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Section A7.4.3.3.2

Bioconcentration in invertebrates-Marine water, Oyster

Annex Point IIIA XIII.2.3

		1 REFERENCE	Official use only
1.1 Reference		<u>Reference Type: Test report</u> <u>Year: 2002</u> <u>Report date: 4 October 2002</u> [REDACTED]	
1.2 Data protection		Yes	
1.2.1 Data owner		Rohm and Haas Company	
1.2.2			
1.2.3 Criteria for data protection		[REDACTED] [REDACTED]	
		2 GUIDELINES AND QUALITY ASSURANCE	Official use only
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	Official use only
3.1 Test material		¹⁴ C-DCOIT(RH-287). [REDACTED]	
3.1.1 Lot/Batch number		[REDACTED]	
3.1.2 Specification		[REDACTED]	
3.1.3 Radiopurity		[REDACTED]	

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3.1.4 Further relevant
properties

[REDACTED]

x

[REDACTED]

3.1.5 Radiolabelling

[REDACTED]

3.1.6 Method of analysis

[REDACTED]

**3.2 Reference
substance**

[REDACTED]

3.2.1 Method of analysis
for reference
substance

[REDACTED]

**3.3 Testing/estimation
procedure**

3.3.1 Test system/
performance

[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) and indicates that the compound will not bioaccumulate.

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

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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 $0.45 \pm 0.12\%$ throughout the entire 56 day bioconcentration test (uptake and depuration phases). |

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4.1.3	Concentrations of test material during test	<p>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 nominal 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.</p> <p>Depuration of ¹⁴C-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 ¹⁴C-activity in the water.</p>	x																																	
4.1.4	Bioconcentration factor (BCF)	<p>The BCF's for the individual sampling days are:</p> <table><thead><tr><th>Day</th><th colspan="2">BCF (mL/g)</th></tr><tr><th></th><th><u>0.0027 µg/L</u></th><th><u>0.031 µg/L</u></th></tr></thead><tbody><tr><td>7</td><td>3.67</td><td>5.0</td></tr><tr><td>14</td><td>12.0</td><td>5.6</td></tr><tr><td>21</td><td>8.1</td><td>14.7</td></tr><tr><td>28</td><td>16.3</td><td>15.9</td></tr></tbody></table> <p>The calculated steady-state BCF's are</p> <table><thead><tr><th></th><th colspan="2">BCF (mL/g)</th></tr><tr><th></th><th><u>0.0027 µg/L</u></th><th><u>0.031 µg/L</u></th></tr></thead><tbody><tr><td>BIOFAC</td><td>44 ± 23</td><td>19 ± 4.9</td></tr><tr><td>k₁/k₂</td><td>43.8</td><td>19.5</td></tr><tr><td>Tissue conc./Water conc.</td><td>11.1</td><td>10.6</td></tr></tbody></table> <p>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 (k₁) and depuration (k₂) rate constants. The tissue and water concentrations are the mean measured values following initiation of steady state (Days 14 through 28).</p>	Day	BCF (mL/g)			<u>0.0027 µg/L</u>	<u>0.031 µg/L</u>	7	3.67	5.0	14	12.0	5.6	21	8.1	14.7	28	16.3	15.9		BCF (mL/g)			<u>0.0027 µg/L</u>	<u>0.031 µg/L</u>	BIOFAC	44 ± 23	19 ± 4.9	k ₁ /k ₂	43.8	19.5	Tissue conc./Water conc.	11.1	10.6	x
Day	BCF (mL/g)																																			
	<u>0.0027 µg/L</u>	<u>0.031 µg/L</u>																																		
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Tissue conc./Water conc.	11.1	10.6																																		
4.1.5	Uptake and depuration rate constants	<p>The uptake (k₁) and depuration (k₂) rate constants were calculated using BIOFAC and are tabulated below.</p> <table><thead><tr><th></th><th><u>0.0027 µg/L</u></th><th><u>0.031 µg/L</u></th></tr></thead><tbody><tr><td>k₁</td><td>570 ± 88 day⁻¹</td><td>720 ± 120 day⁻¹</td></tr><tr><td>k₂</td><td>13 ± 6 day⁻¹</td><td>37 ± 7 day⁻¹</td></tr></tbody></table>		<u>0.0027 µg/L</u>	<u>0.031 µg/L</u>	k ₁	570 ± 88 day ⁻¹	720 ± 120 day ⁻¹	k ₂	13 ± 6 day ⁻¹	37 ± 7 day ⁻¹																									
	<u>0.0027 µg/L</u>	<u>0.031 µg/L</u>																																		
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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.									
		<table><tr><td></td><td><u>0.0027 $\mu\text{g/L}$</u></td><td><u>0.031 $\mu\text{g/L}$</u></td></tr><tr><td>DT_{50} (days)</td><td>42</td><td>15.6</td></tr><tr><td>DT_{90} (days)</td><td>83.2</td><td>32</td></tr></table>		<u>0.0027 $\mu\text{g/L}$</u>	<u>0.031 $\mu\text{g/L}$</u>	DT_{50} (days)	42	15.6	DT_{90} (days)	83.2	32
	<u>0.0027 $\mu\text{g/L}$</u>	<u>0.031 $\mu\text{g/L}$</u>									
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 $\mu\text{g/L}$ treatment is -0.7194 and -0.00038, respectively. The correlation coefficient and slope for the 0.031 $\mu\text{g/L}$ 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.									

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

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		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	Juvenile oysters (<i>Crassostrea virginica</i>) 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	<u>Experimental studies/Relevant test material specific properties</u>	
		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	

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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	<p>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.</p> <p>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.</p> <p>Comment (5.2): The water solubility of DCOIT at pH7 and 20°C is 3.47 mg/l.</p> <p>Depuration at the low dose level does not seem to continue after day 42, indicating that ¹⁴C-labelled metabolites might have been incorporated into tissues of the oysters.</p>
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.

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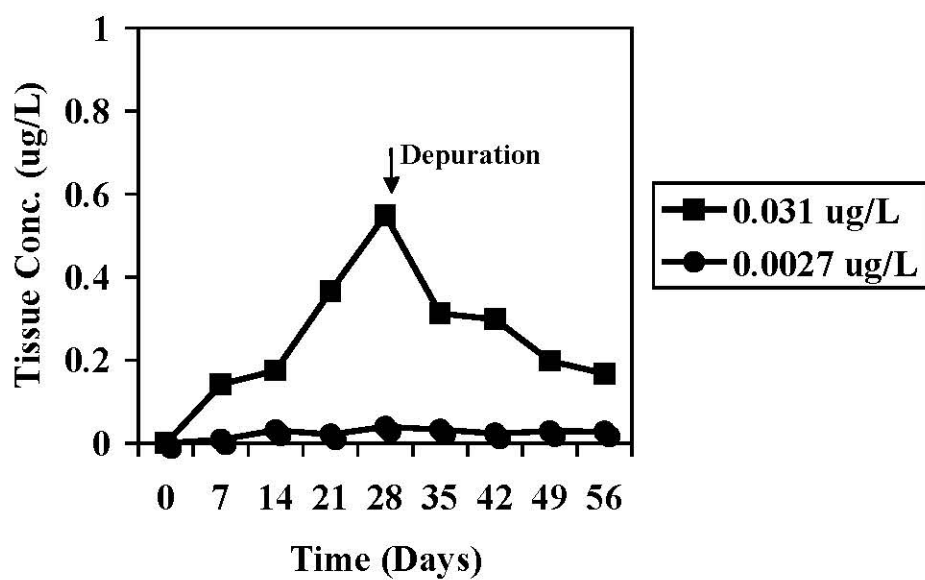
Bioconcentration in invertebrates-Marine water, Oyster – TABLES
AND FIGURES

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 ($\mu\text{g/L}$) ¹			
	Water		Tissue	
	0.0027 $\mu\text{g/L}$ ²	0.031 $\mu\text{g/L}$ ³	0.0027 $\mu\text{g/L}$ ²	0.031 $\mu\text{g/L}$ 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 $\mu\text{g/L}$ ³ Average concentration of ^{14}C -activity in water during the 28 day uptake phase. Nominal concentration was 0.08 $\mu\text{g/L}$ 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

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

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Section A7.4.3.4.a

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Effects on reproduction and growth rate with an invertebrate species-Freshwater, *Daphnia magna*Official
use only

1 REFERENCE

1.1 Reference

Reference type: Test report

Year: 1990

Report date: 30 November 1990

[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]

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Effects on reproduction and growth rate with an invertebrate species-Freshwater, *Daphnia magna*

	test substances				
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	■			
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.			
		<table><tr><td>mean measured</td><td>% of nominal</td><td>range (µg DCOIT/L)</td></tr></table>	mean measured	% of nominal	range (µg DCOIT/L)
mean measured	% of nominal	range (µg DCOIT/L)			

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Effects on reproduction and growth rate with an invertebrate species-Freshwater, *Daphnia magna*

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 EC₅₀ 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	Effects on reproduction and growth rate with an
Annex Point IIIA XIII.2.4	invertebrate species-Freshwater, <i>Daphnia magna</i>

5.3	Conclusion	see table A7.4.3.4.a/01-8	x
5.3.1	Reliability	<p>(2), reliable with restrictions.</p> <p>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.</p> <p>Therefore the chronic <i>Daphnia magna</i> 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).</p>	
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.

Document III-A / Section A7.4.3**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 $\geq 80\%$ 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 $\mu\text{g/l}$.

Reliability

2, reliable with restrictions

Acceptability

Acceptable with the restrictions noted above

Remarks

-

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

[REDACTED]	
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

[REDACTED]	
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

[REDACTED]	
[REDACTED]	[REDACTED]
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[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

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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

	NOEL	LOEL	ug a.i./L 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 fulfilled
Mortality of parent animals < 20% at test termination	yes	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60		yes

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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

Official
use only

1 REFERENCE

1.1 Reference

Reference Type: test report

Year: 2000

Report date: 1 June 2000

[REDACTED]

1.2 Data protection

Yes

1.2.1 Data owner

Rohm and Haas Company

1.2.2

1.2.3 Criteria for data
protection

[REDACTED]

[REDACTED]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

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]

Document III-A / Section A7.4.3**Section A7.4.3.4.b/01 Effects on reproduction and growth rate with an
Annex Point IIIA XIII.2.4 invertebrate species-Marine water, Mysid****3.4 Testing procedure**

3.4.1	Dilution water	[REDACTED]
3.4.2	Test organisms	[REDACTED]
3.4.3	Handling of offspring	[REDACTED]
3.4.4	Test system	[REDACTED]
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	[REDACTED]
3.4.9	Monitoring of TS concentration	[REDACTED]
3.4.10	Statistics	[REDACTED]

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 sublethal effects in surviving mysids. Gravid females were observed

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Section A7.4.3.4.b/01 **Effects on reproduction and growth rate with an**
Annex Point IIIA XIII.2.4 **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

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

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 **Effects on reproduction and growth rate with an**
Annex Point IIIA XIII.2.4 **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	<p>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 <u>per day</u>. This criterion can therefore not be considered fulfilled. It is recognised that this requirement might be difficult to achieve. 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 (Techniques for the Laboratory Culture of <i>Mysidopsis</i> 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 <i>M. bahia</i> a number of 1.3 offspring/female/day was reported by Charles L. McKenny, Jr. (Diseases of Aquatic Organisms, Vol. 1: 131-139, 1986).</p> <p>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.</p> <p>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.</p>
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	-

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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 – TABLES AND FIGURES

[illegible][illegible]

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[REDACTED]	
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
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[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

Document III-A / Section A7.4.3

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

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

	fulfilled	Not fulfilled
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

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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

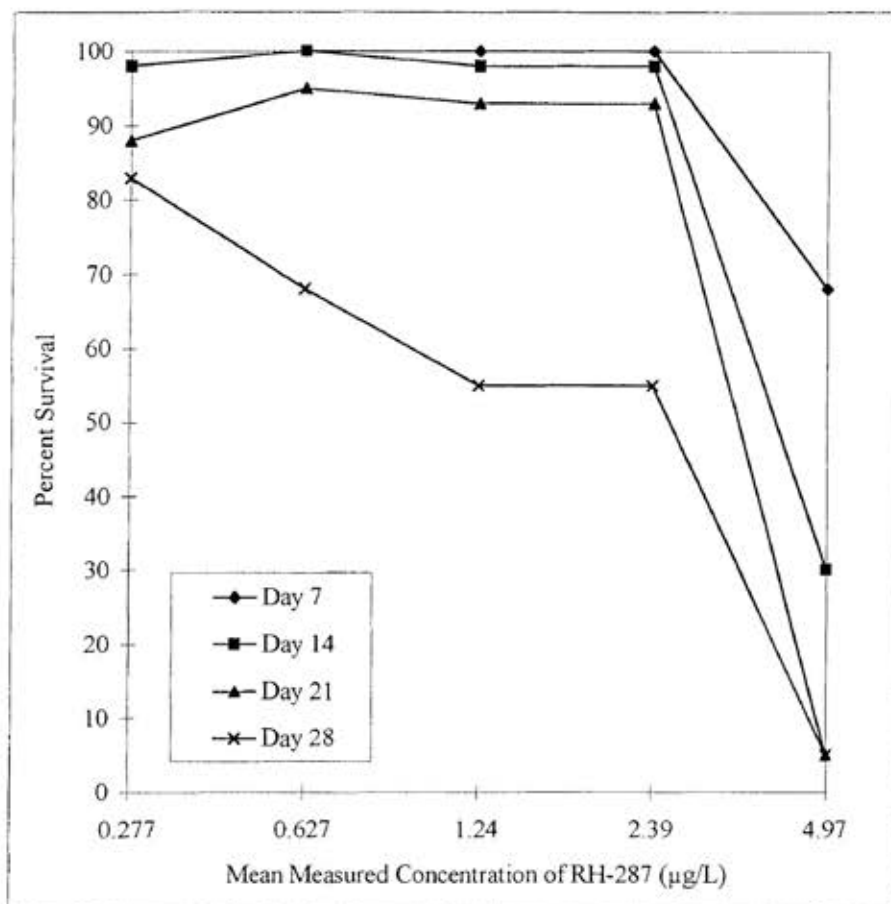


Figure 1 Survival of first generation mysids, *Americamysis bahia*, exposed to RH-287 for 7, 14, 21, and 28 days.

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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

		1 REFERENCE	Official use only
1.1 Reference		<u>Reference Type: test report</u> <u>Year: 2007</u> <u>Report date: 4 June 2007</u> [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] [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]	

Document III-A / Section A7.4.3

**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	[REDACTED]
3.4.2	Test organisms	[REDACTED]
3.4.3	Test system	[REDACTED]
		[REDACTED]
3.4.4	Test conditions	see table A7.4.3.5.1.a/01-5
3.4.5	Duration of the test	10 days
3.4.6	Test parameter	[REDACTED]
3.4.7	Sampling	[REDACTED]
3.4.8	Monitoring of TS concentration	[REDACTED]
3.4.9	Statistics	[REDACTED]

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

Document III-A / Section A7.4.3

**Section A7.4.3.5.1.a/01 Acute toxicity to sediment dwelling organisms-
Annex Point IIIA XIII.3.4 Freshwater, *Chironomus tentans***

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, <i>Chironomus tentans</i> , 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	<p>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.</p> <p>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.</p>
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
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

x

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 (5.2.2): 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	-

Document III-A / Section A7.4.3

Section A7.4.3.5.1.a/01

Acute toxicity to sediment dwelling organisms-Freshwater, *Chironomus tentans* – TABLES AND FIGURES

[REDACTED]

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

[REDACTED]

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Document III-A / Section A7.4.3

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[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

Document III-A / Section A7.4.3

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

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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/ μ g)	Total [14 C] (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

Document III-A / Section A7.4.3**Pore water:**

Nominal concentration DCOIT (mg/kg)	Sampling time (days)	Specific activity (dpm/ μ g)	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

Document III-A / Section A7.4.3**Sediment samples:**

Nominal concentration DCOIT (mg/kg)	Sampling time (days)	Specific activity (dpm/ μ g)	Total [^{14}C] (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 fulfilled
Mortality of control animals <10%	yes	
Concentration of test substance \geq 80% of initial concentration during test	yes*	

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

Document III-A / Section A7.4.3

Section A7.4.3.5.1a/02 Chronic toxicity to sediment dwelling organisms-
Annex Point IIIA XIII.3.4 Freshwater, *Chironomus riparius*

		1 REFERENCE	Official use only
1.1 Reference		<u>Reference Type: test report</u> <u>Year: 2003</u> <u>Report date: 1 October 2003</u> [REDACTED]	
1.2 Data protection		Yes	
1.2.1 Data owner		Rohm and Haas Company	
1.2.2			
1.2.3 Criteria for data protection		[REDACTED] [REDACTED]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study		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]	

Document III-A / Section A7.4.3

**Section A7.4.3.5.1a/02 Chronic toxicity to sediment dwelling organisms-
Annex Point IIIA XIII.3.4 Freshwater, *Chironomus riparius*****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

[REDACTED]

3.4.3 Test system

[REDACTED]

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

[REDACTED]

3.4.8 Monitoring of TS
concentration

[REDACTED]

3.4.9 Statistics

[REDACTED]

4 RESULTS**4.1 Limit Test**

Not performed

**4.2 Results test
substance**4.2.1 Initial
concentrations of test
substance0 (control), 0 (9.3 mL/kg acetone control), 5.0, 10, 20, 40, and 80 mg
DCOIT/kg dry sediment4.2.2 Actual
concentrations of test
substanceTS 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 sedimentMean Measured Sediment
Concentrations (LSC): <MQL (control), <MQL (9.3 mL/kg acetone
control), 4.9, 9.7, 20, 40, and 73 mg ¹⁴C

Document III-A / Section A7.4.3

**Section A7.4.3.5.1a/02 Chronic toxicity to sediment dwelling organisms-
Annex Point IIIA XIII.3.4 Freshwater, *Chironomus riparius***

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

Document III-A / Section A7.4.3

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	28 Januray 2008
Materials and Methods	Agree with applicant's version
Results and discussion	<p>Comment (5.2): For unknown reasons the lethality of <i>C. riparius</i> 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.</p> <p>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 <i>C. riparius</i> up to 40 mg DCOIT per kg dry sediment (nominal).</p>
Conclusion	Agree with applicant's version
Reliability	1, reliable without restrictions
Acceptability	Acceptable
Remarks	-

Document III-A / Section A7.4.3

Section A7.4.3.5.1a/02

Chronic toxicity to sediment dwelling organisms-Freshwater,
Chironomus riparius – TABLES AND FIGURES

[REDACTED]	
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

[REDACTED]	
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

[REDACTED]	
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

Document III-A / Section A7.4.3

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

Document III-A / Section A7.4.3

Table A7.4.3.5.1.a/02-6: Analytical measured concentration: results 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	< 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

Measured RH-287 Technical Concentration as mg 14[C] equivalents/kg Dry Sediment (Percent of Nominal) Based on LSC Analysis						
Mean Measured Sediment Concentrations (mg 14[C] equivalents per kg dry sediment)	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)

Document III-A / Section A7.4.3

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.

Document III-A / Section A7.4.3**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
<u>Expressed as mg DCOIT per kg Dry Sediment</u>				
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
<u>Expressed as mg ¹⁴C-DCOIT Equivalents per kg Dry Sediment</u>				
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
<u>Expressed as µg DCOIT per Liter of Pore Water</u>				
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
<u>Expressed as µg ¹⁴C-DCOIT Equivalents per Liter of Pore Water</u>				
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 fulfilled
70% emergence in the control	yes	
Emergence period within days 12-23		yes¹
O ₂ concentration > 60 % at temperature.		yes²
pH in overlaying water 6-9	yes	
Water temperature should not differ by more than 1 degree	yes	

¹Emergence occurred between days 17-28.² O₂ 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

		1 REFERENCE	
1.1	Reference	<u>Reference Type: test report</u> <u>Year: 2003</u> <u>Report date: 2 October 2003</u> [REDACTED]	
1.2	Data protection	Yes	
1.2.1	Data owner	Rohm and Haas Company	
1.2.2			
1.2.3	Criteria for data protection	[REDACTED] [REDACTED]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	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]	

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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

3.2 Preparation of TS solution for poorly soluble or volatile test substances

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

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.

x

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

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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

control), 4.6, 10, 19, 38, and 78 mg ¹⁴C-DCOIT equivalents/kg dry sediment

Results of the analytical measurements can be found in Tables A7.4.3.5.1.b/01-6 and A7.4.3.5.1.b/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 ¹⁴C equivalent/kg dry sediment), 124 (19 mg ¹⁴C equivalent/kg dry sediment) and 539 (78 mg ¹⁴C equivalent/kg dry sediment) µg ¹⁴C-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 ¹⁴C equivalent/kg dry sediment), 121 (19 mg ¹⁴C equivalent/kg dry sediment) and 384 (78 mg ¹⁴C equivalent/kg dry sediment) µg ¹⁴C-DCOIT equivalent/L

4.2.3 Effect data see table A7.4.3.5.1.b/01-8 and see table A7.4.3.5.1.b/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 A7.4.3.5.1.b/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

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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

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 LC₅₀ 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 -

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Section A7.4.3.5.1b/01

Chronic toxicity to sediment dwelling organisms-Marine water,
Leptocheirus plumulosus – TABLES AND FIGURES

[REDACTED]

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

[REDACTED]

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	0.01-0.5
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

[REDACTED]

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]