevant properties

3.1.5 Radiolabelling

3.1.6 Method of analysis

3.2 Reference substance

3.2.1 Method of analysis for reference substance

3.3 Testing/estimation procedure

3.3.1 Test system/ performance [redacted]

Section A7.4.3.3.1.a Bioconcentration in fish-Freshwater, Bluegill Sunfish

Annex Point IIIA XIII.2.3

[redacted]

3.3.2	Estimation of bioconcentration	The log P_{ow} , 2.8 was determined by the shake flask method (U.S. EPA 40 CFR § 158, Pesticide Assessment Guidelines Subdivision D § 63-11 and OECD 107) indicating that the compound will not present a bioaccumulation hazard (*i.e.*, log $P_{ow} < 3$).

4 RESULTS

4.1 Experimental data

4.1.1	Mortality/behaviour	Reference 1, 2, and 3: No mortalities or adverse behaviors were observed in the control or treated organisms (test material from these three studies came from a single in-life dosing). Reference 4: There was one mortality in the control treatment and four in those treated with the test material.
4.1.2	Lipid content	Lipid content was not determined.
4.1.3	Concentrations of test material during test	Reference 1, 2, 3 (single in-life study): Radioanalysis showed that concentration in water (based on parent compound) ranged from 0.76 µg/L (Day 0.17) to 1.5 µg/L (Day 3) with an average DCOIT concentration during the uptake phase of 1.2 ± 0.24 µg/L (Day 0-28). This compares favorably with the expected nominal concentration, 1.2 µg/L of ^{14}C DCOIT. During the uptake phase, the concentration in fillets, viscera, and whole fish ranged from 6.9-230 µg/L, 110-1300 µg/l, and 56-770 µg/L, respectively (Table A7.4.3.3.1.a-1 and Figure A7.4.3.3.1.a-1). Steady state uptake of parent compound was reached within 28 days. The depuration data is presented in Table A7.4.3.3.1.a-2 and Figure A7.4.3.3.1.a-1. As discussed below, very little if any of the ^{14}C-activity measured in the organisms is parent and thus depuration kinetics is primarily a measure of metabolite depuration. The depuration half-life for ^{14}C-activity is 11.6 days and the time for 90% depuration is 38 days. Reference 4: This study was performed to generate additional test material for metabolite identification. All the dosed fishes were removed on Day 28, separated into fillets and viscera, and homogenized. The average concentration in fillets and viscera were 130 µg/l and 690 µg/l, respectively. Since there were no interval sampling periods, uptake kinetics could not be determined. Radioanalysis showed the concentration in water (based on parent compound; aliquots removed at 0, 3, 7, 14, 21, and 28 days after dosing), ranged from 0.71 µg/ml (Tank A, Day 3) to 1.3 µg/ml (Tank B, Day 0) with an average DCOIT concentration of 0.99 ± 0.18 µg/ml. The interval water concentrations are presented in Table A7.4.3.3.1a-3. There was no depuration phase in this study.
4.1.4	Bioconcentration factor (BCF)	Reference 1, 2, and 3: BCF for sampling Days 0.17, 1, 3, 7, 14, 21, and 28 for whole fish, viscera, and fillets appear in Table A7.4.3.3.1.a-4. These BCF values were determined as the ratio of ^{14}C-activity in tissue to the in water. The steady state bioconcentration factor, $K_{uptake}/K_{depuration}$ (K_1/K_2) was 750. Reference 4: The BCF at the only tissue sampling day (Day 28) was 130x and 700x for fillets and viscera, respectively.
4.1.5	Uptake and depuration rate constants	Reference 1, 2, and 3: The uptake rate constant (K_1) was 45 ppb in fish/ppb in water/day. The depuration rate constant (K_2) was 0.060 day^{-1}. The kinetic rates were

determined from the slope of a linear regression line derived from the natural log transformation of the tissue concentrations (both uptake and depuration) versus the study days. The steady state BCF was determined as the ratio of the uptake rate constant to the depuration rate constant (K_1/K_2) and the value was 750.

Reference 4:

This study was designed to generate sufficient tissue for metabolite identification. Kinetic analysis was not possible since there was only one sample date.

4.1.6 Depuration time

Reference 1, 2, and 3:

$DT_{50} = \ln 2/K_2 = 11.6$ days and $DT_{90} = \ln 10/K_2 = 38.4$ days. K_2 is 0.060 day^{-1} as described above.

Reference 4:

No depuration study was performed. This study was designed to generate metabolites only.

4.1.7 Metabolites

Reference 2:

The results in Table A7.4.3.3.1.a-5 show that in both fillets and viscera, parent comprised less than 1% of the total ^{14}C-residues on Days 21 and 28.

Reference 3:

As described above in section 3.3.1, a number of procedures were employed to enhance the extraction of ^{14}C-residues. Aqueous based solvents yielded the highest extraction efficiency indicating that the metabolites are primarily polar compounds. The metabolites are equally distributed between anionic, cationic and neutral molecules. No evidence was found for glucuronide conjugation.

Reference 4:

No 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (parent), 5-chloro-2-n-octyl-4-isothiazolin-3-one, or 2-n-octyl-4-isothiazolin-3-one were detected in extracted whole fish tissue (spiking viscera with these three compounds yielded a recovery of 92%, 74%, and 74% respectively and spiking fillets with parent compound yielded a recovery of 74%).

Metabolite identification was complicated since the total residue in fish tissue was very low (*e.g.*, fillets contained 0.13 ppm total residue and viscera 0.69 ppm total residue). Typical chromatographic isolation proved an unsatisfactory method because of the massive matrix interference arising from the low residue quantity. A different approach was necessary. Viscera and fillets were extracted with water and the soluble fraction (82% in viscera and 35% in fillets) treated with trichloroacetic acid (TCA) to precipitate proteins. In viscera, 73% of the ^{14}C- soluble residue precipitated with TCA and in fillets 50%. A molecular weight distribution of the water soluble extract was undertaken using centrifugal ultrafiltration membranes. From viscera and fillets, 27% and 36% of the ^{14}C-activity, respectively, had a molecular weight greater than 100,000. Nearly all the remaining activity (*e.g.*, molecular weight of less than 100,000) had a molecular weight of less than 10,000. Thus a large majority of the ^{14}C-activity is associated with proteins that are either very large or very small.

Acid hydrolysis of the isolated protein fraction released very little of the ^{14}C-residue. Treatment with proteases solubilized practically all of the protein-associated activity. This indicates that ^{14}C-amino acid adducts were formed. Amino acid analysis indicated that the activity is primarily associated with cysteine (*e.g.*, a cysteine adduct). Additionally, treatment of the TCA precipitate with dithiothrietol (a sulfhydryl reagent) released about 20% of the ^{14}C-activity thus indicating the presence of disulfide (-S-S-) bonds.

		Insignificant ^{14}C-activity was associated with carbohydrates and lipids. Thus based on TCA precipitation, incorporation of most of the activity from fillets in water insoluble residues (fillets being primarily proteinaceous tissue), molecular weight distribution by centrifugal ultrafiltration, and amino acid analysis of TCA precipitate, the ^{14}C-activity is primarily associated with proteins and specifically as isothiazolone ring cleaved amino acid adducts. Incorporation of these adducts into a growing biopolymer would be severely restricted due to the specificity of transfer-RNA. Direct conjugation of ring cleaved metabolites with proteins would lead to inactivation and subsequent proteolytic degradation (possibly explaining the observation of high and low molecular weight fractions).	
4.1.8	Other Observations	None	
4.2	**Estimation of bioconcentration**	The bioaccumulation potential of parent compound at expected environmental concentrations is low. Expected environmental concentrations, based on preliminary monitoring and modeling are less than the measured NOEC in aquatic organisms and less than those showing biocidal effects. The log P is 2.8, indicating that bioaccumulation should be low.	x

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Initially Bluegill sunfish were dosed with ^{14}C DCOIT at 1.2 ppb (References 1-3) and water and tissue samples taken on Days 0, 0.17, 3, 7, 14, 21, and 28 (uptake phase). On day 28 fish were transferred to water free of the active substance and water and tissue samples taken on Days 29, 32, 35, 38, 42, and 49 (depuration phase). Tissue was quantitated for parent compound by TLC. The metabolites were characterized by solvent extractability, ion exchange chromatography and enzyme hydrolysis. In a separate study (Reference 4), fishes were dosed at 0.99 ppb and all the tissue sampled on Day 28 for metabolite characterization. Metabolite characterization involved precipitation with trichloroacetic acid, molecular weight determination, and amino acid analysis. U.S. EPA 40 CFR § 158, Pesticide Assessment Guidelines Subdivision N § 165-4 were followed. However, most of these studies were performed prior to implimentation the US EPA GLP guidelines but were performed in the spirit of the forth coming law.

5.2 Results and discussion

x

Experimental studies/Relevant test material specific properties

Solubility:	4.7 ppm
Volatility:	9.8×10^{-6} hPA at 25°C (vapor pressure)
Log Pow:	2.8
BCF (parent):	less than 13x in whole tissue, viscera and fillets*
BCF (total residue):	fillets, 7-200; viscera, 110-1100; whole fish, 57-660
BCF (steady state):	750
Uptake rate constant:	45 ppb in fish/ppb in water/day
Depuration rate constant:	0.060 day^{-1}
Depuration DT_{50}:	11.6 days

* DCOIT in fish is found to be less than 1% of a.r. by day 28. Taking the highest recorded ^{14}C-BCF of 1300 and multiplying it by 1% gives a parent BCF of less than 13.

5.3 Conclusion

x

The studies provided fulfil the requirement for fish bioaccumulation. At environmentally relevant concentrations, the bioaccumulation of parent compound will be significantly less than the toxicity. Parent compound is rapidly metabolized, both in the water and fish, to compounds that are orders of magnitude less toxic (rainbow trout studies) than parent compound. Thus at environmentally relevant concentrations, the active substance and its metabolites are below toxic thresholds and will have minimal effect on aquatic organisms.

5.3.1 Reliability

2-as a result of being performed prior to GLP guideline introduction.

5.3.2 Deficiencies

The studies are scientifically sound but the initial BCF studies were performed prior to the introduction of GLP guidelines. However, they were carried out in the spirit of GLP guidelines and no significant deficiencies were noted. Repeating these studies following GLP guidelines would not alter the results (especially since the log octanol:water partition coefficient is less than 3) and thus there is no justification for the sacrifice of additional vertebrates.

Document III-A / Section A7.4.3

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	19 December 2007
Materials and Methods	**Comment (3.1.4):** The water solubility of DCOIT at pH7 and 20°C is 3.47 mg/l.
Results and discussion	**Comment (4.2):** The ^{14}C-BCF values calculated from this study are quite high for a compound with a log Kow of 2.8. Also the depuration rate is slow. The latter could be seen in relation to the fact that metabolites of the active compound seem to be incorporated into protein of the fish via aminoacid adducts. **Comment (5.2):** The water solubility of DCOIT at pH7 and 20°C is 3.47 mg/l.
Conclusion	**Comment (5.3):** It can not be concluded from this test that the bioaccumulation of parent compound will be significantly less than its toxicity. The conclusion from this test is, that DCOIT together with its breakdown products has a relatively high BCF even thought the log K_{ow} is only 2.8.
Reliability	2, reliable with restrictions
Acceptability	Acceptable with the restrictions noted above
Remarks	-

Document III-A / Section A7.4.3

Section A7.4.3.3.1.a	Bioconcentration in fish-Freshwater, Bluegill Sunfish – **TABLES AND FIGURES**

Table A7.4.3.3.1.a-1: Uptake Phase Concentration in Water, Fillets, Viscera, and Whole Fish (Reference 1, 2, and 3)

Uptake Day	Concentration based on ^{14}C DCOIT Equivalents (ppb)			
	Water	Fillet	Viscera	Whole Fish
0	1.2			
0.17	0.76	6.9	110	56
1	1.0	31	370	240
3	1.5	82	810	530
7	1.1	110	910	480
14	1.4	100	1000	560
21	1.3	200	1400	800
28	1.0	230	1300	770

Table A7.4.3.3.1.a-2: Depuration phase concentration and percent depuration of ^{14}C DCOIT Equivalents in fillets, Viscera, and whole fish (Reference 1, 2, and 3).

Depuration Day	Concentration based on ^{14}C DCOIT Equivalents					
	Fillets		Viscera		Whole Fish	
	ppb	%	ppb	%	ppb	%
1	200	13	880	32	550	29
3	180	22	610	53	420	45
7	150	35	480	63	350	55
10	140	39	450	65	300	61
14	120	48	360	72	280	64
21	93	60	280	78	180	77

Table A7.4.3.3.1.a-3: Concentration (Determined by Radioanalysis) in the Water Phase from Reference 4.

Uptake Day	Concentration based on ^{14}C DCOIT Equivalents (µg/l)		
	Tank A	Tank B	Average
0	1.1	1.3	1.2
3	0.71	0.74	0.73
7	1.1	0.94	1.02
14	1.1	1.1	1.1
21	1.1	1.0	1.1
28	0.90	0.82	0.86

Table A7.4.3.3.1.a-4: Bioconcentration Factors from Reference 1, 2, and 3 for Fillets, Viscera and Whole Fish based on total ^{14}C.

Day	Bioconcentration Factor*		
	Fillets	Viscera	Whole Fish
0.17	7	110	57
1	31	380	240
3	74	730	480
7	99	818	430
14	86	860	480
21	170	1200	680
28	200	1100	660

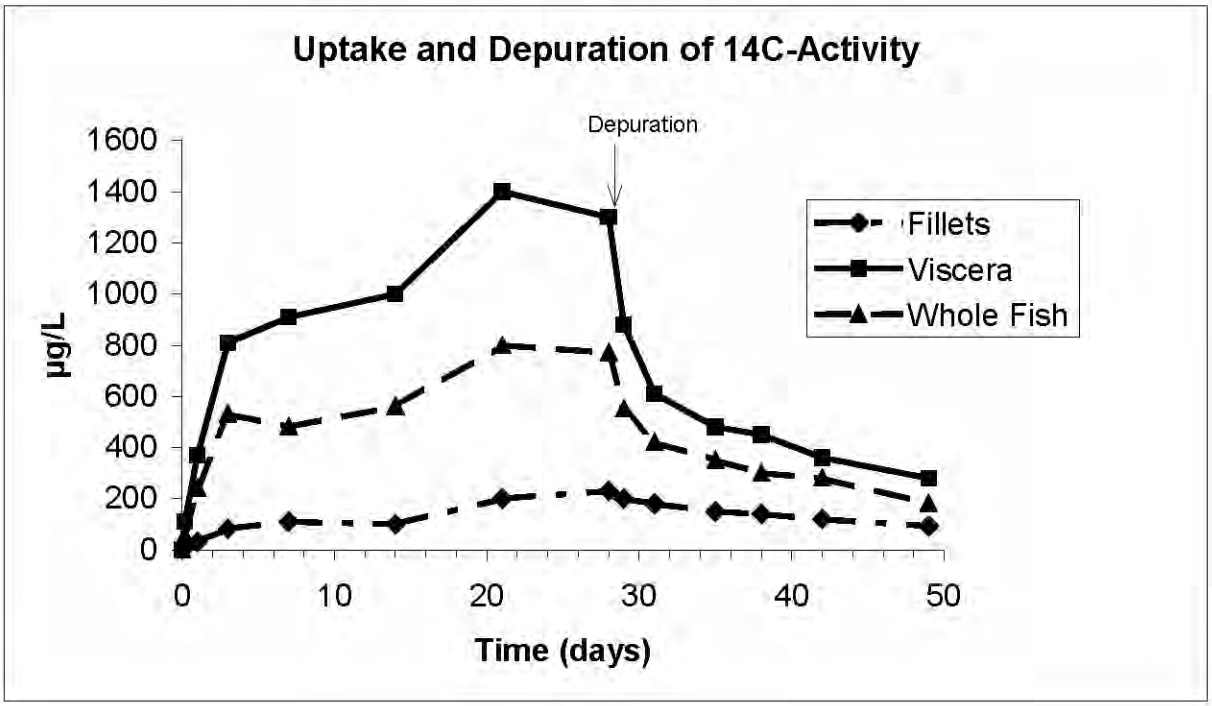
* Calculated by dividing the tissue concentrations by the mean measured water concentration of 1.16 µg/L (day 28)

Tables A7.4.3.3.1.a-5: Quantitation of parent compound in water, fillets, and viscera from Reference 2.

Day	Quantitation of ^{14}C DCOIT					
	Water		Fillets		Viscera	
	ppb	%[1]	ppb	%[1]	ppb	%[1]
21	0.03	4.5	1.4	0.67	8.1	0.62
28	0.004	0.55	0.79	0.32	6.9	0.53

[1] Percent of total ^{14}C-residues determined by radioassay

Figure A7.4.3.3.1.a-1: Uptake and Depuration of ^{14}C-Activity from Fillets, Viscera, and Whole Fish for References 1, 2, and 3.



Section A7.4.3.3.1.b Bioconcentration in fish-Freshwater, Carp

Annex Point IIIA XIII.2.3

Official use only

1 REFERENCE

1.1	**Reference**	[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**	Yes. Guidelines used were the Japanese Ministry of International Trade and Industry described in "Chemical Substance Control Law" (Japanese Law No. 117, 1974).
2.2	**GLP**	Yes per Good Laboratory Practice Regulations recognized by Basic Industries Bureau, Japanese Ministry of International Trade and Industry.
2.3	**Deviations**	No listed deviations.

3 MATERIALS AND METHODS

3.1	**Test material**	^{14}C –DCOIT (RH 287)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.3	Purity	

Section A7.4.3.3.1.b Bioconcentration in fish-Freshwater, Carp

Annex Point IIIA XIII.2.3

3.1.4	Further relevant properties	[redacted]
3.1.5	Radiolabelling	
3.1.6	Method of analysis	
3.2	**Reference substance**	
3.2.1	Method of analysis for reference substance	
3.3	**Testing/estimation procedure**	
3.3.1	Test system/ performance	
3.3.2	Estimation of bioconcentration	The log P_{ow} , 2.8 was determined by the shake flask method (U.S. EPA 40 CFR § 158, Pesticide Assessment Guidelines Subdivision D § 63-11 and OECD 107) indicating that the compound will not be a bioaccumulation hazard.

4 RESULTS

4.1 Experimental data

Document III-A / Section A7.4.3

Section A7.4.3.3.1.b Bioconcentration in fish-Freshwater, Carp

Annex Point IIIA XIII.2.3

4.1.1	Mortality/behaviour	No mortalities or abnormalities were observed in either the treatment or control aquaria during the study.
4.1.2	Lipid content	The fat content was 4.4 ± 0.3% based on measurement of 4 fishes.
4.1.3	Concentrations of test material during test	Reference 1: The nominal dosing concentrations of DCOIT were 0.02 µg/l and 0.2 µg/l. The measured concentrations of total ^{14}C-residues (DCOIT equivalents) in the water , based on radioassay ranged from 0.0132 µg/l (Day7) to 0.0256 µg/l (Day 49) for the low dose and 0.187 µg/l (Day 3 and 31) to 0.234 (Day 14) for the high dose with the averages being 0.0183 ± 0.00388 µg/l and 0.208 ± 0.0170 µg/l, respectively. The results are presented in Table A7.4.3.3.1b-1. The concentration of total ^{14}C-residues (LSC) in whole fish during the uptake phase for the low dose ranged from 3.6 ng/g to 20.4 ng/g. In the high dose it ranged from 48.9 ng/g to 170 ng/g. The results are presented in Table A7.4.3.3.1b-1 and graphically in Figure A7.4.3.3.1.b-1. Steady state uptake was reached by the end of the uptake phase. The depuration data for total ^{14}C-residues is also presented in Table A7.4.3.3.1.b-1and Figure A7.4.3.3.1.b-1. During depuration, elimination of the radiolabel by whole fish reached 35% on Day 7, 64% on Day 14, and 70% on Day 28 for the high dose, and 47% on Day 7, 75% on Day 14, and 82% on Day 28 for the low dose. The depuration half-life is 11.2 days and 16.1 days ($\ln 2/K_{depuration}$) for the low and high doses, respectively. The time for 90% depuration is 37.1 days and 53.5 days ($DT_{90}=\ln 10/K_{depuration}$) for the low and high doses, respectively. Reference 2: Water was partitioned with ethyl acetate and ^{14}C DCOIT isolated from the organic fraction by HPLC and quantitated by radioassay. The results appear in Table A7.4.3.3.1.b-2. The percent of ethyl acetate extractable radiolabeled ranged from 87.4% - 38.5% and 74.0%-34.0% in the low and high dose, respectively. The percentage of DCOIT ranged from 83.2%- 29.4% of total activity (or 95.2%-71.1% of the activity in the ethyl acetate fraction) for the low dose and 70.5% - 26.9% of the total activity (or 98.7% - 74.1% of the activity in the ethyl acetate fraction) for the high dose. Additionally, two low and high dose fish from Day 56 were dissected and the activity in various tissues was quantitated by radioassay. The results are presented in Table A7.4.3.3.1.b-3. The concentration of total ^{14}C-residues (measured by liquid scintillation counting) ranged from a low in the muscle of 5.7 or 43.1 ng/g (low and high dose, respectively) to a high of 319 or 1578.5 (low and high dose, respectively) in the gall bladder.
4.1.4	Bioconcentration factor (BCF)	The low and high dose BCF for sample Days 3, 7, 14, 28, 42, and 56 for whole fish are presented in Table A7.4.3.3.1.b-4. Also presented are the BCF's for the dissected organs (Table A7.4.3.3.1.b-3). The BCF's were determined as the ratio of ^{14}C-activity in tissue to that in water. The steady state BCF in whole fish, calculated as $K_{uptake}/K_{depuration}$, was 735 and 713 for the low and high dose respectively.
4.1.5	Uptake and depuration rate	The uptake rate constant (K_{uptake}) for the low and high dose was 45.6 and 30.7 ppb in fish/ppb in water/day, respectively. The depuration rate

	constants	constant ($K_{depuration}$) was 0.062 and 0.043 day^{-1} for the low and high dose, respectively. The rate constants were determined from the following equation: $$K_{uptake} = \frac{C_f K_d}{C_w (1 - e^{-K_d t})}$$ where C_f = concentration of test material in fish at mid-point of uptake; $K_{depuration}$ = depuration rate constant (day^{-1}). This is determined from the slope derived from linear regression of the natural log of the depuration concentration in fish versus time; C_w = mean water concentration of the test material; t = midpoint time of uptake curve. For the low dose: $$K_{uptake} = \frac{(10.3)(0.062)}{0.0194(1 - e^{(-0.062)(21)})}$$ For the high dose: $$K_{uptake} = \frac{(102.2)(0.043)}{0.196(1 - e^{(-0.043)(21)})}$$	
4.1.6	Depuration time		

	DT_{50}	DT_{90}
Low Dose	11.2 days	37.1 days
High Dose	16.1 days	53.3 days

Where $DT_{50} = \ln 2/K_d$ and $DT_{90} = \ln 10/K_d$

4.1.7	Metabolites	No metabolite identification was undertaken in this study.	
4.1.8	Other Observations	None.	
4.2	**Estimation of bioconcentration**	The bioaccumulation of parent compound at expected environmental concentrations are expected to be moderately low. This correlates with the log P_{ow} which is 2.8. Expected environmental concentrations, based on preliminary monitoring and modelling, are less than the measured NOEC in aquatic organisms and less than those that show biological effects. The LC_{50} for higher tropic level species, Mallard Duck and Bobwhite Quail, are greater than 4000 mg/kg indicating little potential for toxic bioaccumulation.	x

Section A7.4.3.3.1.b Bioconcentration in fish-Freshwater, Carp

Annex Point IIIA XIII.2.3

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The test guidelines followed were Japanese Ministry of International Trade and Industry described in "Chemical Substance Control Law" (Japanese Law No. 117, 1974).

Carp were dosed with ^{14}C DCOIT at 0.02 µg/L and 0.2 µg/L. Water samples were taken on Days -7, -2, 0. 3, 7, 10, 14, 17, 21, 24, 28, 31, 42, 45, 49, 52, and 56 (uptake phase). Tissue samples were taken on Days 3, 7, 14, 28, 42, and 56. On Day 56 the fish were transferred to water free of the active substance and tissue samples taken on depuration Days 7, 14, and 28. The water was radioassayed and parent compound quantitated by HPLC. Tissue was homogenized and radioassayed. On the last day of the uptake phase (Day 56) two fish were removed, the blood drained and the organs removed. The organs were homogenized and radioassayed.

5.2 Results and discussion

x

Experimental studies/Relevant test material specific properties

Solubility:	4.7 ppm
Volatility:	9.8×10^{-6} hPa at 25°C (vapor pressure)
Log P_{ow}:	2.8
BCF Whole Fish	Low Dose, 198 - 1126
(total residue):	High Dose, 237 - 816
Uptake rate constant:	Low Dose, 45 .6 ppb in fish/ppb in water/day
	High Dose, 30.7 ppb in fish/ppb in water/day
BCF (steady state)	High Dose, 735
	Low Dose, 713
Depuration rate constant:	Low Dose, 0.062 day^{-1}
	High Dose, 0.043 day^{-1}
Depuration DT_{50}:	Low Dose, 11.2 days
	High Dose, 16.1 days

5.3 Conclusion

x

This study is supplemental to the Bluegill Sunfish Bioaccumulation Study. ^{14}C-Activity is rapidly absorbed and depurated in carp. At environmentally relevant concentrations, the active substance and its metabolites apparently do not bioaccumulate sufficiently to be toxic (below the toxic thresholds) and will have minimal effect on aquatic organisms.

5.3.1 Reliability: 2-Valid with restrictions

5.3.2 Deficiencies: No metabolite analysis.

Document III-A / Section A7.4.3

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	19 December 2007
Materials and Methods	**Comment (3.1.4):** The water solubility of DCOIT at pH7 and 20°C is 3.47 mg/l.
Results and discussion	**Comment (4.2):** The bioaccumulation of parent compound is expected to be low out from the log K_{ow} of 2.8. However, measured BCF values based on total radioactivity (parent and metabolites) are quite high and the depuration rate is relatively slow. **Comment (5.2):** The water solubility of DCOIT at pH7 and 20°C is 3.47 mg/l.
Conclusion	**Comment (5.3):** It can not be concluded from this test that at relevant environmental concentrations, the active substance and its metabolites apparently do not bioaccumulate sufficiently to be toxic and will have minimal effect on aquatic organisms. The conclusion from this test is that DCOIT (including its breakdown products) has a relatively high BCF even thought the log K_{ow} is only 2.8.
Reliability	2, reliable with restrictions
Acceptability	Acceptable with the restrictions noted above
Remarks	-

Section A7.4.3.3.1.b	Bioconcentration in fish-Freshwater, Carp – **TABLES AND FIGURES**

Table A7.4.3.3.1.b-1: Uptake Phase Concentration in Water, Fillets, Viscera, and Whole Fish (Reference1)

Day	Concentration based on ^{14}C DCOIT (µg/L or µg/kg)			
	0.02 µg/L Dosing Level		0.2 µg/L Dosing Level	
	Water	Tissue	Water	Tissue
Uptake				
-7	0.0220		0.222	
-2	0.0210		0.213	
0	0.0199		0.224	
3	0.0159	3.6	0.187	48.9
7	0.0132	10.0	0.201	89.8
10	0.0190		0.227	
14	0.0133	10.1	0.234	125
17	0.0136		0.189	
21	0.0165		0.246	
24	0.0174		0.206	
28	0.0172	11.3	0.208	143
31	0.0254		0.187	
35	0.0190		0.205	
38	0.0183		0.192	
42	0.0194	19.7	0.196	170
45	0.0246		0.199	
49	0.0256		0.204	
52	0.0163		0.212	
56	0.0161	20.4	0.215	165
Average	0.0183±0.00388		0.208	0.208±0.0170
Depuration				
7		10.9		107
14		5.1		59.1
28		3.6		50.2

Table A7.4.3.3.1.b-2: Percent of Ethyl Acetate Extractable ^{14}C-Activity and ^{14}C DCOIT in Water (Reference 2).

Day	Percent of Total ^{14}C-Activity			
	0.2 µg/L Dosing Level		0.02 µg/L Dosing Level	
	Ethyl Acetate	DCOIT	Ethyl Acetate	DCOIT
-2	74.0	70.5	73.9	68.4
0	63.6	62.8	87.4	83.2
7	34.0	26.9	49.3	43.8
28	40.6	30.1	38.5	29.4
56	37.1	31.6	79.6	56.6

Table A7.4.3.3.1.b-3: Concentration and Bioconcentration Factor (BCF) in tissue and organs at Day 56 (Reference 2).

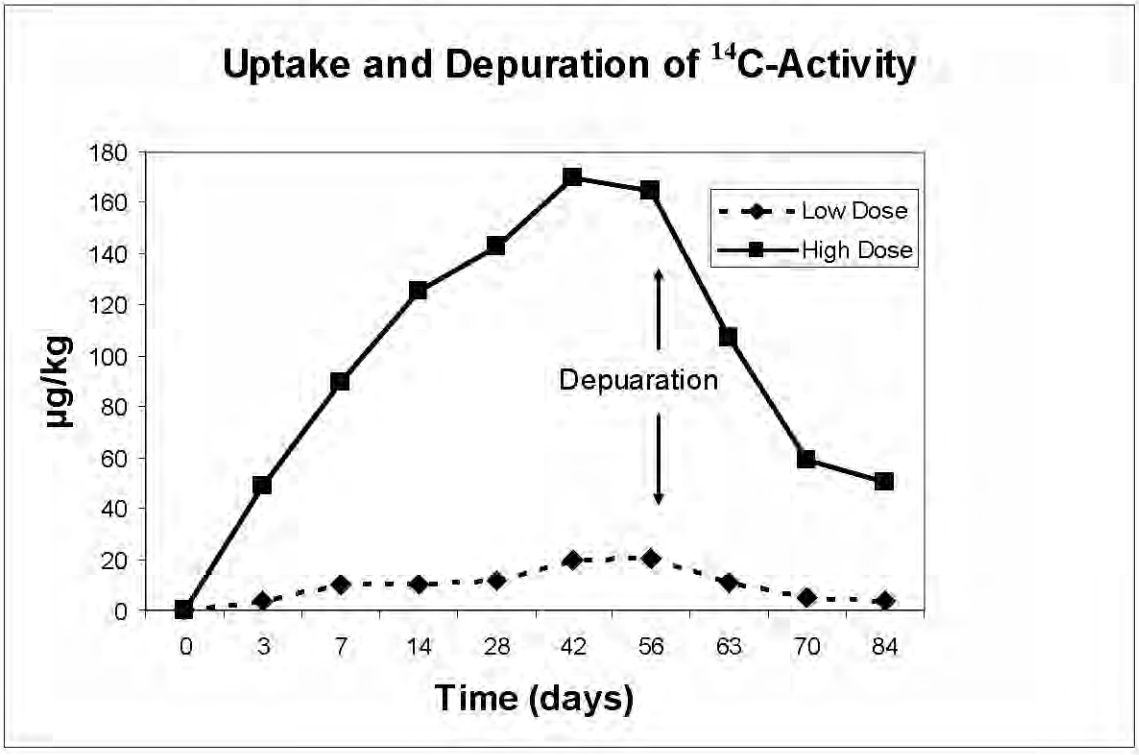
Tissue/Organ	0.02 µg/L Dose Level		0.2 µg/L Dose Level	
	Concentration (µg/kg)[1]	BCF[1]	Concentration (µg/kg)[1]	BCF[1]
Whole Fish	21	1145	148	696
Carcass	18	986	118.5	568
Skin and Scale	15.6	851	146.5	705
Muscle	5.7	313	43.1	207
Gill	124	6759	1361.5	6545
Blood	81.2	5078	592	2846
Gonad	30.9	1687	[2]	
Heart	42.5	2318	265	1275
Brain	14.6	793	95.2	458
G.I. Tract	43.6	2382	443.5	2124
Spleen	68	3712	509	2447
Kidney	153	8351	1053	5064
Gall Bladder	319	17425	1578.5	7588
Hepatopancreas	81.9	4474	695.5	2344

[1] Average of two fish
[2] Sample lost during combustion

Table A7.4.3.3.1.b-4: Bioconcentration Factor (BCF) in Fish Dosed at 0.02µg/L and 0.2 µg/L (Reference1).

Day	Bioconcentration Factor (BCF)	
	0.02 µg/L Dose Level	0.2 µg/L Dose Level
3	198	237
7	611	441
14	623	579
28	698	667
42	1126	816
56	1115	795

Figure A7.4.3.3.1.b-1: Uptake and Depuration of ^{14}C-Activity in Carp



Document III-A / Section A7.4.3

Section A7.4.3.3.2 Bioconcentration in invertebrates-Marine water, Oyster

Annex Point IIIA XIII.2.3

Official use only

1.1	**Reference**	[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**	Yes, This is a guideline study OECD305 E and U.S. EPA OPPTS 850.1710.
2.2	**GLP**	Study conducted in compliance with US EPA FIFRA GLP and OECD guidelines.
2.3	**Deviations**	The calibration of the pH meter used to measure acidity on 14 March 2000, calibration of the thermometer used during the wet/dry tissue ratio analysis, and calibration of the thermometer used to measure daily temperatures from Days 1 to 5 can not be verified. The stability of the test substances under test conditions was assumed but not verified.

3 MATERIALS AND METHODS

3.1	**Test material**	^{14}C-DCOIT(RH-287). [redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.3	Radiopurity	

Section A7.4.3.3.2 Bioconcentration in invertebrates-Marine water, Oyster

Annex Point IIIA XIII.2.3

3.1.4	Further relevant properties	[redacted]
3.1.5	Radiolabelling	
3.1.6	Method of analysis	
3.2	**Reference substance**	
3.2.1	Method of analysis for reference substance	
3.3	**Testing/estimation procedure**	
3.3.1	Test system/ performance	
3.3.2	Estimation of bioconcentration	The log P_{ow} , 2.8 was determined by the shake flask method (U.S. EPA 40 CFR § 158, Pesticide Assessment Guidelines Subdivision D § 63-11 and OECD 107) and indicates that the compound will not bioaccumulate.

Section A7.4.3.3.2 Bioconcentration in invertebrates-Marine water, Oyster

Annex Point IIIA XIII.2.3

4 RESULTS

4.1 Experimental data

4.1.1	Mortality/behaviour	Survival of oysters averaged 96-100% in each treatment and control vessel during the uptake and depuration period. No sublethal effects were observed during the bioconcentration test.
4.1.2	Lipid content	The percent lipid averaged 0.45 ± 0.12% throughout the entire 56 day bioconcentration test (uptake and depuration phases).

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Section A7.4.3.3.2 Bioconcentration in invertebrates-Marine water, Oyster

Annex Point IIIA XIII.2.3

4.1.3 Concentrations of test material during test

Radioanalysis showed the concentration in water (based on parent compound) for the 0.008 µg/L nominal dose ranged from 0.002 µg/L (Day 0, replicate 1) to 0.0039 µg/L (Day 4 after cleaning). For the 0.08 µg/L nominal dose the range was from 0.022 µg/L (Day 14, replicate 1) to 0.044 µg/L (Day 4 before cleaning). The DCOIT concentration during the uptake phase of the 0.008 µg/L dose averaged 0.0027 ± 0.0006 µg/L which represented 34% of the nomina dose. For the 0.08 µg/L dose the average DCOIT concentration during the uptake phase was 0.031 ± 0.008 µg/L which represented 39% of the nominal dose. During the uptake phase the tissue concentration ranged from 0.0080 µg/L to 0.046 µg/L for the 0.008 µg/L nominal dose and 0.079 µg/L to 0.67 µg/L for the 0.08 µg/L nominal dose. These results are in Table A7.4.3.3.2-1 and Figure A7.4.3.3.2-1. Mean tissue concentrations on Days 14, 21, and 28 were not significantly different (P = 0.05), indicating that tissue concentrations were at steady state.

Depuration of ^{14}C-activity from oysters occurred relatively slowly (Table A7.4.3.3.2-1 and Figure A7.4.3.3.2-1). As expected there was no detectable ^{14}C-activity in the water.

x

4.1.4 Bioconcentration factor (BCF)

The BCF's for the individual sampling days are:

Day	BCF (mL/g)	
	0.0027 µg/L	0.031 µg/L
7	3.67	5.0
14	12.0	5.6
21	8.1	14.7
28	16.3	15.9

The calculated steady-state BCF's are

	BCF (mL/g)	
	0.0027 µg/L	0.031 µg/L
BIOFAC	44 ± 23	19 ± 4.9
k_1/k_2	43.8	19.5
Tissue conc./Water conc.	11.1	10.6

The BIOFAC computer program (BIOFAC-PC, Dow Chemical Company, Midland, MI USA, 1991) utilizes non-linear parameter estimation methods to calculate the BCF and uptake (k_1) and depuration (k_2) rate constants. The tissue and water concentrations are the mean measured values following initiation of steady state (Days 14 through 28).

x

4.1.5 Uptake and depuration rate constants

The uptake (k_1) and depuration (k_2) rate constants were calculated using BIOFAC and are tabulated below.

	0.0027 µg/L	0.031 µg/L
k_1	570 ± 88 day^{-1}	720 ± 120 day^{-1}
k_2	13 ± 6 day^{-1}	37 ± 7 day^{-1}

Section A7.4.3.3.2 Bioconcentration in invertebrates-Marine water, Oyster

Annex Point IIIA XIII.2.3

4.1.6	Depuration time	Depuration of ^{14}C RH-287 from exposed oysters occurred slowly. The DT_{50} and DT_{90} for depuration are tabulated below.

	0.0027 µg/L	0.031 µg/L
DT_{50} (days)	42	15.6
DT_{90} (days)	83.2	32

The above kinetics were calculated from a two variable trend line analysis and the DT_{50} and DT_{90} estimated from the resulting linear equation. The correlation coefficient and slope for the 0.0027 µg/L treatment is -0.7194 and -0.00038, respectively. The correlation coefficient and slope for the 0.031 ug/L treatment is -0.9247 and -0.012, respectively.

4.1.7	Metabolites	No metabolites were identified because the BCF was significantly less than 100. Additionally, total residue levels were less than 1 ppb making metabolite identification essentially impossible.
4.1.8	Other Observations	None
4.2	**Estimation of bioconcentration**	With the BCF for total ^{14}C residues being less than 100, the bioaccumulation potential of parent compound and metabolites at expected environmental concentrations is low. Expected environmental concentrations, based on preliminary monitoring and modeling are less than the measured NOEC in aquatic organisms and less than those showing biocidal effects. The log P is 2.8, also indicating that bioaccumulation should be low.

Document III-A / Section A7.4.3

Section A7.4.3.3.2 Bioconcentration in invertebrates-Marine water, Oyster

Annex Point IIIA XIII.2.3

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Juvenile oysters (*Crassostrea virginica*) were dosed with ^{14}C-DCOIT at nominal 0.008 µg/L and 0.08 µg/L (actual dose, 0.0027 µg/L and 0.031 µg/L) in a flow-through system. Water and tissue samples were taken on Days 0. 7, 14, 21, and 28 (uptake phase). On day 28, oysters were exposed to treatment water free of the active substance and water and tissue samples taken on Days 35, 42, 49, and 56 (depuration phase). Water samples were partitioned with hexane and quantitated by radioassay. Tissue samples were homogenized and solubilized prior to radioassay quantitation. Dissolved oxygen, pH, salinity and temperature were recorded daily. The test guidelines were OECD 305 E and US EPA OPPTS 850.1710. The study was conducted in compliance with GLP guidelines.

5.2 Results and discussion

x

Experimental studies/Relevant test material specific properties

Solubility: 4.7 ppm

Volatility: 9.8 x 10^{-6} hPa at 25°C (vapor pressure)

Log P_{ow}: 2.8

	0.0027 µg/L	0.031 µg/L
BCF (BIOFAC)	44 ± 23	19 ± 4.9
BCF (k_1/k_2)	43.8	19.5
BCF (Tissue conc./Water conc.)	11.1	10.6
Uptake rate constant (k_1)	570±88 day^{-1}	720±120 day^{-1}
Depuration rate constant (k_2)	13.6±6 day^{-1}	37±7 day^{-1}
DT_{50} (days)	42	15.6

5.3 Conclusion

The studies provided fulfil the requirement for invertebrate bioaccumulation. At environmentally relevant concentrations, the active substance and its metabolites are below toxic thresholds and will have minimal effect on aquatic organisms.

5.3.1 Reliability: 1-Valid without restrictions

5.3.2 Deficiencies: None

Document III-A / Section A7.4.3

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	19 December 2007; revised 5 August 2009
Materials and Methods	**Comment (3.1.4):** The water solubility of DCOIT at pH7 and 20°C is 3.47 mg/l.
Results and discussion	**Comment (4.1.3):** From figure 7.4.3.3.2-1 it is quite clear that the steady state uptake for tissue is not reached at day 28. This is more prominent at the higher dose level but also at the lower dose level steady state seemed not to have been reached within this time period. **Comment (4.1.4):** The result from this study is a calculated BCF based on uptake and depuration rate constants and not a steady state BCF, as steady state for uptake is not reached at day 28. **Comment (5.2):** The water solubility of DCOIT at pH7 and 20°C is 3.47 mg/l. Depuration at the low dose level does not seem to continue after day 42, indicating that ^{14}C-labelled metabolites might have been incorporated into tissues of the oysters.
Conclusion	Agree with applicant's version
Reliability	Agree with applicant's version
Acceptability	Acceptable
Remarks	The test was conducted according to the guideline and there it is explicitly required stopping the uptake phase at day 28 and in case a plateau was not reached to calculate the maximum BCF. This has been done in the study.

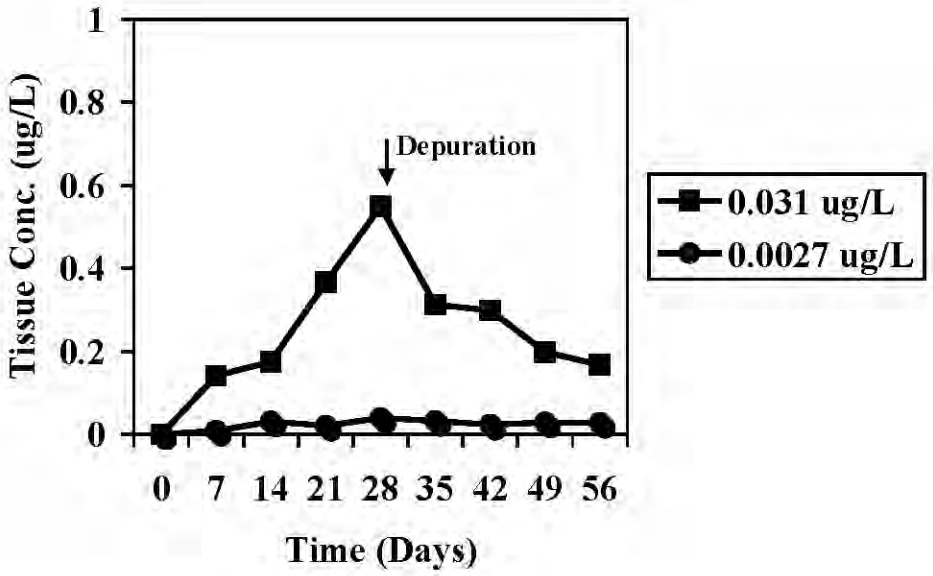
Section A7.4.3.3.2	Bioconcentration in invertebrates-Marine water, Oyster – **TABLES AND FIGURES**

Table A7.4.3.3.2-1: DCOIT Concentration in Water and Tissue during Uptake and Depuration

Study Day	Concentration based on ^{14}C DCOIT (µg/L)[1]			
	Water		Tissue	
	0.0027µg/L[2]	0.031 µg/L[3]	0.0027µg/L[2]	0.031 µg/L[3]
Uptake				
0	0.0029	0.0415	NA[4]	NA
4a[5]	0.0035	0.044	NA	NA
4b[6]	0.0039	0.034	NA	NA
7	0.00225	0.0265	0.00825	0.1325
14	0.00255	0.0260	0.0305	0.1445
21	0.0026	0.0235	0.021	0.345
28	0.0024	0.0320	0.039	0.51
Depuration				
35	<LOD[7]	<LOD	0.032	0.28
42	<LOD	<LOD	0.0235	0.275
49	<LOD	<LOD	0.0285	0.17
56	<LOD	<LOD	0.0275	0.14

[1] Results in table are the average of two aquaria per dose.
[2] Average concentration of ^{14}C-activity in water during the 28 day uptake phase. Nominal concentration was 0.008 µg/L
[3] Average concentration of ^{14}C-activity in water during the 28 day uptake phase. Nominal concentration was 0.08 µg/L
[4] NA = not applicable. No tissue quantitated at this time point.
[5] Quantitation of water prior to cleaning of aquaria.
[6] Quantitation of water after cleaning of aquaria.
[7] LOD : Limit of quantification

Figure A7.4.3.3.2-1: Uptake and depuration of ^{14}C-activity in oysters



Document III-A / Section A7.4.3

Section A7.4.3.4.a **Effects on reproduction and growth rate with an invertebrate species-Freshwater, *Daphnia magna***
Annex Point IIIA XIII.2.4

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	1 REFERENCE	
1.1	**Reference**	[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**	Yes, US EPA FIFRA 72-4
2.2	**GLP**	Yes
2.3	**Deviations**	Yes. Reproductive output of adult daphnids in the control was less than the guideline-required minimum. Additionally, despite the very fast turnover rate, measured test concentrations were > 30 % lower than nominal.
	3 METHOD	
3.1	**Test material**	RH-287 Technical
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	96.9% DCOIT
3.1.4	Composition of Product	[redacted]
3.1.5	Further relevant properties	[redacted]
3.1.6	Method of analysis	[redacted]
3.2	**Preparation of TS solution for poorly soluble or volatile**	[redacted]

Section A7.4.3.4.a **Effects on reproduction and growth rate with an invertebrate species-Freshwater, *Daphnia magna***
Annex Point IIIA XIII.2.4

	test substances	[redacted]
3.3	**Reference substance**	
3.4	**Testing procedure**	
3.4.1	Dilution water	
3.4.2	Test organisms	
3.4.3	Handling of offspring	
3.4.4	Test system	
3.4.5	Test conditions	see table A7.4.3.4.a/01-5
3.4.6	Duration of the test	21 days
3.4.7	Test parameter	[redacted]
3.4.8	Examination / Sampling	
3.4.9	Monitoring of TS concentration	
3.4.10	Statistics	

4 RESULTS

4.1	**Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal (µg DCOIT/L): 1.2, 1.9, 3.2, 4.8, 8.0
4.2.2	Actual concentrations of test substance	Please see results of the analytical measurements in Table A7.4.3.4.a/01-6.

mean measured	% of nominal	range (µg DCOIT/L)

Section A7.4.3.4.a **Effects on reproduction and growth rate with an invertebrate species-Freshwater, *Daphnia magna***
Annex Point IIIA XIII.2.4

		0.63	52	0.42 – 0.90
		1.1	58	0.43 – 1.6
		1.8	56	1.3 – 2.8
		3.1	64	1.3 – 4.1
		5.4	68	2.3 – 6.7

4.2.3	Effect data	No sublethal effects were noted during the test. See Table A7.4.3.4.a/01-7.
4.2.4	Concentration / response curve	Not described in report
4.2.5	Other effects	Not applicable
4.3	**Results of controls**	Mean control and solvent control survival was 92.5% after 21 d. First generation control daphnids produced an average of 19 young/surviving female. First generation solvent control daphnids produced an average of 44 young/surviving female.
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	US EPA FIFRA 72-4, Aquatic invertebrate life-cycle studies with analytical confirmation of TS concentrations.
5.2	**Results and discussion**	First generation control daphnids produced an average of 19 young/surviving female. First generation solvent control daphnids produced an average of 44 young/surviving female. The controls did not produce well because of the rapid turnover rate that was required to maintain the concentrations of the TS. This turnover rate reduced the concentration of available food, but increasing the feeding rate would have served to increase the biodegradation of test substance. The adequate production by the solvent controls is believed to have resulted from the increased productivity of the microbial community from the presence of TEG. Insoluble material was not observed in any test vessels during the test.
5.2.1	NOEC	0.63 µg DCOIT/L, first generation survival, number of young produced-total per replicate (treatment versus both controls) x 1.1 µg DCOIT/L, number of young produced-mean per surviving female (treatment versus both controls) 1.8 µg DCOIT/L, dry weight of first generation
5.2.2	LOEC	1.1 µg DCOIT/L, first generation survival, number of young produced-total per replicate (treatment versus both controls) 1.8 µg DCOIT/L, number of young produced-mean per surviving female (treatment versus both controls)
5.2.3	EC_{50}	1.2 µg DCOIT/L, first generation survival
5.2.4	MATC	0.83 µg DCOIT/L, first generation survival, number of young produced-total per replicate (treatment versus both controls) 1.4 µg DCOIT/L, number of young produced-mean per surviving female (treatment versus both controls)

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Section A7.4.3.4.a Annex Point IIIA XIII.2.4	Effects on reproduction and growth rate with an invertebrate species-Freshwater, *Daphnia magna*

5.3	**Conclusion**	see table A7.4.3.4.a/01-8	x
5.3.1	Reliability	(2), reliable with restrictions. Reproductive output of adult daphnids in the control was less than the guideline-required minimum apparently because of the lack of an adequate food supply. This was due to the rapid rapid turn over rate required to maintain the concentration of test substance. Additionally, despite the very fast turn over, measured test concentrations were > 30 % lower than nominal. Therefore the chronic *Daphnia magna* study was repeated (Rohm and Haas report N°01RC-0138). This repeat study was not successful for the same reasons that created problems in the first study. Reduced food availability due to the necessary high diluter turnover rate led to a few adult daphnid mortalities in the controls, on the last day of the study (day 21), which lowered survival to 78 % in the control and 80 % in the solvent control. Additionally, analytical results from the second study suggested that low measured test substance concentrations were likely due to increased bioadsorption resulting from efforts to provide sufficient food to counteract the fast turnover rate. The study being unvalid due to the control mortality, reproduction data were not analyzed statistically. It is summarized as a non key study (see Appendix II, A7.4.3.4.a/02 and A7.4.3.4.a/03).	
5.3.2	Deficiencies	Yes, see above.	

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	19 December 2007
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (5.2.1):** Due to the low reproduction in the control no NOEC reproduction can be derived from this test but only a NOEC first generation survival.

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Conclusion	**Comment (5.3):** A NOEC reproduction can not be established from this test due to insufficient reproduction of the control daphnids. The test has been accepted nevertheless because the applicant tried already once to repeat the test and faced the same problems as in the current test. It seems that it is technically not possible to conduct this test. The turnover rate was high to maintain adequate test substance concentrations. This high turnover rate leads to insufficient reproduction of the controls and could not prevent biodegradation of DCOIT in the test system. Moreover, animals in this test were feeded with algae and DCOIT is known to react with algae and thus disappears from the test system. In the repeated test the turnover rate was reduced; however, concentrations of DCOIT were not kept ≥ 80% of nominal, the total number of young produced in the control was not satisfactory and there were 22% mortality in the control after 21 days. The NOEC survival of first generation is 0.63 µg/l.
Reliability	2, reliable with restrictions
Acceptability	Acceptable with the restrictions noted above
Remarks	-

Section A7.4.3.4.a	Effects on reproduction and growth rate with an invertebrate species-Freshwater, *Daphnia magna* – **TABLES AND FIGURES**

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Table A7.4.3.4.a/01-5: Test conditions

Criteria	Details
Test temperature	20.4 ± 0.4 °C
Dissolved oxygen	> 75% saturation
pH	8.3 ± 0.1
Adjustment of pH	Not described
Conductivity	1400 ± 100 µmhos/cm
Aeration of dilution water	Yes
Quality/Intensity of irradiation	10 $Es^{-1}m^{-2}$
Photoperiod	16 h light:8 h dark with 15 minute transition periods

Table A7.4.3.4.a/01-6: Analytical measurements

Dose	Rep	0	7	8	15	21	Mean +/- SD	% of Nominal
0.0 Control	1	ND	ND	ND	ND	ND	-	-
	2	ND	ND	ND	ND	ND	-	-
0.0 Solvent Control	1	ND	ND	ND	ND	ND	-	-
	2	ND	ND	ND	ND	ND	-	-
1.2	1	0.90	0.35	0.63	0.42	0.60	0.63 +/- 0.2	52
	2	0.87	0.45	0.62	0.44	0.55		
1.9	1	1.60	0.17	1.00	0.92	0.43	1.1 +/- 0.4	58
	2	1.60	0.50	1.20	0.89	1.20		
3.2	1	2.80	0.77	1.90	1.50	1.30	1.8 +/- 0.6	56
	2	2.60	1.20	1.70	1.40	1.50		
4.8	1	4.10	2.80	3.40	3.00	3.50	3.1 +/- 0.9	64
	2	4.00	2.00	2.70	2.90	1.30		
8	1	6.70	5.50	5.60	5.20	5.30	5.4 +/- 1.4	68
	2	6.60	5.80	5.90	2.30	6.00		
Diluter Stock Solution	1	7.30	6.00	6.50	6.10	7.70	6.9 +/- 0.7	86

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Table A7.4.3.4.a/01-7: Effect data

Mean measured concentration (µg DCOIT/L)	% survival at 21 days	Total number of young produced	Day of first brood	Mean young per surviving female	Average dry weight of surviving adults (mg)
0 (control)	90	676	10-11	19	0.30
0 (solvent control)	95	1687	10	44	0.26
0.63	82	720	9-14	25	0.29
1.1	62	405	9-14	18	0.38
1.8	25	84	10	7	0.30
3.1	5	64	12-14	32	0.25
5.4	0	--	--	--	--

Table A7.4.3.4.a/01-8: Conclusion

	ug a.i./L			
	NOEL	LOEL	MATC	EC50
Biological Endpoint				
First Generation Survival	0.63	1.1	0.83	1.2
Treatment v Control	0.63	1.1	0.83	
Treatment v Solvent Control	0.63	1.1	0.83	
Number of Young Produced				
1. Total per Replicate				
Treatment v Control	1.1	1.8	1.4	
Treatment v Solvent Control	<0.63	0.63	<0.63	
Treatment v both Controls	0.63	1.1	0.83	
2. Mean per Surviving Female				
Treatment v Control	1.8	>1.8	>1.8	
Treatment v Solvent Control	0.63	1.1	0.83	
Treatment v both Controls	1.1	1.8	1.4	
Dry Weight of First Generation	1.8	-	-	

Table A7.4.3.4.a/01-9: Validity criteria for invertebrate reproduction test according

	fulfilled	Not fullfilled
Mortality of parent animals < 20% at test termination	**yes**	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60		**yes**

Section A7.4.3.4.b/01 Effects on reproduction and growth rate with an invertebrate species-Marine water, Mysid

Annex Point IIIA XIII.2.4

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	1 REFERENCE	
1.1	**Reference**	[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**	Yes, US EPA OPPTS 850.1350
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 METHOD	
3.1	**Test material**	RH-287 Technical
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	100.3%
3.1.4	Composition of Product	[redacted]
3.1.5	Further relevant properties	[redacted]
3.1.6	Method of analysis	[redacted]
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	[redacted]
3.3	**Reference substance**	[redacted]

Section A7.4.3.4.b/01 **Effects on reproduction and growth rate with an invertebrate species-Marine water, Mysid**
Annex Point IIIA XIII.2.4

3.4	**Testing procedure**	
3.4.1	Dilution water	[redacted]
3.4.2	Test organisms	
3.4.3	Handling of offspring	
3.4.4	Test system	
3.4.5	Test conditions	see table A7.4.3.4.b/01-5
3.4.6	Duration of the test	28 days
3.4.7	Test parameter	[redacted]
3.4.8	Examination / Sampling	
3.4.9	Monitoring of TS concentration	
3.4.10	Statistics	
	4 RESULTS	
4.1	**Range finding test**	Performed
4.1.1	Concentrations	0 control and solvent control, 0.70, 1.5, 2.4, 5.0 and 10.0 µg/L
4.1.2	Number/ percentage of animals showing adverse effects	After 17 d of exposure, there was at least 80% survival at 0 µg/L, 100% survival at 0.70 and 1.5 µg/L, 90% survival at 2.4 and 5.0 µg/L and 0% survival at 10.0 µg/L.
4.1.3	Nature of adverse	No sublethel effects in surviving mysids. Gravid females were observed

Section A7.4.3.4.b/01
Annex Point IIIA XIII.2.4

Effects on reproduction and growth rate with an invertebrate species-Marine water, Mysid

	effects	in test vessels containing 0, 0.7, 1.5 and 2.4 µg/L
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal µg/L: 0.47, 0.91, 1.8, 3.5, 7.0
4.2.2	Actual concentrations of test substance	mean measured µg/L: 0.277, 0.627, 1.24, 2.39, 4.97, concentrations were consistent throughout the test Analytical results are presented in Table A7.4.3.4.b/01-6.
4.2.3	Effect data	Number of offspring produced by first generation mysids: LOEC = 2.39 µg/L NOEC = 1.24 µg/L MATC = 1.72 µg/L Survival of second generation mysids, sublethal effects, length and weight of first and second generation mysids: LOEC = 4.97 µg/L NOEC = 2.39 µg/L MATC = 3.45 µg/L 7 d LC_{50} > 4.97 µg/L 14 d LC_{50} = 4.1 µg/L (95% CI = 2.39 to 4.97 µg/L) 21 d LC_{50} = 3.4 µg/L (95% CI = 2.39 to 4.97 µg/L) 28 d LC_{50} = 2.5 µg/L (95% CI = 0.627 to 4.97 µg/L) Deaths of first generation mysids: control – 1 day 7, 1 day 17, 1, day 18, 1 day 20, 2 day 23, 1 day 28 solvent control – 1 day 17, 2 day 18, 2 day 20, 3 day 23 0.277 µg/L – 1 day 5, 2 day 16, 2 day 18, 1 day 23, 1 day 25 0.627 µg/L – 1 day 17, 1 day 21, 2 day 22, 2 day 23, 2 day 24, 1 day 25, 2 day 26, 1 day 28 1.24 µg/L – 1 day 10, 2 day 19, 1 day 20, 21 and 22, 2 day 23, 1 day 24, 2 day 25, 2 day 26, 3 day 27, 2 day 28 2.39 µg/L – 1 day 10, 1 day 17, 1 day 19, 5 day 22, 1 day 24, 5 day 25, 3 day 26, 1 day 27 4.97 µg/L - 7 day 3, 4 day 5, 2 day 7, 1 day 10, 9 day 12, 5 day 14. 4 day 17, 5 day 18, 1 day 20 Number of young produced at test termination: control – 59 solvent control – 73 0.277 µg/L – 67 0.627 µg/L – 67 1.24 µg/L – 48

2.39 µg/L – 24
4.97 µg/L – 0

0.0 Control	Number of Surviving Females Days 14 - 28	12.27
	Total Number of Offspring Days 14 - 28	59
	Average Number of Offspring per Female	4.81
0.0 Solvent Control	Number of Surviving Females Days 14 - 28	14.0
	Total Number of Offspring Days 14 - 28	73
	Average Number of Offspring per Female	5.2
Combined Controls	Number of Surviving Females Days 14 - 28	26.27
	Total Number of Offspring Days 14 - 28	132
	Average Number of Offspring per Female	5.0

4.2.4	Concentration / response curve	See Figure A7.4.3.4.b/01-1.	
4.2.5	Other effects	No survival and sublethal effects observed at highest test concentration, 4.97 µg/L, at first generation on day 21 and 12, respectively. Sublethal effects included: lethargic, visually smaller than controls, swimming erratically and/or immobilized.	
4.3	**Results of controls**	Mean control and solvent control survival at the end of the test were 83 and 80%, respectively. Each control and solvent control replicate produced offspring. Mean offspring production was 4.7 young per female in the control and 5.3 young per female in the solvent control and 100% of the retained offspring survived to the end of the test. No sublethal effects were noted in the control or solvent control during the test.	x
4.4	**Test with reference substance**	Not performed	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	US EPA OPPTS 850.1350, Flow-through chronic toxicity to estuarine invertebrates with analytical confirmation of TS.	
5.2	**Results and discussion**	No insoluble test material was observed during the test.	x
5.2.1	NOEC	0.627 µg/L, based on most sensitive parameter, survival of first generation mysids	
5.2.2	LOEC	1.24 µg/L, based on most sensitive parameter, survival of first generation mysids	
5.2.3	MATC	0.882 µg/L, based on most sensitive parameter, survival of first generation mysids	
5.2.4	EC_{50}	2.5 µg/L (28 days)	
5.3	**Conclusion**	see table A7.4.3.4.b/01-6	x
5.3.1	Reliability	(1), reliable without restriction	x

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Section A7.4.3.4.b/01
Annex Point IIIA XIII.2.4

Effects on reproduction and growth rate with an invertebrate species-Marine water, Mysid

5.3.2	Deficiencies	No	x

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	19 December 2007
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (4.3):** Mean offspring production was 4.7 young per female in the control and 5.3 young per female in the solvent control. However, the validity criterion of OPPTS 850.1350 guideline is that the average number of young produced per female in the controls should not be less than three per day. This criterion can therefore not be considered fulfilled. It is recognised that this requirement might be difficult to achive. A figure of 2.5 offspring per female and day seems to be more realistic applying figures given in a publication by Suzanne M. Lussier (Technicques for the Laboratory Cultur of *Mysidopsis* Species (Crustacea: Mysidacea); Environmental Toxicology and Chemistry, vol 7, pp. 969-977, 1988). There it was assumed that the first brood is produced after 2 weeks, the number of offspring per brood is 7-10 and time between single broods is 5-7 days. Regarding *M. bahia* a number of 1.3 offspring/female/day was reported by Charles L. McKenny, Jr. (Diseases of Aquatic Organisms, Vol. 1: 131-139, 1986). The total number of offspring produced in the test with DCOIT is low compared to these figures and this indicates that the adults were not in a good shape in this test. **Comment (5.2):** Due to the fact that the number of offspring produced is low in this test, an establishment of a NOEC reproduction is not possible. However, the NOEC survival of first generation mysids, which was the most sensitive endpoint in this study, can be used as an endpoint from this study.
Conclusion	**Comment (5.3):** The validity criterion with respect to the production of young is not fulfilled.
Reliability	**Comment (5.3.1 and 5.3.2):** Due to the restrictions described, the reliability is changed from 1 to 2, reliable with restrictions.
Acceptability	Acceptable with the restrictions noted above
Remarks	-

Section A7.4.3.4.b/01 Annex Point IIIA XIII.2.4	Effects on reproduction and growth rate with an invertebrate species- Marine water, Mysid – **TABLES AND FIGURES**

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Table A7.4.3.4.b/01-5: Test conditions

Criteria	Details
Test temperature	25 ± 2 °C
Dissolved oxygen	6.6 to 8.0 mg/L
pH	7.6 to 8.0
Adjustment of pH	Not described
Aeration of dilution water	Yes
Quality/Intensity of irradiation	cool white fluorescent lights, 32 footcandles
Photoperiod	14 h light and 10 h dark with 15 minute transition periods

Table A7.4.3.4.b/01-6: Test conditions

Dose	Rep	0	7	8	15	21	Mean +/- SD	% of Nominal
0.0 Control	1	ND	--	--	ND	--		
	2	--	ND	--	--	ND		
	3	ND	--	ND	--	--		
	4	--	ND	--	ND	--		
	5	--	--	ND	--	ND	ND	--
0.0 Solvent Control	1	ND	--	--	ND	--		
	2	--	ND	--	--	ND		
	3	ND	--	ND	--	--		
	4	--	ND	--	ND	--		
	5	--	--	ND	--	ND	ND	--
0.47	1	0.328	--	--	0.244	--		
	2	--	0.313	--	--	0.192		
	3	0.366	--	0.308	--	--		
	4	--	0.286	--	0.237	--		
	5	--	--	0.300	--	0.192	0.277	60
0.91	1	0.685	--	--	0.586	--		
	2	--	0.656	--	--	0.536		
	3	0.673	--	0.647	--	--		
	4	--	0.660	--	0.592	--		
	5	--	--	0.696	--	0.540	0.627	69
1.8	1	1.35	--	--	1.20	--		
	2	--	1.20	--	--	1.02		
	3	1.31	--	1.40	--	--		
	4	--	1.23	--	1.20	--		
	5	--	--	1.39	--	1.09	1.24	69
3.5	1	2.68	--	--	2.39	--		
	2	--	2.57	--	--	2.13		
	3	2.55	--	2.69	--	--		
	4	--	2.34	--	1.88	--		
	5	--	--	2.74	--	1.93	2.39	68
7.0	1	4.69	--	--	4.99	--		
	2	--	5.35	--	4.61	4.32		
	3	4.41	--	5.47	4.92	--		
	4	--	5.22	--	4.85	--		
	5	--	--	5.86	--	--	4.97	71

Table A7.4.3.4.b/01-7: Validity criteria for invertebrate reproduction test

	fulfilled	Not fullfilled
Mortality of parent animals < 20% at test termination	yes	
Average number of young produced per female in the controls more than three per day		yes

Figure A7.4.3.4.b/01-1: Survival of first generation mysids, Americamysis bahia, exposed to DCOIT for 7, 14, 21 and 28 days

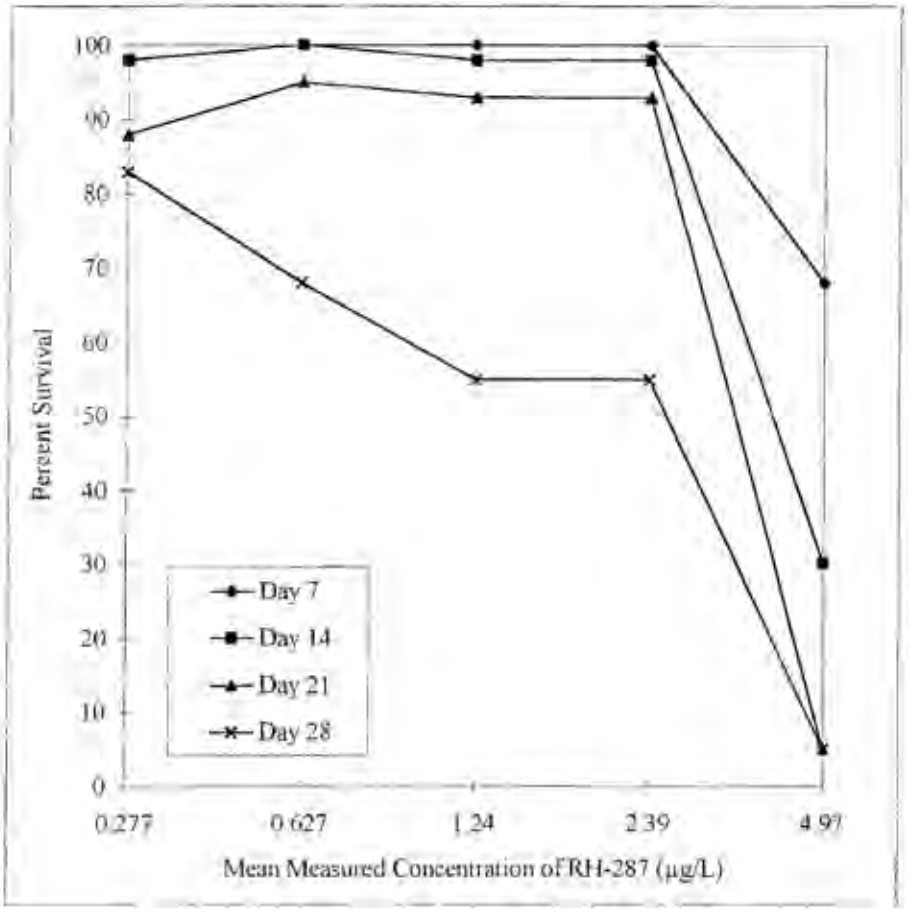

Figure 1 Survival of first generation mysids, *Americamysis bahia*, exposed to RH-287 for 7, 14, 21, and 28 days.

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Section A7.4.3.5.1.a/01 Acute toxicity to sediment dwelling organisms- Freshwater, *Chironomus tentans*

Annex Point IIIA XIII.3.4

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1 REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2	Companies with letter of access	
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, US EPA OPPTS 850.1735
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	The test substance was radiolabelled. Unlabelled DCOIT specification was as given in section 2
3.1.3	Purity	Purity ^{12}C-DCOIT: 99.3%; radiopurity ^{14}C-DCOIT : 95.8 %
3.1.4	Composition of Product	[redacted]
3.1.5	Further relevant properties	[redacted]
3.1.6	Method of analysis	[redacted]
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	[redacted]

Section A7.4.3.5.1.a/01 **Acute toxicity to sediment dwelling organisms-Freshwater, *Chironomus tentans***

Annex Point IIIA XIII.3.4

3.3	**Reference substance**	[redacted]
3.4	**Testing procedure**	
3.4.1	Dilution water	
3.4.2	Test organisms	
3.4.3	Test system	
3.4.4	Test conditions	
3.4.5	Duration of the test	
3.4.6	Test parameter	
3.4.7	Sampling	
3.4.8	Monitoring of TS concentration	
3.4.9	Statistics	

4 RESULTS

4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	6.3, 13, 25, 50 and 100 mg DCOIT/kg dry sediment.
4.2.2	Actual concentrations of test substance	TS was measured in the overlying water, the pore water and in the sediment at test initiation and termination. See table A7.4.3.5.1.a/01-8
4.2.3	Effect data	see table A7.4.3.5.1.a/01-6 and see table A7.4.3.5.1.a/01-7
4.2.4	Concentration / response curve	Not described in report

Section A7.4.3.5.1.a/01 Acute toxicity to sediment dwelling organisms-Freshwater, *Chironomus tentans*

Annex Point IIIA XIII.3.4

4.2.5	Other effects	Not applicable	
4.3	**Results of controls**	see table A7.4.3.5.1.a/01-6	
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	US EPA OPPTS 850.1735, Acute toxicity study in whole sediment to midge larvae with analytical confirmation of TS concentrations. Midges, *Chironomus tentans*, were exposed to negative control (untreated sediment), solvent control (sediment spiked with acetone), and 6.3, 13, 25, 50 and 100 mg DCOIT/kg dry sediment under flow-through conditions for 10 days.	
5.2	**Results and discussion**	The overlying water appeared clear and colourless in all test compartments at test initiation and at test termination. All water quality parameters were within acceptable limits during the test. There were a few observations of organisms on the surface of the sediment or climbing the walls of the test compartments in all treatment groups and controls, but occurrences were more frequent in the higher DCOIT treatment groups (13, 25, 50 and 100 mg/kg). There was smaller organisms after 10 days in the 25, 50 and 100 mg/kg treatment groups. Percent survival at test termination was 100, 100, 100, 73, 38, 28 and 16% in the negative control solvent control, 6.3, 13, 25, 50 and 100 mg/kg treatment groups, respectively. The ash-free dry weight of the 50 and 100 mg/kg treatment groups was statistically different ($p<0.05$) from the pooled controls.	
5.2.1	LC_0	6.3 mg DCOIT/kg dry sediment.	
5.2.2	LC_{50}	19.9 mg DCOIT/kg dry sediment with 95% confidence interval of 13 and 25 mg DCOIT/kg based on nominal concentrations	x
5.2.3	LC_{100}	Not applicable	
5.3	**Conclusion**	The no-observed-effect concentration (NOEC) was 6.3 mg DCOIT/kg dry sediment and the lowest observed effect concentration was 13 mg DCOIT/kg dry sediment, based on survival.	
5.3.1	Reliability	(1), reliable without restriction	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	19 December 2007
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (5.2.2):** Agree with applicant's version. However, the correct unit of the LC_{50} is mg ^{14}C eqv. / kg dwt and not mg DCOIT / kg dwt as DCOIT itself was not monitored during the test.
Conclusion	Agree with applicant's version
Reliability	1, reliable without restrictions
Acceptability	Acceptable
Remarks	-

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Section A7.4.3.5.1.a/01	Acute toxicity to sediment dwelling organisms-Freshwater, *Chironomus tentans* – **TABLES AND FIGURES**

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[redacted]	[redacted]

Table A7.4.3.5.1.a/01-5: Test conditions

Criteria	Details
Test temperature	22.1 to 23.6 °C
Dissolved oxygen	≥ 5.6 mg/L (66% of saturation)
pH	water = 8.0 – 8.4
Adjustment of pH	No
Aeration of dilution water	Yes, with spray nozzles
Quality/Intensity of irradiation	Fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). Light intensity at test initiation was 287 lux at the surface of the water.
Photoperiod	16 h daylight, 8 h dark with 30 minute transition periods

Table A7.4.3.5.1.a/01-6: Effect and Mortality data

Test-Substance Concentration (nominal) [1] [mg DCOIT /kg]	Number Dead Day 10	Percent Survival Day 10	Mean ash-free dry weight Day 10 (mg)
untreated control	0/80	100	1.66
acetone control	0/80	100	1.57
6.3	0/80	100	1.66
13	22/80	73	1.67
25	50/80	38	1.83
50	58/80	28	1.25
100	67/80	16	1.08

[1] specify, if TS concentrations were nominal or measured

Table A7.4.3.5.1.a/01-7: Effect data

	LC_{50} [1]	95 % c.l.	LC_0 [1]	LC_{100} [1]
10 d [mg DCOIT/kg]	19.9 (n)	NA	6.3 (n)	Not applicable

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.4.3.5.1.a/01-8: Measured concentrations of DCOIT

Overlying water

Nominal concentration DCOIT (mg/L)	Sampling time (days)	Specific activity (dpm/µg)	Total [14C] (dpm)	Measured concentration mg ^{14}C equiv / L
Background	day 0	No data	31.66	No data
	day 10	No data	32.05	
Negative control	day 0	3971	37.02	<LOQ
	day 10	3971	33.04	<LOQ
Solvent control	day 0	3971	29.06	<LOQ
	day 10	3971	30.33	<LOQ
6.3	day 0	3971	932.36	<LOQ
	day 10	3971	133.55	<LOQ
13	day 0	1925	970.84	<LOQ
	day 10	1925	118.60	<LOQ
25	day 0	1001	1005.58	<LOQ
	day 10	1001	126.21	<LOQ
50	day 0	500	635.27	0.121
	day 10	500	96.49	<LOQ
100	day 0	250	482.92	0.180
	day 10	250	64.56	<LOQ

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Pore water:

Nominal concentration DCOIT (mg/kg)	Sampling time (days)	Specific activity (dpm/µg)	Total [14C] (dpm)	mg ^{14}C equiv / L
Background	day 0	No data	31.66	No data
	day 10	No data	32.05	
Negative control	day 0	3971	26.03	<LOQ
	day 10	3971	31.32	<LOQ
Solvent control	day 0	3971	26.43	<LOQ
	day 10	3971	36.25	<LOQ
6.3	day 0	3971	45404.60	2.28
	day 10	3971	33586.90	1.69
13	day 0	1925	44992.90	4.67
	day 10	1925	34368.40	3.57
25	day 0	1001	36300.30	7.25
	day 10	1001	28636.80	5.72
50	day 0	500	24877.60	9.93
	day 10	500	16370.80	6.53
100	day 0	250	15892.40	12.7
	day 10	250	12175.40	9.71

Sediment samples:

Nominal concentration DCOIT (mg/kg)	Sampling time (days)	Specific activity (dpm/µg)	Total [14C] (dpm)	Measured concentration mg ^{14}C equiv /kg dwt	Percent of nominal
Background	day 0	No data	25.69	No data	n.a.
	day 10	No data	31.72	No data	
Negative control	day 0	3971	29.66	<LOQ	n.a.
	day 10	3971	27.00	<LOQ	
Solvent control	day 0	3971	37.04	<LOQ	n.a.
	day 10	3971	36.59	<LOQ	
6.3	day 0	3971	5418.40	5.64	89.6
	day 10	3971	5579.91	4.40	69.8
13	day 0	1925	5803.52	11.9	91.8
	day 10	1925	7272.35	14.3	110
25	day 0	1001	5406.27	23.3	93.2
	day 10	1001	6382.46	23.4	93.5
50	day 0	500	6326.86	47.6	95.3
	day 10	500	5471.51	45.9	91.9
100	day 0	250	6142.68	95.2	95.2
	day 10	250	5949.91	86.9	86.9

Table A7.4.3.5.1.a/01-9: Validity criteria

	fulfilled	Not fullfilled
Mortality of control animals <10%	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**yes***	

* only total radioactivity (^{14}C) was monitored and not parent DCOIT

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Section A7.4.3.5.1a/02 **Chronic toxicity to sediment dwelling organisms-Freshwater, *Chironomus riparius***

Annex Point IIIA XIII.3.4

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	1 REFERENCE	
1.1	**Reference**	[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**	Yes, Draft OECD Guideline 218, Biocidal Products Directive (98/8/EC) Technical Guidance Document
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	DCOIT (RH-287 Technical); ^{14}C-DCOIT
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	The test substance was radiolabelled. Unlabelled DCOIT specification was as given in section 2
3.1.3	Purity	99.3%; specific activity of the ^{14}C sample was 24.50 mCi/g, radiopurity = 96.80%.
3.1.4	Composition of Product	[redacted]
3.1.5	Further relevant properties	[redacted]
3.1.6	Method of analysis	[redacted]

Section A7.4.3.5.1a/02 **Chronic toxicity to sediment dwelling organisms-Freshwater, *Chironomus riparius***

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3.2 Preparation of TS solution for poorly soluble or volatile test substances	[redacted]
3.3 Reference substance	[redacted]
3.4 Testing procedure	
3.4.1 Dilution water	[redacted]
3.4.2 Test organisms	
3.4.3 Test system	
3.4.4 Test conditions	see table A7.4.3.5.1.a/02-5
3.4.5 Duration of the test	28 days
3.4.6 Test parameter	[redacted]
3.4.7 Sampling	
3.4.8 Monitoring of TS concentration	
3.4.9 Statistics	

4 RESULTS

4.1 Limit Test	Not performed
4.2 Results test substance	
4.2.1 Initial concentrations of test substance	0 (control), 0 (9.3 mL/kg acetone control), 5.0, 10, 20, 40, and 80 mg DCOIT/kg dry sediment
4.2.2 Actual concentrations of test substance	TS was measured in the overlying water, the pore water and in the sediment at test initiation and days 2, 7 and 28. Mean Measured Sediment Concentrations (HPLC): <MQL (control), <MQL (9.3 mL/kg acetone control), 3.09, 6.59, 14.6, 31.3, and 63.7 mg DCOIT/kg dry sediment Mean Measured Sediment Concentrations (LSC): <MQL (control), <MQL (9.3 mL/kg acetone control), 4.9, 9.7, 20, 40, and 73 mg ^{14}C

Section A7.4.3.5.1a/02 **Chronic toxicity to sediment dwelling organisms-Freshwater, *Chironomus riparius***

Annex Point IIIA XIII.3.4

		equivalents/kg dry sediment
		Details of analytical measurements can be found in Tables A7.4.3.5.1.a/02-6 and A7.4.3.5.1.a/02-7.
4.2.3	Effect data	see table A7.4.3.5.1.a/02-8 and see table A7.4.3.5.1.a/02-9
4.2.4	Concentration / response curve	Not described in report
4.2.5	Other effects	Not applicable
4.3	**Results of controls**	see table A7.4.3.5.1.a/02-8
4.4	**Test with reference substance**	Not performed
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Draft OECD Guideline 218, Biocidal Products Directive (98/8/EC) Technical Guidance Document, Chronic midge toxicity study in a sediment-water system with analytical confirmation of TS concentrations.
5.2	**Results and discussion**	
5.2.1	LOEC	see table A7.4.3.5.1.a/02-9
5.2.2	NOEC	see table A7.4.3.5.1.a/02-9
5.2.3	LC_{50}	see table A7.4.3.5.1.a/02-9
5.2.4	MATC	see table A7.4.3.5.1.a/02-9
5.3	**Conclusion**	
5.3.1	Reliability	(1), reliable without restriction
5.3.2	Deficiencies	No

x

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	28 Januray 2008
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (5.2):** For unknown reasons the lethality of *C. riparius* was higher in those replicates that were terminated after 10 days than after 28 days. Due to this fact the 10 day survival is the most sensitive endpoint. OECD Guideline 218 includes development rate as an additional endpoint to be calculated. This endpoint has not been addressed. However, analysis of the data shows that development rate is not a sensitive endpoint in this test. DCOIT did not have adverse effects on the development rate of C. riparius up to 40 mg DCOIT per kg dry sediment (nominal).
Conclusion	Agree with applicant's version
Reliability	1, reliable without restrictions
Acceptability	Acceptable
Remarks	-

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Section A7.4.3.5.1a/02	Chronic toxicity to sediment dwelling organisms-Freshwater, *Chironomus riparius* – **TABLES AND FIGURES**

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Table A7.4.3.5.1.a/02-5: Test conditions

Criteria	Details
Test temperature	19.3 to 20.0°C
Dissolved oxygen	4.9 to 9.4 mg/L (56 to 108 % saturation)
pH	overlying water = 7.58 to 8.75 sediment = 6.68
Adjustment of pH	sediment = yes, $CaCO_3$
Total hardness	overlying water = 136 to 160 mg $CaCO_3$/L
Ammonia	overlying water = 0.012 to 400 µg/L pore water = 0.015 to 4.5 µg/L
Aeration of dilution water	Yes. Aeration was provided at an initial rate of 60-100 bubbles per minute to each test chamber through a glass pipet. The pipet was inserted such that its tip was 2-3 cm from the sediment surface.
Quality/Intensity of irradiation	fluorescent
Photoperiod	16 h daylight, 8 h dark with 30 minute transition periods

Table A7.4.3.5.1.a/02-6: Analytical measured concentration: results of HPLC measurements

Mean Measured Sediment Concentrations (mg 14[C] equivalents per kg dry sediment)	Measured Concentrations as mg RH-287 Technical/kg Dry Sediment (Percent of Nominal) Based on HPLC Analysis				
	Day 0	Day 2	Day 7	Day 28	Mean
0.0 Negative Control	< MQL	--	--	< MQL	< MQL
0.0 Acetone Control	< MQL	< MQL	< MQL	< MQL	< MQL
4.9	4.59 (92)	3.76 (75)	2.39 (48)	1.62 (32)	3.09 (62)
9.7	8.54 (85)	--	--	4.64 (46)	6.59 (66)
20	16.2 (81)	--	--	13.0 (65)	14.6 (73)
40	34.3 (86)	--	--	28.2 (71)	31.3 (78)
73	70.3 (88)	69.9 (87)	65.8 (82)	48.9 (61)	63.7 (80)

Table A7.4.3.5.1.a/02-7: Analytical measured concentration: results of LSC measurements

Mean Measured Sediment Concentrations (mg 14[C] equivalents per kg dry sediment)	Measured RH-287 Technical Concentration as mg 14[C] eqivalents/kg Dry Sediment (Percent of Nominal) Based on LSC Analysis					
	Day 0	Day 7	Day 14	Day 21	Day 28	Mean
0.0 Negative Control	< MQL	< MQL	< MQL	< MQL	< MQL	< MQL
0.0 Acetone Control	< MQL	< MQL	< MQL	< MQL	< MQL	< MQL
4.9	5.0 (100)	5.1 (102)	4.7 (94)	5.3 (106)	4.2 (84)	4.9 (98)
9.7	10 (100)	9.5 (95)	10 (100)	9.7 (97)	9.4 (94)	9.7 (97)
20	20 (100)	22 (110)	20 (100)	19 (95)	20 (100)	20 (100)
40	41 (103)	40 (100)	41 (103)	38 (95)	38 (95)	40 (100)
73	80 (100)	82 (103)	68 (85)	76 (95)	57 (71)	73 (91)

Table A7.4.3.5.1.a/02-8: Effect and Mortality data

Test-Substance Concentration (nominal) [1] [mg DCOIT/kg dry sediment]	Day 10 Mean Survival [a]	Day 10 Percent Survival	Day 10 Mean ash-free dry weight (mg/animal)	Day 28 Treatment Percent Emergence	Day 28 Percent Survival
control	15.5	78	0.273	69	70
acetone control	14.8	74	0.245	70	70
5	14.8	74	0.337	81	81
10	9.3	46 *	0.312	78	78
20	4.3	21 *	0.322	45 *	45 *
40	0	0 * [b]	--- [c]	8 * [b]	8 * [b]
80	0	0 *	--- [c]	--- [c]	--- [c]

[1] specify, if TS concentrations were nominal or measured

[a] The chironomid larvae that were not found were treated as dead.

[b] No surviving larvae present within these replicates. Two replicates used for emergence had live animals.

* Statistically significant (p <0.05) reduction in the survival of this treatment as compared to the pooled control value (i.e., 76%).

[c] No live animals were collected from this replicate or treatment after 10 days of exposure.

Table A7.4.3.5.1.a/02-9: Effect data

Biological Parameter	Statistical Endpoints [a]			
	LC_{50} or EC_{50} (95% CI [b])	NOEC	LOEC	MATC
Expressed as mg DCOIT per kg Dry Sediment				
10-day Survival	8.91 (7.89 to 10.1)	3.09	6.59	4.51
10-day Ash-free Dry Weight	>14.6 (N/C [c])	14.6	>14.6	N/C
Percent Adult Emergence	16.6 (14.8 to 18.6)	6.59	14.6	9.81
Expressed as mg ^{14}C-DCOIT Equivalents per kg Dry Sediment				
10-day Survival	13 (11 to 14)	4.9	9.7	6.9
10-day Ash-free Dry Weight	>14 (N/C)	14	>14	N/C
Percent Adult Emergence	22 (20 to 25)	9.7	20	14
Expressed as µg DCOIT per Liter of Pore Water				
10-day Survival	30.9 (27.1 to 35.2)	9.46	23.3	14.8
10-day Ash-free Dry Weight	>52.3 (N/C)	52.3	>52.3	N/C
Percent Adult Emergence	60.0 (53.4 to 67.5)	23.3	52.3	34.9
Expressed as µg ^{14}C-DCOIT Equivalents per Liter of Pore Water				
10-day Survival	245 (213 to 283)	91	>91	N/C
10-day Ash-free Dry Weight	>333 (N/C)	333	>333	N/C
Percent Adult Emergence	>333 (N/C)	N/C	N/C	N/C

[a] based on mean measured concentrations
[b] CI = confidence interval.
[c] N/C – Could not be calculated.

Table A7.4.3.5.1.a/02-8: Validity criteria

	fulfilled	Not fullfilled
70% emergence in the control	**yes**	
Emergence period within days 12-23		**yes**[1]
O_2 concentration > 60 % at temperature.		**yes**[2]
pH in overlaying water 6-9	**yes**	
Water temperature should not differ by more than 1 degree	**yes**	

[1]Emergence occurred between days 17-28.

[2] O_2 saturation ranged from 56-108%

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Section A7.4.3.5.1b/01 **Chronic toxicity to sediment dwelling organisms-Marine water, *Leptocheirus plumulosus***

Annex Point IIIA XIII.3.4

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	1 REFERENCE	
1.1	**Reference**	[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**	Yes, U.S. EPA/U.S. Army Corps of Engineers Method EPA/600/R-01-020, Biocidal Products Directive (98/8/EC) Technical Guidance Document
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	DCOIT (RH-287 Technical), ^{14}C-DCOIT
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	The test substance was radiolabelled. Unlabelled DCOIT specification was as given in section 2
3.1.3	Purity	99.3%; specific activity of the sample was 24.50 mCi/g and radiopurity = 96.80%
3.1.4	Composition of Product	[redacted]
3.1.5	Further relevant properties	[redacted]
3.1.6	Method of analysis	[redacted]

Section A7.4.3.5.1b/01 Chronic toxicity to sediment dwelling organisms-Marine water, *Leptocheirus plumulosus*

Annex Point IIIA XIII.3.4

3.2 Preparation of TS solution for poorly soluble or volatile test substances	[redacted]	
3.3 Reference substance		
3.4 Testing procedure		
3.4.1 Dilution water		
3.4.2 Test organisms		
3.4.3 Test system		
3.4.4 Test conditions	see table A7.4.3.5.1.b/01-5	
3.4.5 Duration of the test	28 days	
3.4.6 Test parameter	[redacted]	
3.4.7 Sampling		
3.4.8 Monitoring of TS concentration		
3.4.9 Statistics		
4 RESULTS		
4.1 Limit Test	Not performed	
4.2 Results test substance		
4.2.1 Initial concentrations of test substance	0 (control), 0 (6.3 mL/kg acetone control), 6.0, 12, 24, 48, and 96 mg DCOIT/kg dry sediment	x
4.2.2 Actual concentrations of test substance	TS was measured in the overlying water, the pore water and in the sediment at test initiation and day 28. Mean Measured Sediment Concentrations (HPLC): <MQL (control), <MQL (6.3 mL/kg acetone control), <MQL, <MQL, 0.170, 0.452, and 0.991 mg DCOIT/kg dry sediment Mean Measured Sediment Concentrations (LSC): <MQL (control), <MQL (6.3 mL/kg acetone	x

	control), 4.6, 10, 19, 38, and 78 mg ^{14}C-DCOIT equivalents/kg dry sediment Results of the analytical measurements can be found in Tables A7.4.3.5.1b/01-6 and A7.4.3.5.1b/01-7. Mean Measured Pore water Concentrations (HPLC): <MQL (control), <MQL (6.3 mL/kg acetone <MQL, <MQL, <MQL, <MQL, and <MQL µg DCOIT Mean Measured Pore water Concentrations (LSC): <MQL (control), <MQL (6.3 mL/kg acetone control), 32 (4.6 mg ^{14}C equivalent/kg dry sediment), 124 (19 mg ^{14}C equivalent/kg dry sediment) and 539 (78 mg ^{14}C equivalent/kg dry sediment) µg ^{14}C-DCOIT equivalent/L Mean Measured overlying water Concentrations (HPLC): <MQL (control), <MQL (6.3 mL/kg acetone control), 0.291, 0.578, 1.08, 3.09, 13.2 µg DCOIT/L Mean Measured overlying water Concentrations (LSC): <MQL (control), <MQL (6.3 mL/kg acetone control), 40.9 (4.6 mg ^{14}C equivalent/kg dry sediment), 121 (19 mg ^{14}C equivalent/kg dry sediment) and 384 (78 mg ^{14}C equivalent/kg dry sediment) µg ^{14}C-DCOIT equivalent/L
4.2.3 Effect data	see table A7.4.3.5.1b/01-8 and see table A7.4.3.5.1b/01-9
4.2.4 Concentration / response curve	Not described in report
4.2.5 Other effects	Not applicable
4.3 Results of controls	see table A7.4.3.5.1b/01-8
4.4 Test with reference substance	Not performed
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	U.S. EPA/U.S. Army Corps of Engineers Method EPA/600/R-01-020, Biocidal Products Directive (98/8/EC) Technical Guidance Document, Chronic amphipod, *Leptocheirus plumulosus*, toxicity study in a sediment-water system with analytical confirmation of TS concentrations.
5.2 Results and discussion	The DCOIT recoveries from the natural sediment matrix used in the the survival and reproduction study with *Neanthes arenaceodentata* (Rohm and Haas Report N° 02RC-0052) and with *Leptocheirus plumulosus* (Rohm and Haas Report N° 02RC-0050) were much lower than the recoveries from the formulated sediment used in the survival and emergence study with *Chironomus riparius* (Rohm and Haas Report N° 02RC-0051). The differences in the particle size distribution as well as

Section A7.4.3.5.1b/01 **Chronic toxicity to sediment dwelling organisms-Marine water, *Leptocheirus plumulosus***

Annex Point IIIA XIII.3.4

		the particle size and types of organic carbon within these different matrices most likely attributed to the differing amounts of recoverable DCOIT in the sediment extracts.
5.2.1	LOEC	see table A7.4.3.5.1.b/01-9
5.2.2	NOEC	see table A7.4.3.5.1.b/01-9
5.2.3	LC_{50}	see table A7.4.3.5.1.b/01-9
5.2.4	MATC	see table A7.4.3.5.1.b/01-9
5.3	**Conclusion**	
5.3.1	Reliability	(1), reliable without restriction
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	19 January 2008
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (4.2.1):** The concentrations given here are nominal concentrations of DCOIT.
	Comment (4.2.2): Test concentrations have been measured with HPLC and LSC. From the HPLC measurements it becomes clear that parent DCOIT rapidly disappears from the test system. Measurements already at day 0 show that DCOIT concentrations have declined considerably: MQL (control), <MQL (acetone control), <MQL, 0.146, 0.289, 0.853, and 2.20 mg DCOIT/kg dry sediment.
Conclusion	**Comment (5.3):** Agree with applicant's version
Reliability	1, valid without restrictions
Acceptability	Acceptable
Remarks	-

Section A7.4.3.5.1b/01	Chronic toxicity to sediment dwelling organisms-Marine water, *Leptocheirus plumulosus* – **TABLES AND FIGURES**

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[redacted]

Table A7.4.3.5.1.b/01-5: Test conditions

Criteria	Details
Test temperature	24.2 to 25.3°C
Dissolved oxygen	4.6 to 7.4 mg/L (64 to 103 % saturation)
pH	overlying water = 7.02 to 8.31
Adjustment of pH	Not described
Salinity	20.2 to 20.7 ‰
Total hardness	Not described
Ammonia	overlying water = 0.0088 to 9.0 µg/L pore water = 0.0081 to 48 µg/L
Aeration of dilution water	Yes. Aeration was provided at an initial rate of 60-100 bubbles per minute to each test chamber through a glass pipet. The pipet was inserted such that its tip was 2-3 cm from the sediment surface.
Quality/Intensity of irradiation	fluorescent
Photoperiod	16 h daylight, 8 h dark with 30 minute transition periods

Document III-A / Section A7.4.3

Table A7.4.3.5.1.b/01-6: Analytical measured concentrations: HPLC measurements

Mean Measured Sediment Concentrations (mg 14[C] equivalents per kg dry sediment)	Measured Concentrations as mg RH-287 Technical/kg Dry Sediment (Percent of Nominal) Based on HPLC Analysis				
	Day 0	Day 2	Day 7	Day 28	Mean
0.0 Negative Control	< MQL	--	--	< MQL	< MQL
0.0 Acetone Control	< MQL	< MQL	< MQL	< MQL	< MQL
4.6	< MQL	< MQL	< MQL	< MQL	< MQL
10	0.146 (1)	--	--	< MQL	< MQL
19	0.289 (1)	--	--	< MQL	0.170 (<1)
38	0.853 (2)	--	--	< MQL	0.452 (<1)
78	2.20 (2)	0.762 (1)	0.642 (1)	0.359 (<1)	0.991 (1)

Table A7.4.3.5.1.b/01-7: Analytical measured concentrations: LSC measurements

Mean Measured Sediment Concentrations (mg 14[C] equivalents per kg dry sediment)	Measured RH-287 Technical Concentration as mg 14[C] eqivalents/kg Dry Sediment (Percent of Nominal) Based on LSC Analysis					
	Day 0	Day 7	Day 14	Day 21	Day 28	Mean
0.0 Negative Control	< MQL	< MQL	< MQL	< MQL	< MQL	< MQL
0.0 Acetone Control	< MQL	< MQL	< MQL	< MQL	< MQL	< MQL
4.6	5.9 (98)	4.7 (78)	3.8 (63)	4.4 (73)	4.3 (72)	4.6 (77)
10	11 (92)	10 (83)	8.5 (71)	10 (83)	11 (92)	10 (83)
19	22 (92)	20 (83)	19 (79)	18 (75)	18 (75)	19 (79)
38	38 (79)	40 (83)	39 (81)	37 (77)	35 (73)	38 (79)
78	84 (88)	77 (80)	76 (79)	71 (74)	81 (84)	78 (81)

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Table A7.4.3.5.1.b/01-8: Effect and Mortality data

Test-Substance Concentration (nominal) [1] [mg DCOIT/kg dry sediment]	28 Day Mean Survival	28 Day Mean Juveniles per Adult	28 Day Mean dry weight (mg/animal)	28 Day Mean Growth Rate (mg/day)	Day 28 Treatment Percent Survival
control	17.4	9.8	1.86	0.060	87
acetone control	18.2	7.8	1.73	0.056	90
6.0	17.8	9.6	1.69	0.054	89
12	15.2	10.0	1.81	0.058	76
24	12.4	9.0	1.69	0.054	62 *
48	13	5.6	1.53	0.049	65 *
96	9	7.0	1.46♣	0.046♣	45 *

* Statistically significant (p <0.05) reduction in treatment survival as compared to the pooled control value.

♣ Statistically significant (p <0.05) reduction in treatment biomass as compared to the pooled control value (only for comparisons based on pore water concentration of ^{14}C-DCOIT equivalents)

Note: Pooled control survival = 88%.

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Table A7.4.3.5.1.b/01-9: Effect data

Biological Parameter	LC_{50} or EC_{50} [a] (95% CI [b])	Statistical Endpoints NOEC [c]	LOEC	MATC
	Expressed as mg DCOIT per kg Dry Sediment			
28-day Survival	≥0.991 (N/C[d])	0.0988 [e]	0.170	0.130
Reproductive Output	>0.991 (N/C)	0.991	>0.991	N/C
Dry Weight	>0.991 (N/C)	0.991	>0.991	N/C
Growth Rate	>0.991 (N/C)	0.991	>0.991	N/C
	Expressed as mg ^{14}C-DCOIT Equivalents per kg Dry Sediment			
28-day Survival	≥78 (N/C)	10	19	14
Reproductive Output	>78 (N/C)	78	>78	N/C
Dry Weight	>78 (N/C)	78	>78	N/C
Growth Rate	>78 (N/C)	78	>78	N/C
	Expressed as µg DCOIT per Liter of Pore Water			
28-day Survival	N/C [f]	N/C	N/C	N/C
Reproductive Output	N/C	N/C	N/C	N/C
Dry Weight	N/C	N/C	N/C	N/C
Growth Rate	N/C	N/C	N/C	N/C
	Expressed as µg ^{14}C-DCOIT Equivalents per Liter of Pore Water			
28-day Survival	>539 (N/C)	32	124	63.0
Reproductive Output	>539 (N/C)	539	>539	N/C
Dry Weight	>539 (N/C)	124	539	259
Growth Rate	>539 (N/C)	124	539	259

[a] Median effect or lethal concentration was determined by trimmed Spearman-Karber method.
[b] CI = confidence interval.
[c] NOEC was determined by Dunnet's test and is presented based on mean measured concentrations
[d] N/C – Could not be calculated.
[e] Estimated value. Actual value is less than MQL (0.103 mg/kg).
[f] There were no measurable concentrations of DCOIT within these samples.

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Table A7.4.3.5.1.b/01-10: Validity criteria

	fulfilled	Not fullfilled
Neonate *L. plumulosus*, size-selected (retained between 0.25-mm and 0.6-mm screens) or age selected	yes	
Average survival of amphipods in the negative control sediment must be greater than or equal to 80% at the end of the test, with no single replicate having 60% survival or less.	yes	
Measurable growth and reproduction should be observed in all replicates of the negative control treatment.	yes	
The time-weighted average of daily temperature readings must be within +2°C of the desired temperature. The instantaneous temperature must always be within +3°C of desired temperature.	yes	
The time-weighted average of daily salinity readings must be 5‰ ±2‰ or 20‰ ± 2‰. The instantaneous salinity readings must always be 5‰ ±3‰ or 20‰ ± 3‰.	yes	

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Section A7.4.3.5.1 b/02 Chronic toxicity to sediment dwelling organisms-Marine water, *Neanthes arenaceodentata*

Annex Point IIIA XIII.3.4

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1 REFERENCE

1.1	**Reference**	[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**	Yes, American Society for Testing & Methods (ASTM) E1611 and Biocidal Products Directive (98/8/EC) Technical Guidance Document.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	DCOIT (RH-287 Technical), ^{14}C-DCOIT
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	The test substance was radiolabelled. Unlabelled DCOIT specification was as given in section 2
3.1.3	Purity	DCOIT : 99.3%; ^{14}C-DCOIT specific activity = 24.50 mCi/g, radiopurity = 96.80%.
3.1.4	Composition of Product	[redacted]
3.1.5	Further relevant properties	[redacted]
3.1.6	Method of analysis	[redacted]

Section A7.4.3.5.1 b/02 **Chronic toxicity to sediment dwelling organisms-Marine water, *Neanthes arenaceodentata***

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3.2 Preparation of TS solution for poorly soluble or volatile test substances	[redacted]
3.3 Reference substance	[redacted]
3.4 Testing procedure	
3.4.1 Dilution water	[redacted]
3.4.2 Test organisms	
3.4.3 Test system	
3.4.4 Test conditions	see table A7.4.3.5.1.b/02-5
3.4.5 Duration of the test	28 days
3.4.6 Test parameter	[redacted]
3.4.7 Sampling	
3.4.8 Monitoring of TS concentration	
3.4.9 Statistics	

4 RESULTS

4.1 Limit Test	Not performed	
4.2 Results test substance		
4.2.1 Initial concentrations of test substance	0 (control), 0 (6.3 mL/kg acetone control), 5.0, 10, 20, 40, and 80 mg DCOIT/kg dry sediment	x
4.2.2 Actual concentrations of test substance	Mean Measured Sediment Concentrations (HPLC): <MQL (control), <MQL (6.3 mL/kg acetone control), <MQL, 0.108, 0.223, 0.212, and 1.20 mg DCOIT/kg dry sediment	x

Section A7.4.3.5.1 b/02 **Chronic toxicity to sediment dwelling organisms-Marine water, *Neanthes arenaceodentata***

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	Mean Measured Sediment Concentrations (LSC): <MQL (control), <MQL (6.3 mL/kg acetone control), 4.9, 9.9, 20, 28, and 69 mg ^{14}C-DCOIT equivalents/kg dry sediment
	Analytical results can be found in tables A7.4.3.5.1.b/02-6 and A7.4.3.5.1.b/02-7.
	Mean Measured overlying water Concentrations (HPLC): <MQL (control), <MQL (6.3 mL/kg acetone control), <MQL,, 0.982, 5.48, 13.2 and 20.48 µg DCOIT/L
	Mean Measured overlying water Concentrations (LSC): <MQL (control), <MQL (6.3 mL/kg acetone control), 40.3 (4.9 mg ^{14}C-DCOIT equivalents/kg dry sediment), 122 (20 mg ^{14}C-DCOIT equivalents/kg dry sediment) and 458 (69 mg ^{14}C-DCOIT equivalents/kg dry sediment) µg ^{14}C-DCOIT equivalents/L
	Mean Measured pore water Concentrations (HPLC): <MQL (control), <MQL (6.3 mL/kg acetone control), <MQL,, <MQL, <MQL, <MQL and <MQL
	Mean Measured pore water Concentrations (LSC): <MQL (control), <MQL (6.3 mL/kg acetone control), 72 (4.9 mg ^{14}C-DCOIT equivalents/kg dry sediment), 341 (20 mg ^{14}C-DCOIT equivalents/kg dry sediment) and 1220 (69 mg ^{14}C-DCOIT equivalents/kg dry sediment) µg ^{14}C-DCOIT equivalents/L
4.2.3 Effect data	see table A7.4.3.5.1.b/02-8 and see table A7.4.3.5.1.b/02-9
4.2.4 Concentration / response curve	Not described in report
4.2.5 Other effects	Not applicable
4.3 Results of controls	see table A7.4.3.5.1.b/02-8
4.4 Test with reference substance	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods	Yes, American Society for Testing & Methods (ASTM) E1611 and Biocidal Products Directive (98/8/EC) Technical Guidance Document, Chronic *Neanthes arenaceodentata* toxicity study in a sediment-water system with analytical confirmation of TS concentrations.
5.2 Results and discussion	The DCOIT recoveries from the natural sediment matrix used in the the survival and reproduction study with *Neanthes arenaceodentata* (Rohm and Haas Report N° 02RC-0052) and *Leptocheirus plumulosus* (Rohm and Haas Report N° 02RC-0050) were much lower than the recoveries from the formulated sediment used in the survival and emergence study with *Chironomus riparius* (Rohm and Haas Report N° 02RC-0051). The differences in the particle size distribution as well as the particle

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Section A7.4.3.5.1 b/02 **Chronic toxicity to sediment dwelling organisms-Marine water, *Neanthes arenaceodentata***

Annex Point IIIA XIII.3.4

		size and types of organic carbon within these different matrices most likely attributed to the differing amounts of recoverable DCOIT in the sediment extracts.
5.2.1	LOEC	see table A7.4.3.5.1.b/02-9
5.2.2	NOEC	see table A7.4.3.5.1.b/02-9
5.2.3	LC_{50}	see table A7.4.3.5.1.b/02-9
5.2.4	MATC	see table A7.4.3.5.1.b/02-9
5.3	**Conclusion**	
5.3.1	Reliability	(1), reliable without restriction
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	18 January 2008
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (4.2.1):** The concentrations given here are nominal concentrations of DCOIT.
	Comment (4.2.2): Test concentrations in sediment have been measured with HPLC and LSC. From the HPLC measurements it becomes clear that parent DCOIT rapidly disappears from the test system. Measurements already at day 0 show that DCOIT concentrations have declined considerably: MQL (control), <MQL (acetone control), <MQL, 0.164, 0.394, 0.373, and 2.80 mg DCOIT/kg dry sediment. Therefore, results have to be calculated based on mean measured concentrations.
Conclusion	**Comment (5.3):** No LOEC can be established from this test as no effects have been seen at the highest concentration tested.
Reliability	1, reliable without restrictions
Acceptability	Acceptable
Remarks	-

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Section A7.4.3.5.1 b/02 Annex Point IIIA XIII.3.4	Chronic toxicity to sediment dwelling organisms-Marine water, *Neanthes arenaceodentata* – **TABLES AND FIGURES**

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Table A7.4.3.5.1.b/02-5: Test conditions

Criteria	Details
Test temperature	overlying water: 17.8 to 21.4°C
Dissolved oxygen	overlying water: 3.6 to 8.8 mg/L (49 to 117 % saturation)
pH	overlying water: 7.42 to 8.29
Adjustment of pH	Not described
Salinity	overlying water: 30.7 to 31.5‰
Total hardness	Not described
Ammonia	overlying water: 0.71 to 66 µg/L pore water: 0.0053 to 280 µg/L
Aeration of dilution water	Yes. Aeration was provided at an initial rate of 60-100 bubbles per minute to each test chamber through a glass pipet. The pipet was inserted such that its tip was 2-3 cm from the sediment surface.
Quality/Intensity of irradiation	fluorescent
Photoperiod	16 h daylight, 8 h dark with 30 minute transition periods

Table A7.4.3.5.1.b/02-6: Analytical measurements: result of HPLC measurements

	Measured Concentrations as mg RH-287 Technical/kg Dry Sediment (Percent of Nominal) Based on HPLC Analysis				
Mean Measured Sediment Concentrations (mg 14[C] equivalents per kg dry sediment)	Day 0	Day 2	Day 7	Day 28	Mean
0.0 Negative Control	< MQL	--	--	< MQL	< MQL
0.0 Acetone Control	< MQL	< MQL	0.603*	< MQL	< MQL
4.9	< MQL	< MQL	< MQL	< MQL	< MQL
9.9	0.164 (2)	--	--	< MQL	0.108 (1)
20	0.394 (2)	--	--	< MQL	0.223 (1)
28	0.373 (1)	--	--	< MQL	0.212 (<1)
69	2.80 (4)	1.53 (2)	0.407 (1)	< MQL	1.20 (2)

*Due to contamination and not included in statistical analysis.

Table A7.4.3.5.1.b/02-7: Analytical measurements: result of LSC measurements

Mean Measured Sediment Concentrations (mg 14[C] equivalents per kg dry sediment)	Measured RH-287 Technical Concentration as mg 14[C] eqivalents/kg Dry Sediment (Percent of Nominal) Based on LSC Analysis					
	Day 0	Day 7	Day 14	Day 21	Day 28	Mean
0.0 Negative Control	< MQL	< MQL	< MQL	< MQL	< MQL	< MQL
0.0 Acetone Control	< MQL	< MQL	< MQL	< MQL	< MQL	< MQL
4.9	5.1 (102)	5.0 (100)	4.8 (96)	4.7 (94)	4.9 (90)	4.9 (98)
9.9	11 (110)	10 (100)	9.4 (94)	9.8 (98)	9.5 (95)	9.9 (99)
20	20 (100)	21 (105)	20 (100)	19 (95)	19 (95)	20 (100)
28	28 (70)	28 (70)	28 (70)	28 (70)	27 (68)	28 (70)
69	70 (88)	72 (90)	68 (85)	68 (85)	66 (83)	69 (86)

Table A7.4.3.5.1.b/02-8: Effect and Mortality data

Test-Substance Concentration (nominal) [1] [mg DCOIT/kg dry sediment]	Day 28 Mean growth rate (mg per animal per day)	Day 28 Mean dry weight (mg per animal)	Day 28 Percent mortality
control	0.24	7.29	4
acetone control	0.26	7.87	4
5	0.24	7.14	4
10	0.23	7.09	0
20	0.26	7.88	8
40	0.24	7.19	4
80	0.24	7.19	4

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Table A7.4.3.5.1.b/02-9: Effect data

Biological Parameter	Statistical Endpoints			
	LC_{50} or EC_{50} (95% CI [a])	NOEC [b]	LOEC	MATC
	Expressed as mg DCOIT per kg Dry Sediment			
28-day Survival	>1.20 (N/C[c])	1.20	>1.20	N/C
Dry Weight	>1.20 (N/C)	1.20	>1.20	N/C
Growth Rate	>1.20 (N/C)	1.20	>1.20	N/C
	Expressed as mg ^{14}C-DCOIT equivalents per kg Dry Sediment			
28-day Survival	>69 (N/C)	69	>69	N/C
Dry Weight	>69 (N/C)	69	>69	N/C
Growth Rate	>69 (N/C)	69	>69	N/C
	Expressed as µg DCOIT per Liter of Pore Water			
28-day Survival	N/C[d]	N/C	N/C	N/C
Dry Weight	N/C	N/C	N/C	N/C
Growth Rate	N/C	N/C	N/C	N/C
	Expressed as µg ^{14}C-DCOIT equivalents per Liter of Pore Water			
28-day Survival	>1,220 (N/C)	1,220	>1,220	N/C
Dry Weight	>1,220 (N/C)	1,220	>1,220	N/C
Growth Rate	>1,220 (N/C)	1,220	>1,220	N/C

[a] CI = confidence interval.
[b] NOEC was determined by the Dunnett's test and is presented based on mean measured concentrations
[c] N/C – Could not be calculated.
[d] There were no measurable concentrations of DCOIT within these samples.

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Table A7.4.3.5.1.b/02-10: Validity criteria

	fulfilled	Not fullfilled
The emergence in the controls must be at least 70% at the end of the test (1)(6);	yes	
>/= 90% mean survival for the control animals with >/= 80% control survival for individual replicates	yes	
All test chambers must be identical	yes	
Treatments must be randomly assigned	yes	
Test organisms must be impartially or randomly assigned	yes	
A negative, reference sediment, positive, or solvent controls must be included in the testing	yes	
Test animals must be the same species and from the same population or culture	yes	
Neanthes must be less than two to three weeks post-emergence at test initiation	yes	
DO must be measured in at least one test chamber in each concentration at the beginning and end of the test	yes	
Temperature should be measured in a test chamber from each concentration daily during the test	yes	
Aeration must not be off for an extended time period such that the DO drops below 60%	yes	
The solvent concentration did not adversely affect survival or growth	yes	
The analytical method must be validated prior to initiation of the test	yes	

Section A7.4.3.5.2 Aquatic plant toxicity - Growth inhibition test, *Lemna gibba*

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Official use only

1 REFERENCE

1.1	**Reference**	[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

2.2 Guideline study	Yes, US EPA OPPTS 850.4400, OECD 221, US EPA TSCA 797.1160, US EPA FIFRA 122-2 and 123-2, EC Council Directive 67/548/EEC
2.3 GLP	Yes
2.4 Deviations	No

3 MATERIALS AND METHODS

3.2	**Test material**	DCOIT (RH-287 Technical)
3.2.1	Lot/Batch number	[redacted]
3.2.2	Specification	As given in section 2
3.2.3	Purity	98.42%
3.2.4	Composition of Product	[redacted]
3.2.5	Further relevant properties	[redacted]
3.2.6	Method of analysis	[redacted]
3.3	**Preparation of TS solution for poorly soluble or volatile test substances**	[redacted]
3.4	**Reference substance**	[redacted]
3.5	**Testing procedure**	

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Section A7.4.3.5.2 **Aquatic plant toxicity - Growth inhibition test, *Lemna gibba***

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3.5.1	Culture medium	[redacted]	
3.5.2	Test organisms	[redacted]	
3.5.3	Test system	[redacted]	x
3.5.4	Test conditions	see table A7.4.3.5.2/01-4	
3.5.5	Duration of the test	7 days	
3.5.6	Test parameter	[redacted]	
3.5.7	Sampling	[redacted]	
3.5.8	Monitoring of TS concentration		
3.5.9	Statistics		

4 RESULTS

4.1 Limit Test Not performed

4.2 Results test substance

4.2.1	Initial concentrations of test substance	Nominal (mg DCOIT/L) 0.0078, 0.016, 0.031, 0.063, 0.13, 0.25, 0.5, 1.0, 2.0	
4.2.2	Actual concentrations of test substance	measured day 0 / measured day 7 (mg DCOIT/L): 0.00454 / <0.00241 0.0118 / <0.00241 0.0218 / <0.00241 0.0467 / <0.00241 0.104 / <0.00241 0.196 / <0.00241 0.444 / 0.00932 0.632 / 0.0634 1.37 / 0.169	x
4.2.3	Growth curves	see Figure A7.4.3.5.2/01-1 and -2	x
4.2.4	Concentration /	Not described	

Section A7.4.3.5.2 **Aquatic plant toxicity - Growth inhibition test, *Lemna gibba***
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	response curve		
4.2.5	Frond count data	see table A7.4.3.5.2/01-5	
4.2.6	Effect data (growth inhibition)	see table A7.4.3.5.2/01-6	
4.2.7	Other observed effects	Not applicable	
4.3 Results of controls		Doubling time for the control was 1.8 days indicating acceptable growth in the control. No significant differences between the control and acetone control were detected and they were pooled for comparison to the DCOIT treatments.	
4.4 Test with reference substance		Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods		US EPA OPPTS 850.4400, OECD 221, US EPA TSCA 797.1160, US EPA FIFRA 122-2 and 123-2, EC Council Directive 67/548/EEC, Acute static toxicity test to duckweed with analytical confirmation of TS concentrations.	
5.2 Results and discussion		see table A7.4.3.5.2/01-6	x
5.2.1	EC_5	see table A7.4.3.5.2/01-6	
5.2.2	EC_{50}	see table A7.4.3.5.2/01-6	
5.2.3	EC_{90}	see table A7.4.3.5.2/01-6	
5.3 Conclusion		the test compound was not stable in the test media following a 7-day exposure	x
5.3.1	Reliability	(1), valid with restrictions	x
5.3.2	Deficiencies	No	x

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	8 January 2008
Materials and Methods	**Comment (3.5.5):** A semi-static or flow-through test design should have been chosen as it is known that DCOIT is not stable under aquatic ecotoxicity testing.
Results and discussion	**Comment (4.2.3):** The effect of exposure period on the endpoints frond number and area under growth curve is better visualized by growth curves plotted on an arithmetic rather than a logarithmic scale as shown in figure -2. This is because the endpoints "frond number" and "area under growth curve" do not account for the fact that the growth is exponential. For this reason the relative difference in frond number that are observed after 3 days almost the same as after 7 days, and calculations of these endpoints after 3 days are not essential. **Comment (4.2.4):** Test substance concentrations should have been monitored more closely as it is known that DCOIT rapidly disappears from aquatic test systems. **Comment (5.2):** Figure -1 shows that the control cultures grow exponentially during the entire test period, while the cultures exposed to DCOIT are inhibited mainly during the first three days of the test. The cultures with the highest concentration (nominal 1.37 mg/l) grew at the same rate as the controls between day 3 and 7 (when plotted against on a logarithmic scale straight curves indicate exponential growth and the growth rate is proportional to the slope of the lines). This shows clearly the effect of the disappearance of the test material from the solutions. Since the growth inhibiting effect is declining during the exposure period, the calculations of the endpoints are based on the initial phase of the test, in this case day 0-3. Although the analysis of the data after 7 days did not show a significant difference between the control and solvent control, the increase in frond numbers after 3 days was significantly different between the control and the solvent control. Therefore the data for day 3 should be compared to the solvent control. The calculation of the effect values for the end points frond number and frond weight are not strongly depending on the time period for which they have been calculated. However, the effect can be seen as a result of the exposure during the first three days. The NOEC for frond weight was higher than for frond number. This depends, however, on the definition of the term NOEC. The estimation of the NOEC by hypothesis testing is somewhat in conflict with a basic role of the scientific method, because there is an attempt to "prove" a null hypothesis of no effect. More correctly, the LOEC is estimated as the lowest concentration showing a significant effect and the NOEC is then defined as the test concentration below the LOEC. With this approach, the NOEC for reduction of frond weight in the Lemna test becomes 4.54 µg/l. This is because the nominal concentration 11.8 µg/l showed a significant, 15 % reduction of frond weight, while at the next higher concentration, 21.8 µg/l the reduction was only 13 %, and not significant according to the statistical calculation done in this test. According to Dunnett's test, the difference from the control was not statistically significant at 21.8 µg/l. However, with William's test all concentrations apart from the lowest are significantly different compared to the control; It seems therefore justified to take 4.5 µg/l as the NOEC for the end point frond weight even if the biological relevance of this NOEC value is somewhat uncertain because of the irregular response pattern.

Document III-A / Section A7.4.3

Conclusion	**Comment (5.3):** The scientifically preferred endpoint growth rate, calculated for the exposure period 0-3 days, is considered to be the most relevant endpoint from this study. This results in an EC50 of 0.206 mg/l and a NOEC of 0.00454 mg/L based on initial measured concentrations.
Reliability	**Comment (5.3.1 and 5.3.2):** Due to the restrictions described, the reliability is changed from 1 to 2, reliable with restrictions.
Acceptability	Acceptable with the restrictions noted above.
Remarks	As in the algae tests most of the observed effecst occur within the initial phase of the test, and the differences in frond numbers or weight observed after 7 days are mainly due to growth inhibition in the initial phase of the test. The endpoints are all estimated based on intial measured concentrations. An alternative presentation, suggested by OECD in case test concentrations are declining during the test, is to use the geometric mean concentrations during the exposure period. However, it would not be correct to express the NOECs as geometric mean concentrations over 7 days since the differences seen between the treatments are mainly due to effects of the initial exposure.

Section A7.4.3.5.2	Aquatic plant toxicity-Growth inhibition test *Lemna gibba* – TABLES AND FIGURES

[redacted]

[redacted]

[redacted]

Table A7.4.3.5.2/01-4: Test conditions

Criteria	Details
Test temperature	23.4 to 24.1 °C
pH	4.92 to 6.03
Aeration of dilution water	Not described
Light intensity	9435 ± 149 lux
Photoperiod	continuous "warm-white" fluorescent light

Table A7.4.3.5.2/01-5: Frond count data

Day 0 measured concentrations (mg DCOIT/L)	Day 3 normal fronds	Day 5 normal fronds	Day 7 normal fronds	Day 7 Treatment mean of normal fronds	Percent Difference (compared to pooled control)
control	160	387	824	275	---
acetone control	181	430	902	301	---
pooled control	---	---	---	288	---
0.00454	164	396	840	280	-3
0.0118	148	362	764	255	-11
0.0218	140	358	694	231	-20
0.0467	146	306	581	194	-33
0.104	140	272	504	168	-42
0.196	126	233	396	132	-54
0.444	90	195	357	119	-59
0.632	69	169	354	118	-59
1.37	60	148	297	99	-66

Document III-A / Section A7.4.3

Table A7.4.3.5.2/01-6: Effect data

RESULTS FOR NUMBER OF NORMAL FRONDS AS COMPARED TO VEHICLE CONTROL (1-TAILED DUNNETT'S TEST)

Day 3 Growth Parameter	EC Type	EC Value (mg a.i./L)	95% Confidence Limits (mg a.i./L)	NOEC (mg a.i./L)
Normal Frond Number*	EC_5	0.00612	0.000539-0.0117	0.00450
	EC_{50}	0.438	0.354-0.523	
	EC_{90}	>1.37	---	
Area Under the Growth Curve*	EC_5	0.0210	0.000671-0.0353	0.00454
	EC_{50}	0.206	0.164-0.249	
	EC_{90}	1.13	0.723-1.54	
Growth Rate*	EC_5	0.0454	0.0180-0.0728	0.00454
	EC_{50}	0.336	0.280-0.393	
	EC_{90}	>1.37	---	

* Significant difference between control & vehicle control

"---" Indicates value could not be estimated.

Day 5 Growth Parameter	EC Type	EC Value (mg a.i./L)	95% Confidence Limits (mg a.i./L)	NOEC (mg a.i./L)
Normal Frond Number	EC_5	<0.00454	---	
	EC_{50}	0.310	0.244-0.376	0.00454
	EC_{90}	>1.37	---	
Area Under the Growth Curve*	EC_5	<0.00454	---	
	EC_{50}	0.172	0.134-0.211	0.00454
	EC_{90}	>1.37	---	
Growth Rate	EC_5	<0.00454	---	
	EC_{50}	1.15	0.786-1.51	0.0218
	EC_{90}	>1.37	---	

* Significant difference between control & vehicle control

"---" Indicates value could not be estimated.

Day 7 Growth Parameter	EC Type	EC Value (mg a.i./L)	95% Confidence Limits (mg a.i./L)	NOEC (mg a.i./L)
Normal Frond Number	EC_5	<0.00454	---	
	EC_{50}	0.203	0.152-0.255	0.0118
	EC_{90}	>1.37	---	
Area Under the Growth Curve*	EC_5	<0.00454	---	
	EC_{50}	0.162	0.129-0.194	0.00454
	EC_{90}	>1.37	---	
Growth Rate	EC_5	<0.00454	---	0.0118

* Significant difference between control & vehicle control

"---" Indicates value could not be estimated.

Figure A7.4.3.5.2/01-1: Growth curves for Duckweed, Lemna gibba, during a 7-day exposure to DCOIT

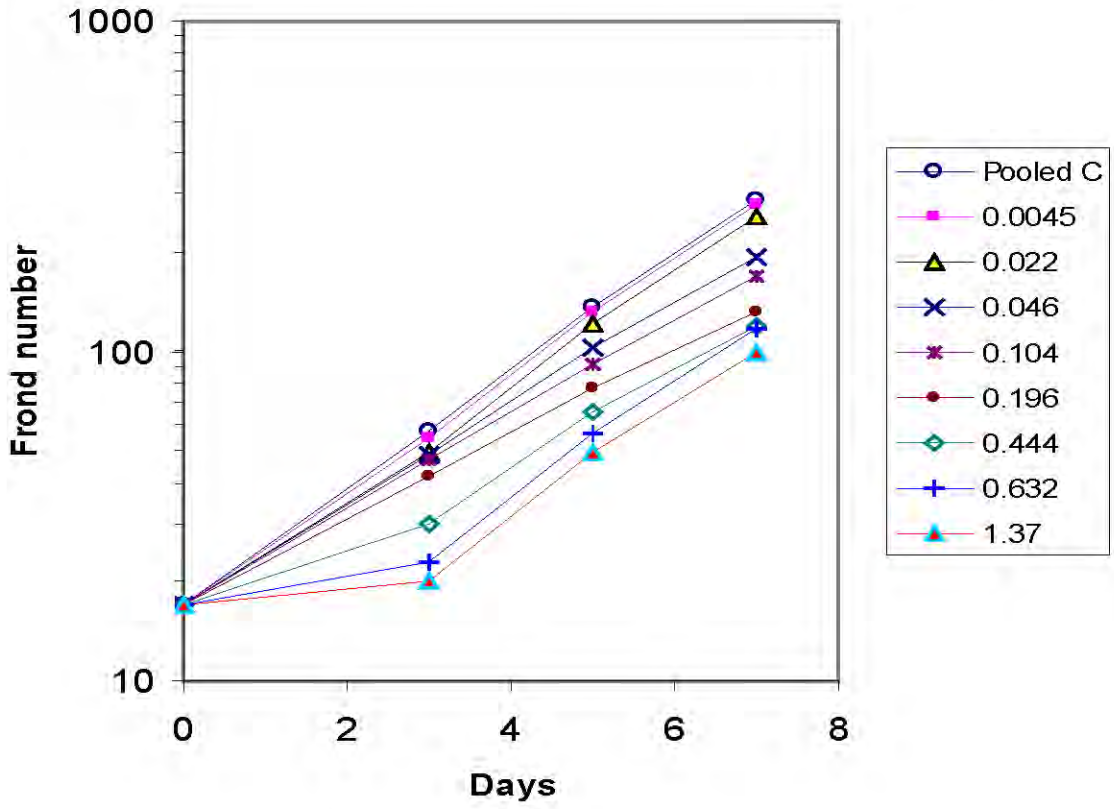
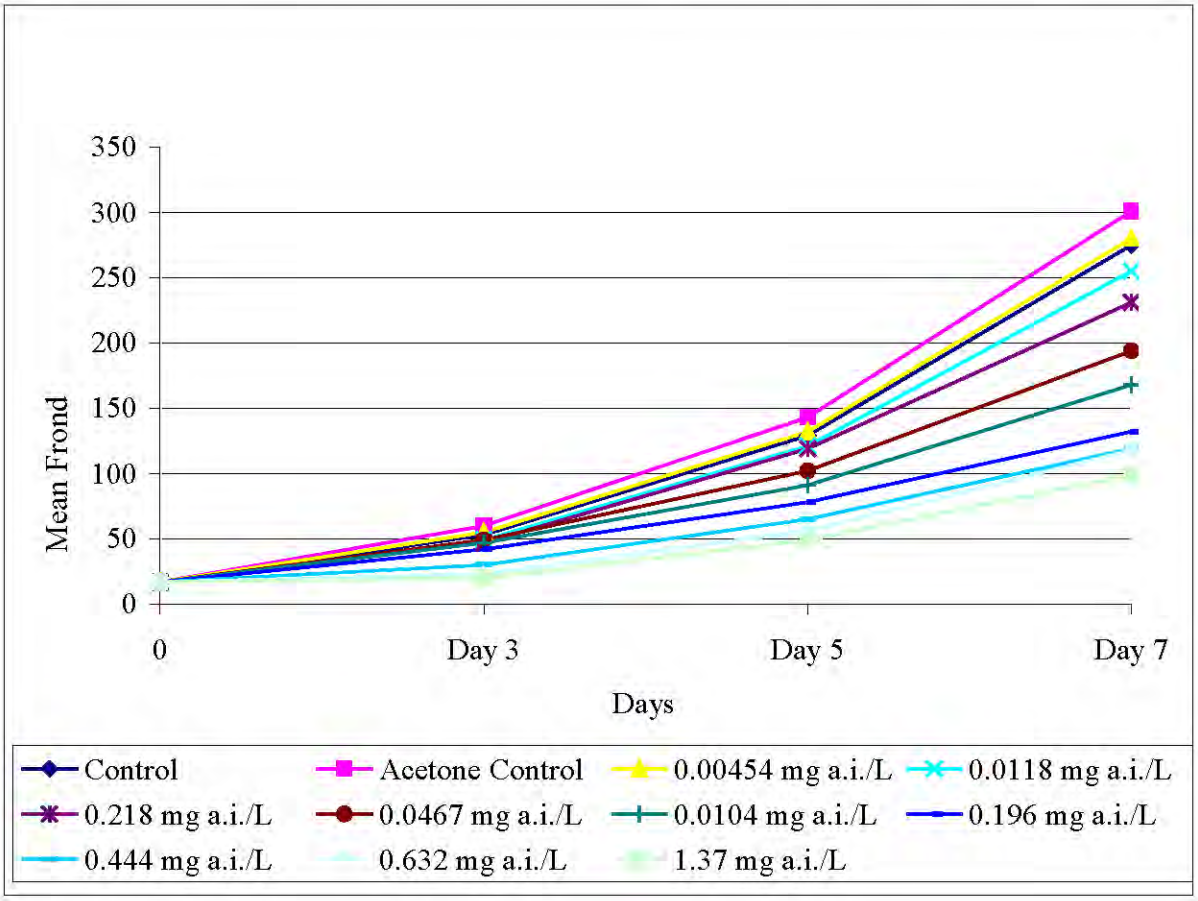


Figure A7.4.3.5.2/01-2: Effect of exposure period on the endpoints frond number and area under growth curve



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 8: Wood preservatives

Document III-A (A7)
Study summaries – Active substance
Ecotoxicological profile including environmental fate and behaviour

Part VI

Fate and behaviour in the environment

Section A7.5: Effects on terrestrial organisms

TABLE OF CONTENTS

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

Official use only

	1 REFERENCE	
1.1	**Reference**	[redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2		
1.2.1	Criteria for data protection	[redacted]
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 216 and OECD 217
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	DCOIT (RH-287 Technical)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	99.3%
3.1.4	Composition of Product	[redacted]
3.1.5	Further relevant properties	
3.1.6	Method of analysis	
3.2	**Reference substance**	
3.2.1	Method of analysis for reference substance	
3.3	**Testing procedure**	
3.3.1	Soil sample /	

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

	inoculum / test organism		
3.3.2	Test system	[redacted]	
3.3.3	Application of TS		
3.3.4	Test conditions	see table A7.5.1.1/01-5	
3.3.5	Test parameter	carbon mineralization and nitrogen mineralization and transformation by soil microflora	
3.3.6	Analytical parameter	CO_2, nitrite, nitrate and ammonium measurements	
3.3.7	Duration of the test	28 days	
3.3.8	Sampling	days 0, 7 and 28 for respiration and nitrification	
3.3.9	Monitoring of TS concentration	[redacted]	
3.3.10	Controls	[redacted]	
3.3.11	Statistics		
		4 RESULTS	
4.1	**Range finding test**	Performed	
4.1.1	Concentration	0 (control), 1, 10, 100, 500, 1000 mg DCOIT/kg dry soil	
4.1.2	Effect data	nitrification data deviated from control: -19, 50, 66, 11, -91% respiration data deviated from control: -11, -33, -67, -78, -78%	x
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	nitrification: 0 (control), 100, 200, 400, 600, 800, 1000 mg DCOIT/kg dry soil respiration: 0 (control), 2.1, 6.2, 19, 56, 167, 500 mg DCOIT/kg dry soil	
4.2.2	Actual concentrations of test substance	Not applicable	x
4.2.3	Growth curves	Not applicable	
4.2.4	Cell concentration data	Not applicable	
4.2.5	Concentration/ response curve	see Figure A7.5.1.1/01-1/5	
4.2.6	Effect data	The initial hourly CO_2 production rates on day 0 and day 28 during the first hours were 0.329 and 0.292 ml CO_2/100 g dry soil, which equals 13.6 and 12.2 mg carbon per 100 g dry soil, respectively. The microbial biomass was between 1.5 and 1.3% of the total soil organic carbon content (0.92% C_{org}) during the study.	x

		see tables A7.5.1.1/01-6 and A7.5.1.1/01-7	
4.2.7	Other observed effects	see tables A7.5.1.1/01-6 and A7.5.1.1/01-7	
4.3	**Results of controls**	see tables A7.5.1.1/01-6 and A7.5.1.1/01-7	
4.4	**Test with reference substance**	Performed: Dinoseb acetate	
4.4.1	Concentrations	33.3 mg ai/kg dry soil	
4.4.2	Results	Effects larger than 25% were found during the 28 day of the respiration part and the nitrification part of the study.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 216 and OECD 217, Effects on soil microflora respiration transformation and nitrification transformation.	
5.2	**Results and discussion**		x
5.2.1	EC_{10}	respiration = 15.3 mg DCOIT/kg dry soil (C.I. 1.20-139) nitrification = 42.9 mg DCOIT/kg dry soil (C.I. 6.94-115)	
5.2.2	EC_{25}	respiration = 67.0 mg DCOIT/kg dry soil (C.I. 7.33-847) nitrification = 77.0 mg DCOIT/kg dry soil (C.I. 16.9-189)	
5.2.3	EC_{50}	respiration = 393* mg DCOIT/kg dry soil (C.I. 46.9-10070), nitrification = 155 mg DCOIT/kg dry soil (C.I. 46.5-363). * value calculated, however effect at 500 mg ai/kg was below 50% inhibition, therefore very broad confidence interval.	
5.3	**Conclusion**	Results of controls and reference substance are in acceptable range. The variations between the replicate control samples in this test were less than $\pm$ 15% for both the respiration rate and nitrate concentration at all sampling intervals, showing the validity of the study. In addition, the results of the reference study, where effects of dinoseb acetate of larger than 25% were found, showed that the methods used were appropriate.	x
5.3.1	Reliability	(1), reliable without restriction	x
5.3.2	Deficiencies	No	x

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	13 September 2007
Materials and Methods	**Comment (3.1.4):** Carbon and nitrogen content of test substance is 47% and 5%, respectively. However, results indicate that DCOIT contribution to respiration or nitrate accumulation is negligible.
Results and discussion	**Comment (4.1.2):** Range finding results were partly misleading with regard to respiration (see comment 4.2.6). **Comment (4.2.2):** Due to the fact that DCOIT is rapidly degraded in soil it can be assumed that test substance concentrations declined during the test (see also comment 5.2). **Comment (4.2.6 and 5.2.3):** For respiration, the effect of the highest concentration was less than 50% in the definitive test, and was determined by extrapolation giving a very broad confidence interval. The upper confidence limit is certainly far beyond the "true" endpoint. Thus, the EC50 for respiration is not valid. However, the reported value of 393 mg/kg could be seen as a conservative estimate. It would be more correct to state that the EC50 (respiration) is > 500 mg/kg. The EC50 for nitrification can be considered valid.
Conclusion	**Comment (5.2):** Nitrification was completely inhibited at all concentrations at 7 days (Table A7.5.1.1/01-7). After 28 days activity was partly resumed in the lower concentration range. This is probably due to the fact that tetst substance concentrations were declining over the course of the test due to biodegradation of the test substance. **Comment (5.3):** Agree with applicant's version. However, the EC50 for soil respiration cannot be used, but the results of the study support the results gained from the nitrification inhibition study.
Reliability	Due to the restrictions noted above the reliability is changed from 1 to 2
Acceptability	Acceptable with the restrictions noted above
Remarks	The great discrepancy between the range finding (4.1) and definite study (Tables A7.5.1.1/01-6&7) may be explained according to the following consideration. Records of the range finding tests are not reported. The definitive study was conducted about 4 weeks after soil sampling. Thus, it must be assumed that the preliminary tests were performed on fresh soil samples. During the first week after soil sampling, microbial activity and community structure may change significantly. Toxicity testing should not be performed until basal respiration is stable, which often awaits one ore two weeks. This condition is probably not met by the range finding test. Immediately after soil disturbance, microbes are less protected and more exposed to chemicals compared to udisturbed soils. This may explain the more toxic response of the respiration range finding test. The results of this inhibition test is partly supported by the degradation studies (Document III-A/Section A7.2.1 Aerobic degradation in soil including extent and nature of bound residues) which stated that 5 ppm DCOIT appeared to partly inhibit microbial activity and degradation (pargraph 4.1 Preliminary studies)

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)– **TABLES AND FIGURES**

[redacted]

[redacted]

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

Table A7.5.1.1/01-5: Test conditions

Criteria	Details
Organic substrate	0.4 g Lucerne meal (containing 3.25% nitrogen) was added to each replicate of the nitrification part of the study
Incubation temperature	19.5 to 21.0 °C
Soil moisture	maintained at 45.5% (20.4 g water/100 g dry soil) of maximum water holding capacity
Method of soil incubation	Not described
Aeration	Not described

Table A7.5.1.1/01-6: Respiration rates

Test Substance Concentration (nominal) [mg DCOIT/kg dry soil]	Measured ($mg O_2$/kg dry soil/hour)			% difference to control		
	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28
0 (control)	4.2	5.2	4.9	---	---	---
2.1	4.4	5.3	4.7	4	3	-3
6.2	3.4	5.2	4.7	-19	0	-3
19	3.6	4.9	4.2	-15	-6	-13
56	3.4	3.4	3.4	-19	-34	-30
167	1.9	2.8	2.6	-54	-47	-47
500	2.1	2.8	2.8	-50	-47	-43

Table A7.5.1.1/01-7: Nitrate Transformation Rates

Test Substance Concentration (nominal) [mg DCOIT/kg dry soil]	Measured ($mg NO_3^-$/kg dry soil/day)		% difference to control	
	Day 7	Day 28	Day 7	Day 28
0 (control)	12.1	8.3	---	---
100	-0.40	5.8	-103	-30
200	-2.4	3.2	-120	-62
400	-2.3	1.7	-119	-79
600	-0.35	0.31	-103	-96
800	1.0	0.57	-92	-93
1000	0.88	0.49	-93	-94

Figure A7.5.1.1/01-1: Glucose induced short term respiration

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9. FIGURES

Figure 1. **Glucose induced short term respiration: Percent deviation of the RH-287 Technical treated samples from the control represented as dose-response track.**