Table A7.4.1.1.c/01-7: Effect data

	48 h [mg NNOMA/l][1]	95 % c.l.	96 h [mg NNOMA/l][1]	95 % c.l.
LC_0	160 (m)	---	160 (m)	---
LC_{50}	250 (m)	160-430	250 (m)	160-430
LC_{100}	430 (m)	---	430 (m)	---

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

Table A7.4.1.1.c/01-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	**yes**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**yes**	

Figure A7.4.1.1.c/01-1: The 96-hour dose-response line for Rainbow Trout (*Oncorhynchus mykiss*) exposed to N-(n-octyl) Malonamic acid

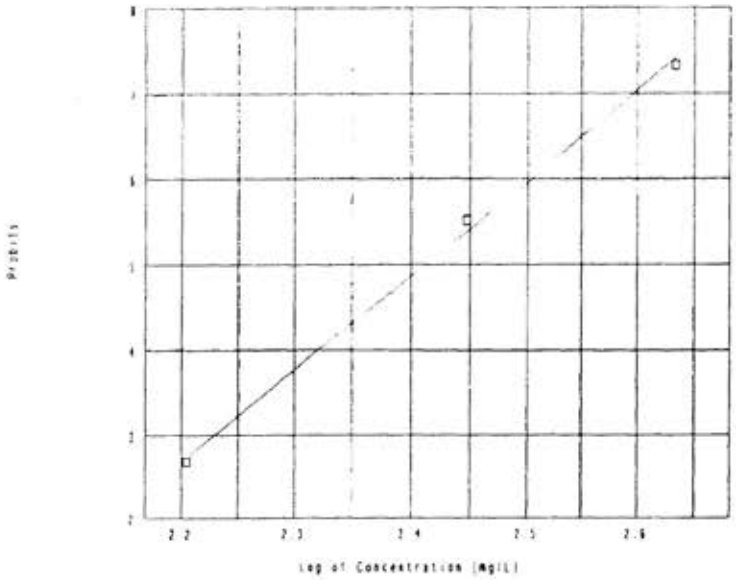

Figure 1: The 96-Hour Dose-Response Line for Rainbow Trout (*Oncorhynchus mykiss*) Exposed to N-(n-octyl) Malonamic Acid

All results were based on the mean measured concentrations of N-(n-octyl) malonamic acid. The 96-hour no-observed effect concentration (NOEC) was 160 mg a.i./L based on the lack of mortality or observed abnormal/sublethal effects at this concentration.

N-(n-octyl) malonamic acid is the initial stable metabolite resulting from metabolism of the industrial biocide, RH-5287 (8,9). Initially, the toxicity of this and other metabolites of RH-5287 were examined by quantitative structure activity relationships (QSAR). To perform this analysis the Numerica QSAR Program (Technical Database Services, Inc., New York, NY) was employed. The table below presents the predicted 96-hour LC_{50}'s in rainbow trout for the metabolites of RH-5287.

Metabolite	Predicted 96-Hour. LC_{50}
N-(n-octyl) malonamic acid	198.92 (mg a.i./L)
N-(n-octyl) acetamide	114.95 (mg a.i./L)
N-(n-octyl) oxamic acid	159.52 (mg a.i./L)
N-(n-octyl)-β-hydroxy propionamide	316.31 (mg a.i./L)

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Section A7.4.1.1.c/02 — Acute toxicity of N-(n-octyl) acetamide to fish-Fresh water, Rainbow trout

Annex Point IIA VII.7.1

Official use only

		1 REFERENCE
1.1	**Reference**	Reference type: Study report Year: 2002 Report date: 21 June 2002 [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 203, US EPA OPPTS 850.1075, US EPA TSCA 797.1400, US EPA FIFRA 72-1, EC Council Directive 91/414/EEC
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	[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 A7.4.1.1.c/02 — Acute toxicity of N-(n-octyl) acetamide to fish-Fresh water, Rainbow trout

Annex Point IIA VII.7.1

3.3.1	Method of analysis for reference substance	[redacted]	
3.4	**Testing procedure**		
3.4.1	Dilution water	[redacted]	x
3.4.2	Test organisms	[redacted]	x
3.4.3	Test system	[redacted]	
3.4.4	Test conditions	see table A7.4.1.1.c/02-5	x
3.4.5	Duration of the test	96 hr	
3.4.6	Test parameter	[redacted]	x
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	Nominal (mg NNOA/L) 2.5, 5.0, 10, 20, 40
4.2.2	Actual concentrations of test substance	measured concentrations (mg NNOA/L)

0 hr	96 hr	mean
2.96	2.4	2.7
4.72	4.31	4.5
11.5	10	11
18.9	19.2	19
30.5	36.9	34

x

4.2.3	Effect data (Mortality)	see table A7.4.1.1.c/02-6; see table A7.4.1.1.c/02-7
4.2.4	Concentration /	The slope of the concentration-response curve is 18. See Figure

	response curve	A7.4.1.1.c/02-1.	
4.2.5	Other effects	discoloration, loss of equilibrium, fish resting on bottom of test chamber, irregular respiration	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	control and acetone (0.10 mL/L) control no adverse effects	
4.3.2	Nature of adverse effects	not applicable	
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 203, US EPA OPPTS 850.1075, US EPA TSCA 797.1400, US EPA FIFRA 72-1, EC Council Directive 91/414/EEC. Acute static 96h fish study with analytical confirmation of test solution concentrations.	
5.2	**Results and discussion**	96 hr NOEC = 11 mg NNOA/L based on lack of mortality and sublethal effects	
5.2.1	LC_0	96 hr = 11 mg NNOA/L	
5.2.2	LC_{50}	96 hr = 25 mg NNOA/L	
5.2.3	LC_{100}	96 hr = 34 mg NNOA/L	
5.3	**Conclusion**	see validity criteria summarized in table table A7.4.1.1.c/02-8	x
5.3.1	Other Conclusions		
5.3.2	Reliability	(1), reliable without restriction	x
5.3.3	Deficiencies	No	

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

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	29 November 2007
Materials and Methods	**Comment (3.4.1):** According to OECD 203, the oxygen concentration should not be less than 60% of the air saturation value. The lowest value in this test was 58%. **Comment (3.4.2):** According to OECD 203, the recommended total length of the fish should be 5.0±1.0 cm. The test fish in this test were partly shorter (3.3-4.3 cm). **Comment (3.4.4):** According to OECD 203, the test temperature should be 13-17°C and no pH adjustment should be conducted. In this test, the temperature was 11-13°C and pH was adjusted. **Comment (3.4.6):** At the highest concentration there was 100% mortality, while in the next lower concentration 0% mortality was observed. In this case, the LC50 value can be calculated as the geometric mean value of these two concentrations, as it has been done in this study summary. However, no clear dose-response-relationship can be established.
Results and discussion	**Comment (4.2.2):** At the highest tested level the concentration at time 0 was only 78% of nominal.
Conclusion	**Comment (5.3):** Although the test has minor deficiencies, the test result is considered valid.
Reliability	**Comment (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	-

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

Section A7.4.1.1.c/02 — Acute toxicity of N-(n-octyl) acetamide to fish-Fresh water, Rainbow trout – **TABLES AND FIGURES**

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

Table A7.4.1.1.c/02-5: Test conditions

Criteria	Details				
	0 h	24 h	48 h	72 h	96 h
Test temperature	11.8 – 12.2	12.0 – 12.7	12.0 – 12.2	12.0 – 13.0	11.4 – 11.7
Dissolved oxygen	9.9 – 10.2	7.8 – 8.8	8.0 – 9.6	6.0 – 7.8	10.4 – 11.2
pH	8.1 – 8.2	8.0 – 8.1	7.9 – 8.0	7.6 – 7.8	7.4 – 8.1
Adjustment of pH	Yes				
Aeration of dilution water	not described in report (gentle aeration in all treatments starting at 72 hours)				
Intensity of irradiation	648 lux				
Photoperiod	16 h light; 8 h dark				

Table A7.4.1.1.c/02-6: Mortality data

Test-Substance Concentration (mean measured)[1] [mg NNOA/l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0 (control)	0	0	0	0	0	0	0	0
0 (acetone control)	0	0	0	0	0	0	0	0
2.7	0	0	0	0	0	0	0	0
4.5	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
34	20/20	20/20	20/20	20/20	100	100	100	100
Temperature [°C]	12.0 – 12.7	12.0 – 12.2	12.0 – 13.0	11.4 – 11.7				
pH	8.0 – 8.1	7.9 – 8.0	7.6 – 7.8	7.4 – 8.1				
Oxygen [mg/l]	7.8 – 8.8	8.0 – 9.6	6.0 – 7.8	10.4 – 11.2				

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

Table A7.4.1.1.c/02-7: Effect data

	48 h [mg NNOA/l][1]	95 % c.l.	96 h [mg NNOA/l][1]	95 % c.l.
LC_0	11 (m)	--	11 (m)	--
LC_{50}	25 (m)	--	25 (m)	--
LC_{100}	34 (m)	--	34 (m)	--

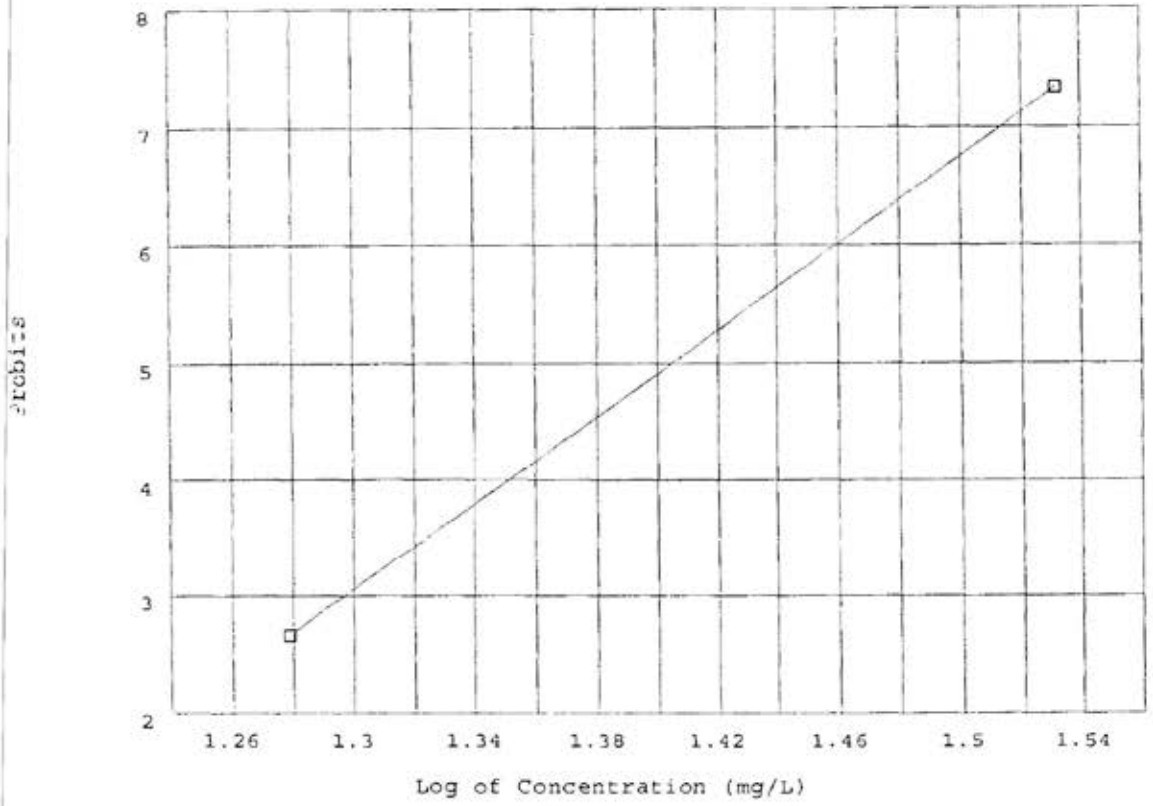
[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.4.1.1.c/02-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	**yes**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**yes**	

Figure A7.4.1.1.c/02-1: Concentration-Response curve for Rainbow Trout (*Oncorhynchus mykiss*) exposed to N-(n-octyl) acetamide

Figure 1. Concentration-Response Curve for Rainbow Trout (*Oncorhynchus mykiss*) Exposed to N-(n-Octyl) Acetamide



The slope of the concentration-response curve is 18.

Rohm and Haas Report No. 01RC-297/ABC Study No. 47175-25

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

Section A7.4.1.2.a/01 **Acute toxicity of DCOIT to invertebrates-Fresh water, *Daphnia magna***

Annex Point IIA VII.7.2

Official use only

		1 REFERENCE
1.1	**Reference**	Reference type: Study report Year: 1990 Report date: 17 July 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 72-2
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	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 test substances**	[redacted]
3.3	**Reference substance**	[redacted]

Section A7.4.1.2.a/01 Acute toxicity of DCOIT to invertebrates-Fresh water, *Daphnia magna*

Annex Point IIA VII.7.2

3.3.1	Method of analysis for 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 A7.4.1.2.a/01-5	
3.4.5	Duration of the test	48 h	
3.4.6	Test parameter	[redacted]	x
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	Nominal (µg DCOIT/L) 0.6, 1.2, 2.5, 5.0 10.0	
4.2.2	Actual concentrations of test substance	measured concentrations (µg DCOIT/L)	x
4.2.3	Effect data (Immobilisation)	see table A7.4.1.2.a/01-6; see table A7.4.1.2.a/01-7	x
4.2.4	Concentration /	The slope of the 48-hour dose-response line was 21 as calculated by the	

0 hr	48 hr	mean
0.47	0.36	0.42
0.68	0.71	0.70
1.3	1.6	1.5
4.5	3.3	3.9
6.7	7.2	7.0

	response curve	least squares regression analysis. See Figure A7.4.1.2.a/01-1.	
4.2.5	Other effects	mortality, daphnids on the bottom of the test chambers and erratic movement	
4.3	**Results of controls**	normal in appearance and behavior	
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	US EPA Guideline 72-2, Acute flow-through 48h *Daphnia magna* study with analytical confirmation of test solution concentrations.	
5.2	**Results and discussion**	48 h NOEC = 3.9 µg DCOIT/L	
5.2.1	EC_0	48 h = 3.9 µg DCOIT/L	
5.2.2	EC_{50}	48 h = 5.2 µg DCOIT/L	
5.2.3	EC_{100}	48 h = 7.0 µg DCOIT/L	
5.3	**Conclusion**	see table A7.4.1.2.a/01-6 and -7	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	29 November 2007
Materials and Methods	**Comment (3.4.6):** At the highest concentration there was no effect after 24 hours and 100% mortality after 48 hours, while in the next lower concentration no mortality was observed at all. In this case, the LC50 value can be calculated as the geometric mean value of these two concentrations, as it has been done in this study summary. However, no clear dose-response-relationship can be established with this approach.
Results and discussion	**Comment (4.2.2):** Measured concentrations at 48 hours were not in all cases ≥80% and that means that this validity criterion is not fulfilled
	Comment (4.2.3): See comment (3.4.6).
Conclusion	**Comment (5.3):** See comment (3.4.6). The results from this study can be accepted, as long-term toxicity data for aquatic organisms are available as well.

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

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 A7.4.1.2.a/01	Acute toxicity of DCOIT to invertebrates-Fresh water, *Daphnia magna* **TABLES AND FIGURES**

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[redacted]	[redacted]

Table A7.4.1.2.a/01-5: Test conditions

Criteria	Details
Test temperature	20 ± 1 °C
Dissolved oxygen	8.1-8.5 mg/L
pH	7.7-8.0
Adjustment of pH	not described
Aeration of dilution water	not described
Quality/Intensity of irradiation	50-70 footcandles
Photoperiod	16 hr daylight

Table A7.4.1.2.a/01-6: Immobilisation data

Test-Substance Concentration (mean measured) [1] [µg DCOIT/l]	Immobile *Daphnia*						
	Number		Percentage (%)		Oxygen [mg/l]	pH	Tempera-ture [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
0 (control)	0	0	0	0	8.2	7.8	20
0 (Triethylene glycol)	0	0	0	0	8.2	7.8	20
0.42	0	0	0	0	8.2	7.8	20
0.70	0	0	0	0	8.2	7.8	20
1.5	0	0	0	0	8.3	7.9	20
3.9	0	0	0	0	8.3	7.9	20
7.0	0	40/40	0	100	8.5	7.9	20

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

Table A7.4.1.2.a/01-7: Effect data

	EC_{50} [1]	95 % c.l.	EC_0 [1]	EC_{100} [1]
24 h [µg DCOIT/l]	---	---	7.0	---
48 h [µg DCOIT/l]	5.2 (m)	3.9 – 7.0	3.9 (m)	7.0 (m)

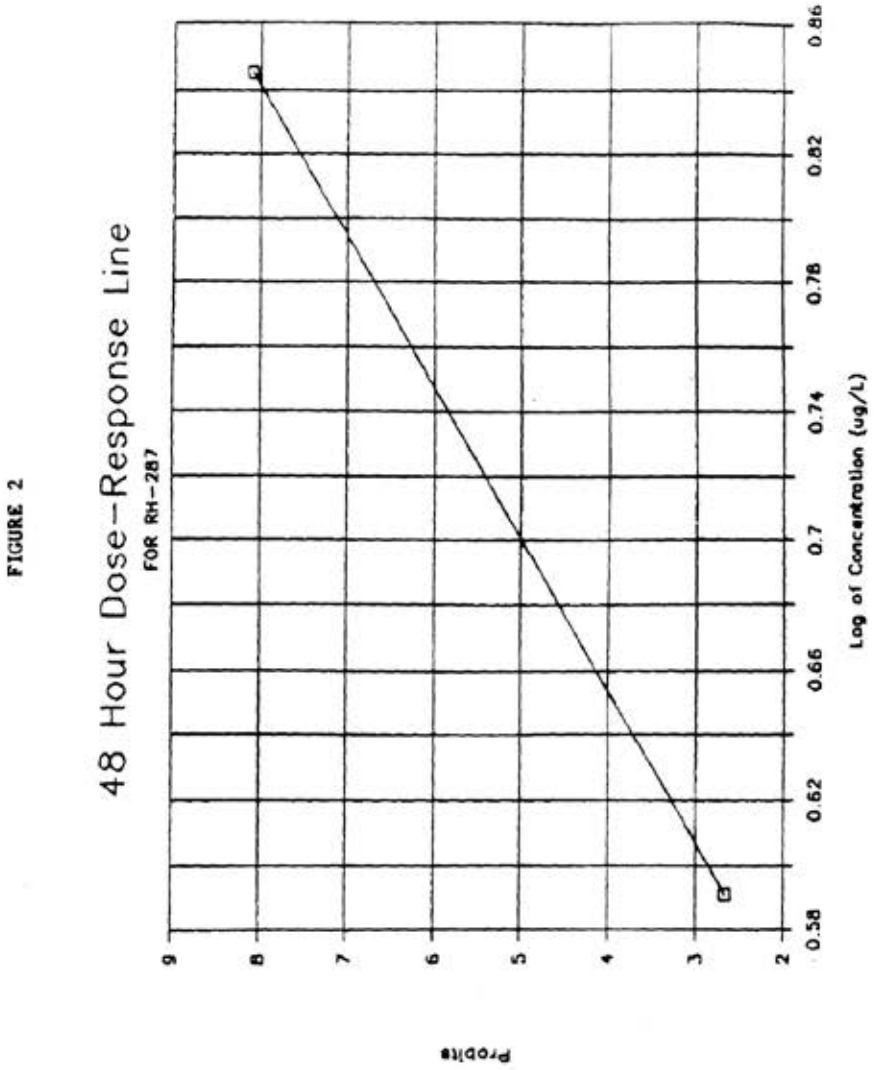
[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.4.1.2.a/01-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	Not fullfilled
Immobilisation of control animals <10%	**yes**	
Control animals not staying at the surface	**yes**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**yes**	

Figure A7.4.1.2.a/01-1: 48-hour dose-response line for *Daphnia magna* exposed to DCOIT

Rohm and Haas Report No. 89RC-0017



ABC LABS NO. 37738-24

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

Section A7.4.1.2.b/01 Acute toxicity of DCOIT to invertebrates-Marine water, Mysid

Annex Point IIA VII.7.2

Official use only

		1 REFERENCE
1.1	**Reference**	Reference type: Study report Year: 1990 Report date: 28 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 72-3
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	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 test substances**	[redacted]
3.3	**Reference substance**	[redacted]
3.4	**Testing procedure**	

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

Section A7.4.1.2.b/01 **Acute toxicity of DCOIT to invertebrates-Marine water, Mysid**

Annex Point IIA VII.7.2

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 A7.4.1.2.b/01-5	
3.4.5	Duration of the test	96 h	
3.4.6	Test parameter	[redacted]	x
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	Nominal (µg DCOIT/L) 2.4, 3.8, 6.2, 9.0, 15.0, 15.0 (secondary stock)	
4.2.2	Actual concentrations of test substance	measured concentration (µg DCOIT/L)	
4.2.3	Effect data (Mortality)	see table A7.4.1.2.b/01-6; see table A7.4.1.2.b/01-7	
4.2.4	Concentration / response curve	The slope of the 96-h dose response line was 5.1. See Figure A7.4.1.2.b/01-1.	
4.2.5	Other effects	mortality	
4.3	**Results of controls**	No adverse effects	x
4.4	**Test with reference substance**	Not performed	

0 hr	96 hr	mean
1.7	1.4	1.6
3.0	2.7	2.8
5.4	5.1	5.2
7.9	7.3	7.6
13	14	13.5

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

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Yes, US EPA FIFRA 72-3, Acute 96h mysid flow-through study with analytical confirmation of test solution concentrations.	
5.2	**Results and discussion**	96 h NOEC = 2.8 µg DCOIT/L	
5.2.1	LC_0	96 h = 1.6 µg DCOIT/L	
5.2.2	LC_{50}	96 h = 4.7 µg DCOIT/L	
5.2.3	LC_{100}	96 h = 7.6 µg DCOIT/L	
5.3	**Conclusion**	see validity criteria summarized in table A7.4.1.2.b/01-8	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	29 November 2007
Materials and Methods	**Comment (3.4.6):** Mortality in the solvent control was 10%. Therefore the validity criterion that mortality of control animals should be <10% could not be considered fulfilled.
Results and discussion	**Comment (4.3):** See comment (3.4.6). Validity criterion of <10% in the control is not fulfilled. The result of this test can nevertheless be accepted, as there are long-term studies available for marine fish, invertebrates and algae.
Conclusion	**Comment (5.3):** See comment (3.4.6). The test result can be accepted, as there are long-term studies available for marine fish, invertebrates and algae.
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 A7.4.1.2.b/01	Acute toxicity of DCOIT to invertebrates-Marine water, Mysid
	TABLES AND FIGURES

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Table A7.4.1.2.b/01-5: Test conditions

Criteria	Details
Test temperature	21.0-22.2 °C
Dissolved oxygen	7.9-8.7 mg/L
pH	7.5-7.8
Adjustment of pH	not described
Salinity	14-16 ppt
Aeration of dilution water	yes
Quality/Intensity of irradiation	9.5 $\mu Es^{-1} m^{-2}$
Photoperiod	16 h light and 8 h dark

Table A7.4.1.2.b/01-6: Mortality data

Test-Substance Concentration (mean measured) [µg DCOIT/l]	Mortality							
	Number				Percentage (%)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0 (control)	1/20	1/20	1/20	1/20	5	5	5	5
0 (TEG control)	0	2/20	2/20	2/20	0	10	10	10
1.6	0	1/20	1/20	1/20	0	5	5	5
2.8	0	2/20	2/20	2/20	0	10	10	10
5.2	0	0	2/20	4/20	0	0	10	20
7.6	2/20	9/20	20/20	20/20	10	45	100	100
13.5	3/20	20/20	20/20	20/20	15	100	100	100
Temperature [°C]	21.4-22.2	21.4-22.2	21.0-21.5	21.0-21.5				
pH	7.5-7.6	7.6	7.6	7.5-7.6				
Oxygen [mg/l]	8.2	7.9-8.1	8.2-8.7	8.1-8.5				
Salinity [ppt]	15	14	15	15				

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

Table A7.4.1.2.b/01-7: Effect data

	LC_{50}[1]	95 % c.l.	LC_0[1]	LC_{100}[1]
24 h [µg DCOIT/l]	> 13.5 (m)	---	5.2 (m)	---
48 h [µg DCOIT/l]	7.9 (m)	5.2-13.5	---	13.5 (m)
72 h [µg DCOIT/l]	5.1 (m)	3.9-6.8	---	7.6 (m)
96 h [µg DCOIT/l]	4.7 (m)	3.4-6.1	---	7.6 (m)

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

Table A7.4.1.2.b/01-8: Validity criteria

	fulfilled	Not fullfilled
Mortality of control animals <10%	**yes**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**yes**	

Figure A7.4.1.2b/01-1: Survival of organisms exposed to the test substance for 96 hours

Amended 11/28/90

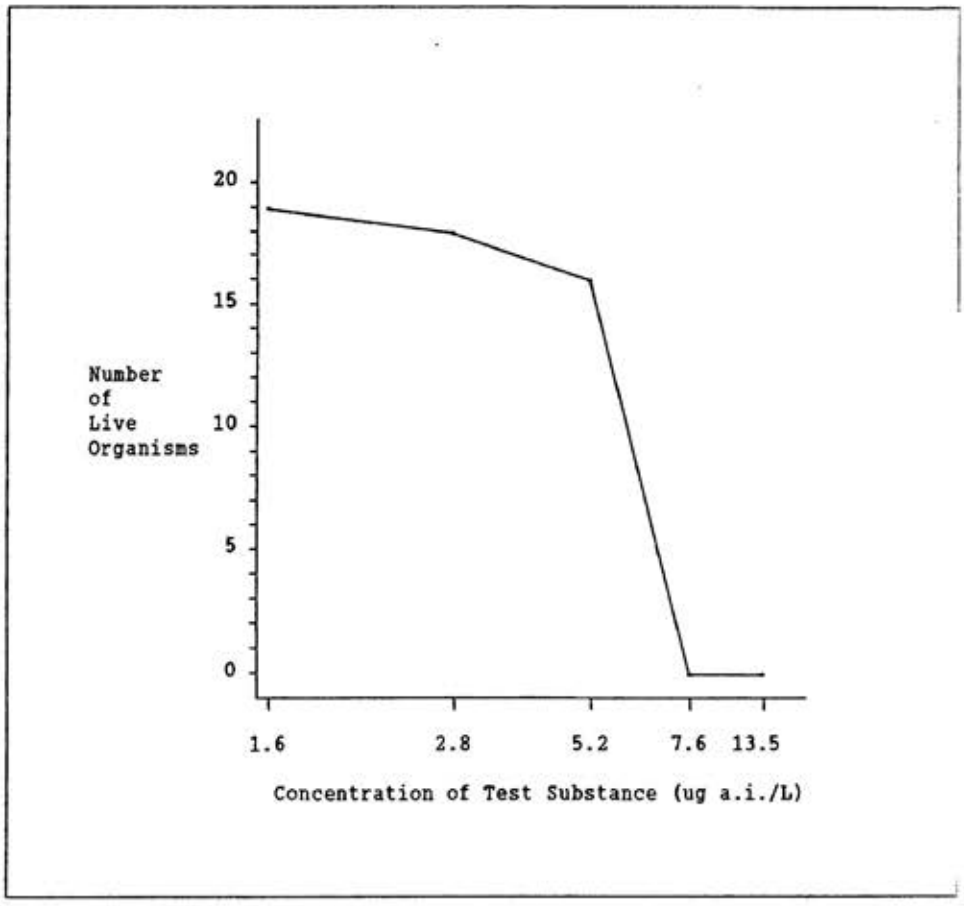

Figure 1. Survival of organisms exposed to the test substance for 96 hours

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Section A7.4.1.2.b/02 Acute toxicity of DCOIT to invertebrates-Marine water, Embryos of American oyster

Annex Point IIA VII.7.2

Official use only

		1 REFERENCE
1.1	**Reference**	Reference type: Study report Year: 1990 Report date: 14 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 72-3
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	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 test substances**	[redacted]
3.3	**Reference substance**	[redacted]
3.4	**Testing procedure**	

Section A7.4.1.2.b/02 **Acute toxicity of DCOIT to invertebrates-Marine water, Embryos of American oyster**

Annex Point IIA VII.7.2

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 A7.4.1.2.b/02-5
3.4.5	Duration of the test	48 h
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	Nominal (µg/L) for natural and synthetic estuarine water 0.56, 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56
4.2.2	Actual concentrations of test substance	Natural estuarine water

Nom.	4-h conc.	48-h conc.	geom. mean (nominal-48h)
0.56	0.086	0.031*	0.13
1	0.25	0.031	0.18
1.8	0.76	0.031	0.24
3.2	1.3	0.031	0.32
5.6	2.8	0.031	0.42
10	5	0.031	0.56
18	13	0.25	2.12
32	22	4.9	12.5
56	48	29	40.3

x

Section A7.4.1.2.b/02 **Acute toxicity of DCOIT to invertebrates-Marine water, Embryos of American oyster**

Annex Point IIA VII.7.2

*LOQ/2

Synthetic estuarine water

Nom.	4-h conc.	48-h conc.	geom. mean (nominal-48h)
0.56	0.34	0.031*	0.13
1	0.83	0.031	0.18
1.8	1.5	0.031	0.24
3.2	2.8	0.077	0.50
5.6	4.6	0.1	0.75
10	8.2	0.71	2.66
18	16	2.7	6.97
32	28	9.9	17.8
56	54	26	38.2

*LOQ/2

4.2.3	Effect data (Mortality, abnormal shell development)	see table A7.4.1.2.b/02-6; see table A7.4.1.2.b/02-7	
4.2.4	Concentration / response curve	In natural estuarine water, there was an extremely rapid die-away of DCOIT in all treatments used in this test. After 48 hr, DCOIT was detectable in only the two highest concentrations. In synthetic estuarine water, the die-away of DCOIT was slower. See Figure A7.4.1.2.b/02-1.	x
4.2.5	Other effects	mortality and abnormal shell development	
4.3	**Results of controls**	normal	x
4.4	**Test with reference substance**	Not performed	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	US EPA FIFRA 72-3, Acute static 48h oyster study with analytical confirmation of test solution concentrations.	
5.2	**Results and discussion**		x
5.2.1	NOEC and LOEC	Based on nominal concentrations: 48h NOEC=10 µg/L (n) synthetic estuarine water; 18 µg/L (n) natural estuarine water	

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Section A7.4.1.2.b/02 **Acute toxicity of DCOIT to invertebrates-Marine water, Embryos of American oyster**

Annex Point IIA VII.7.2

		48h LOEC=18 µg/L (n) synthetic estuarine water; 32 µg/L (n) natural estuarine water	
		Based on geometric mean concentrations: 48h-EC10 = 0.5 µg/L for natural estuarine water (probit analysis) 48h-EC10 = 0.2 µg/L for synthetic estuarine water (probit analysis)	
5.2.2	EC_{50}	Based on nominal concentrations: 48 h = 12.1 µg/L (n) synthetic estuarine water and 24 µg/L (n) natural estuarine water	x
		Based on geometric mean concentrations: 48h-EC50 = 3.2 µg/L for natural estuarine water (probit analysis) 48h-EC50 = 2.1 µg/L for synthetic estuarine water (probit analysis)	
5.2.3	EC_{100}	Based on nominal concentrations 48 h = 56 µg/L (natural estuarine water)	
5.3	**Conclusion**	see validity criteria summarized in table A7.4.1.2.b/02-8	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	29 November 2007
Materials and Methods	**Comment (4.2.2 and 4.2.4):** There was a slower but still consiberable decline in test substance concentrations in the synthetic estuarine water as well.

Results and discussion	**Comment (4.3 and 5.2):** The results for the control do not seem to show a normal pattern (see table A7.4.1.2b/02-6). In the control/solvent control and also at several test concentrations the mean number alive after 48 hours is higher than after 4 hours. In the report it is stated there were some technical errors associated with organisms counts. In addition the counts were performed on 4 x 5 ml aliquots sampled from each treatment solution containing embryos. The aliquots were composited and aliquots of the composited sample were counted. It can be assumed that the variability in the counts was due to the counting issues and the natural variability associated with sampling culture solutions that may or may not be homogeneous at the time of the sampling. In the test with synthetic estuarine water no abnormal shell was observed. However, there are no data in the report to refute the information suggesting that there were no embryos in the synthetic sea water with abnormal shell development. It is quite possible that because the die-away of the test material in synthetic sea-water was slower than in the natural sea water that the toxicity response as a function of increased exposure to the test substance was increased to the point where mortality was the notable effect.
Conclusion	**Comment (5.2.2):** The 48h-EC50 of 3.2 µg/L for natural estuarine water and the 48h-EC50 of 2.1 µg/L for synthetic estuarine water are used as endpoints from this study. These values are obtained by conducting a probit analysis using the mean measured concentrations presented in the tables under point 4.2.2. **Comment (5.3):** The validity criterion that the concentration of the test substance should be ≥80% of in initial concentrations is not fulfilled.
Reliability	**Comment (5.3.1 and 5.3.2):** Due to the deficiencies described, the reliability is changed from 1 to 2, valid with restrictions.
Acceptability	Acceptable with the restrictions noted above.
Remarks	-

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

Section A7.4.1.2.b/02 Annex Point IIA VII.7.2	Acute toxicity of DCOIT to invertebrates-Marine water, American Oyster Embryo – **TABLES AND FIGURES**

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

Table A7.4.1.2.b/02-5: Test conditions

Criteria	Details
Test temperature	25 °C
Dissolved oxygen	7.12 (90.7% saturation)
pH	7.8
Adjustment of pH	Not described
Salinity	16 g/kg
Aeration of dilution water	Yes
Quality/Intensity of irradiation	Not described
Photoperiod	Not described

Table A7.4.1.2.b/02-6: Mortality data – natural estuarine water

Test-Substance Concentration (nominal) [1] [µg/l]	Mean Number Alive/20 mL		Mean Number Abnormal Shell	Oxygen [mg/l]	Salinity [ppt]	pH	Temperature [°C]
	4 h	48 h		48 h	48 h	48 h	48 h
0 (control)	221	339	6.3	6.9	18	8	26
0 (solvent control)	263	323	8.0	6.9	18	8	26
0.56	248	271	5.3	6.8	18	8	26
1.0	323	320	5.0	6.9	18	8	26
1.8	310	271	7.7	6.9	18	8	26
3.2	297	269	10.7	6.8	18	8	26
5.6	289	332	9.3	6.9	18	8	26
10	269	323	6.7	6.9	18	8	26
18	211	280	9.0	6.8	18	8	26
32	280	0.3	1.3	6.9	18	8	26
56	283	0.0	0.0	7.0	18	8	26

Mortality data – synthetic estuarine water

Test-Substance Concentration (nominal) [1] [µg/l]	Mean Number Alive/20 mL		Abnormal Shell	Oxygen [mg/l]	Salinity [ppt]	pH	Temperature [°C]
	4 h	48 h		48 h	48 h	48 h	48 h
0 (control)	196	131	0.0	6.9	16	7.5	25
0 (solvent control)	131	109	0.0	6.9	16	7.8	25
0.56	219	103	0.0	6.9	16	7.7	25
1.0	197	131	0.0	6.9	16	7.8	25
1.8	150	119	0.0	7.0	16	7.8	25
3.2	225	89	0.0	6.8	16	7.8	26
5.6	155	93	0.0	6.9	16	7.8	25
10	166	90	0.0	6.9	16	7.8	25
18	184	14	0.0	7.0	16	7.7	25
32	200	4.0	0.0	6.9	16	7.8	25
56	168	1.7	0.0	6.9	16	7.8	25

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

Table A7.4.1.2.b/02-7: Effect data-Synthetic estuarine water

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
4 h [µg/l]	---	---	---	---
48 h [µg/l]	12.1 (n)	7.2 – 17.1 (n)	10 (n)	56 (n)

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

Table A7.4.1.2.b/02-8: Validity criteria

	fulfilled	Not fullfilled
Mortality of control animals <10%	**yes**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**yes**	
Concentration of test substance ≥80% of initial concentration during test		**yes**

Figure A7.4.1.2b/02-1: Semi-logarithmic plot of DCOIT against time for definitive trial with natural estuarine water (Figure 1) and synthetic estuarine water (Figure 2).

Rohm and Haas Report Number 89RC-0037

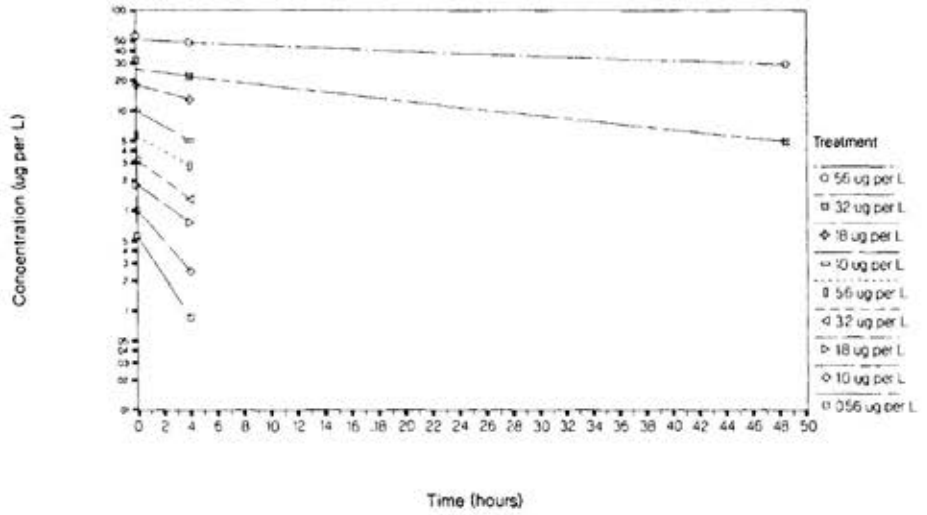

Figure 1. Semi-logarithmic plot of RH-287 concentration against time for definitive trial with natural estuarine water. RH-287 was not detected in treatments with concentrations below 32 µg/L after 48 hr

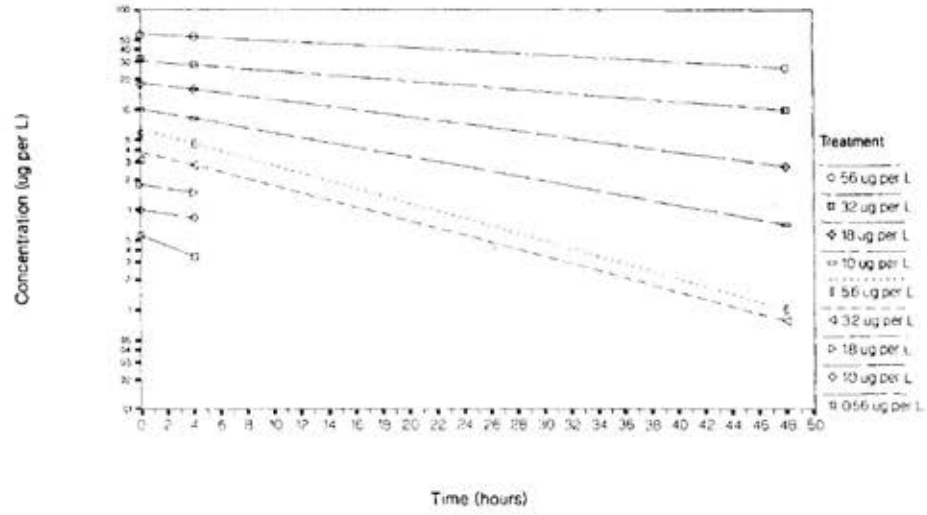

Figure 2. Semi-logarithmic plot of RH-287 concentration against time during definitive trial with synthetic estuarine water

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Section A7.4.1.2.b/03 Acute toxicity of DCOIT to invertebrates-Marine water, embryos of bay mussel

Annex Point IIA VII.7.2

Official use only

		1 REFERENCE
1.1	**Reference**	Reference type: Study report Year: 2001 Report date: 9 November 2001 [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, EPA OPPTS 850.1055
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	99.3 % 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 test substances**	[redacted]
3.3	**Reference substance**	[redacted]
3.4	**Testing procedure**	

Section A7.4.1.2.b/03 Acute toxicity of DCOIT to invertebrates-Marine water, embryos of bay mussel

Annex Point IIA VII.7.2

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 A7.4.1.2.b/03-5
3.4.5	Duration of the test	48 h
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	Nominal (mg DCOIT/L) 0.33, 0.65, 1.3, 2.5, 5.0
4.2.2	Actual concentrations of test substance	measured concentration (mg DCOIT/L)

0 hr	48 hr	Mean
0.241	0.173	0.207
0.442	0.411	0.426
0.753	0.817	0.785
0.760	1.47	1.11
0.727	2.19	1.46

Low recoveries were possibly due to solubility of the TS in dilution water.

4.2.3	Effect data (Mortality	see table A7.4.1.2.b/03-6; see table A7.4.1.2.b/03-7
4.2.4	Concentration / response curve	The slope of the 48 hour concentration-response curve is 5.6. See Figure A7.4.1.2.b/03-1.
4.2.5	Other effects	mortality
4.3	**Results of controls**	No adverse effects Chemical analysis showed quantifiable amounts of DCOIT in both

x

		control samples (98 µg/l and 5.9 µg/l on day 0). The quantifiable level of test substance found in several control and solvent control samples at 0 and 48-hours seems to be the result of contamination of the analytical samples during sample preparation. This is substantiated by acceptable control responses in both the control and solvent control test vessels. The mean measured concentration of the test substance in the control and solvent control treatment groups equalled < 0.0508 and < 0.00489 mg a.i./L, respectively. It is assumed that the testing laboratory had substantiating evidence regarding control survivability in their historical database. Further the loss of embryos constituted a small percentage equalling 9.6 and 8% in the control and solvent control treatment groups, respectively, from the time zero initial embryo counts of 25 per treatment group.
4.4	**Test with reference substance**	Performed

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	EPA OPPTS 850.1055, Acute static toxicity study with analytical confirmation of test solution concentrations.	
5.2	**Results and discussion**	48 h NOEC = 0.207 mg DCOIT/L	
5.2.1	EC_0	48 h EC_{50} = 0.207 mg DCOIT/L	
5.2.2	EC_{50}	48 h EC_{50} = 0.411mg DCOIT/L	
5.2.3	EC_{100}	48 h EC_{50} = 1.11 mg DCOIT/L	
5.3	**Conclusion**	The issue regarding the analytical test substance determinations in both controls was significant. However, based on the clear dose response the study is considered valid nevertheless. See also table A7.4.1.2.b/03-8	
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	29 November 2007
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (4.2.2):** At the two highest tested concentrations the values measured at 48 hours are 200-300% of the initial measured concentrations. Considering the fact that DCOIT is rapidly degradable in the aquatic environment and that this test is a static test, there seemed to be problems with the analytical method. However, the EC50 is well below these concentration levels and results are based on mean measured concentrations.
Conclusion	Agree with applicant's version
Reliability	**Comment (5.3.1 and 5.3.2):** Based on the fact that measureable concentrations of DCOIT were found in the control cultures and the deficiencies described under comment 4.2.2, the reliability is changed from 1 to 2, reliable with restictions.
Acceptability	Acceptable with the restrictions noted above.
Remarks	-

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

Section A7.4.1.2.b/03	Acute toxicity of DCOIT to invertebrates-Marine water, Bay Mussel embryos – **TABLES AND FIGURES**

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Table A7.4.1.2.b/03-5: Test conditions

Criteria	Details
Test temperature	15.9 to 17.4 degrees C
Dissolved oxygen	6.7 to 7.9 mg/L
pH	7.9 tp 8.1
Adjustment of pH	No
Salinity	34 to 35 ppt
Aeration of dilution water	No
Quality/Intensity of irradiation	40 footcandles
Photoperiod	16 h daylight, 8 h dark with 15 minute transition period

Table A7.4.1.2.b/03-6: Mortality data

Test-Substance Concentration (mean measured) [1] [mg DCOIT/l]	Mortality	
	Mean Number embryos/mL 48 h	Percentage (%) 48 h
0 (control)	23	8
0 (acetone control)	23	8
0.207	22	12
0.426	10	60
0.785	2	92
1.11	0	100
1.46	0	100

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

Table A7.4.1.2.b/03-7: Effect data

	EC_{50} [1]	95 % c.l.	EC_0 [1]	EC_{100} [1]
48 h [mg DCOIT/l]	0.411 (m)	0.380 – 0.443	0.207 (m)	1.11 (m)

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

Table A7.4.1.2.b/03-8: Validity criteria

	fulfilled	Not fullfilled
Mortality of control animals <10%	**yes**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**yes**	
Concentration of test substance ≥80% of initial concentration during test		**yes**

Figure A7.4.1.2b/03-1: Survival of embryos of the bay mussel, *Mytilus edilus*, exposed to DCOIT for 48 hours

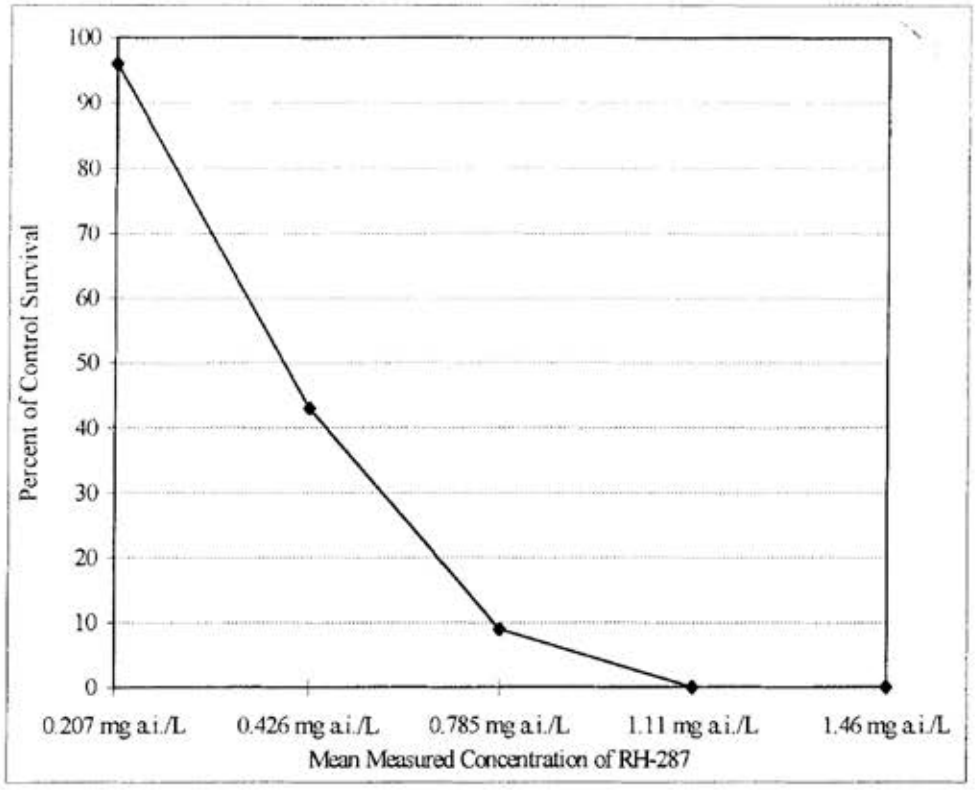

Figure 1. Survival of embryos of the bay mussel, *Mytilus edulis*, exposed to RH-287 Technical for 48 hours.

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

Section A7.4.1.2.c/01 Acute toxicity of N-(n-octyl) malonamic acid to invertebrates-Fresh water, *Daphnia magna*

Annex Point IIA VII.7.2

Official use only

		1 REFERENCE
1.1	**Reference**	Reference type: Study report Year: 2002 Report date: 22 May 2002 [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 Guideline 850.1010 and OECD Guideline 202
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% NNOMA
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]
3.3.1	Method of analysis for reference	[redacted]

Section A7.4.1.2.c/01 **Acute toxicity of N-(n-octyl) malonamic acid to invertebrates-Fresh water, *Daphnia magna***

Annex Point IIA VII.7.2

	substance	
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 A7.4.1.2.c/01-5
3.4.5	Duration of the test	48 h
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	Nominal (mg NNOMA/L) 9.4, 19, 38, 75, 150, 300
4.2.2	Actual concentrations of test substance	measured concentration (mg NNOMA/L)

0 hr	48 hr	mean
9.0	7.4	8.2
20	17	19
42	28	35
67	70	69
135	115	125
300	263	282

4.2.3	Effect data (Immobilisation)	see table A7.4.1.2.c/01-6); see table A7.4.1.2.c/01-7
4.2.4	Concentration / response curve	The slope of the 48-hour dose-response line was 7.7 as calculated by the regression analysis of percent immobility transformed to probits versus log concentration. See Figure A7.4.1.2.c/01-1.
4.2.5	Other effects	quiescent
4.3	**Results of controls**	normal

x

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Section A7.4.1.2.c/01 — **Acute toxicity of N-(n-octyl) malonamic acid to invertebrates-Fresh water, *Daphnia magna***

Annex Point IIA VII.7.2

4.4	**Test with reference substance**	Not performed
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	US EPA OPPTS Guideline 850.1010 and OECD Guideline 202, Acute 48h *Daphnia magna* static toxicity study with analytical confirmation of test substance concentrations.
5.2	**Results and discussion**	48 h NOEC = 69 mg NNOMA/L
5.2.1	EC_0	48 h = 69 mg NNOMA/L
5.2.2	EC_{50}	48 h = 157 mg NNOMA/L
5.2.3	EC_{100}	48 h = 282 mg NNOMA/L
5.3	**Conclusion**	see table A7.4.1.2.c/01-8
5.3.1	Reliability	(1), reliable without restriction
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	29 August 2006
Materials and Methods	Agree with applicant's version
Results and discussion	**Comment (4.2.2):** At the 38 mg/l test level the measured concentration after 48 hours was only 67% of the initial measured concentration, and strictly speaking, the validity criterion that the concentration of the test substance should be ≥80% of in initial concentrations is not fulfilled. However, this is only at one measurement and the EC50 is above this concentration limit. Therefore the test is still considered valid without restrictions.
Conclusion	Agree with applicant's version
Reliability	1, reliable without restrictions
Acceptability	Acceptable
Remarks	-

Section A7.4.1.2.c/01	Acute toxicity of NNOMA to invertebrates-Fresh water, Daphnia
	TABLES AND FIGURES

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Table A7.4.1.2.c/01-5: Test conditions

Criteria	Details
Test temperature	20 ± 1 °C
Dissolved oxygen	8.7 to 8.8 mg/L at test initiation and remained ≥ 7.7 mg/L throughout the remainder of the test
pH	8.2 to 8.4
Adjustment of pH	Yes
Aeration of dilution water	not described
Quality/Intensity of irradiation	543 Lux
Photoperiod	16-h daylight with 30-minutes simulated dawn and dusk periods

Table A7.4.1.2.c/01-6: Immobilisation data

Test-Substance Concentration (mean measured) [1] [mg NNOMA/l]	Immobile *Daphnia*						
	Number		Percentage (%)		Oxygen [mg/l]	pH	Tempera-ture [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
0 (control)	0	0	0	0	8.3	8.3	19.3
8.2	0	0	0	0	8.4	8.4	19.3
19	0	0	0	0	8.4	8.4	19.3
35	0	0	0	0	8.4	8.4	19.4
69	0	0	0	0	8.4	8.4	19.3
125	0	5/20	0	25	8.4	8.4	19.4
282	0	20/20	0	100	8.4	8.4	19.4

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

Table A7.4.1.2.c/01-7: Effect data

	EC_{50} [1]	95 % c.l.	EC_0 [1]	EC_{100} [1]
24 h [mg NNOMA/l]	---	---	> 282	---
48 h [mg NNOMA/l]	157 (m)	137–180	69 (m)	282 (m)

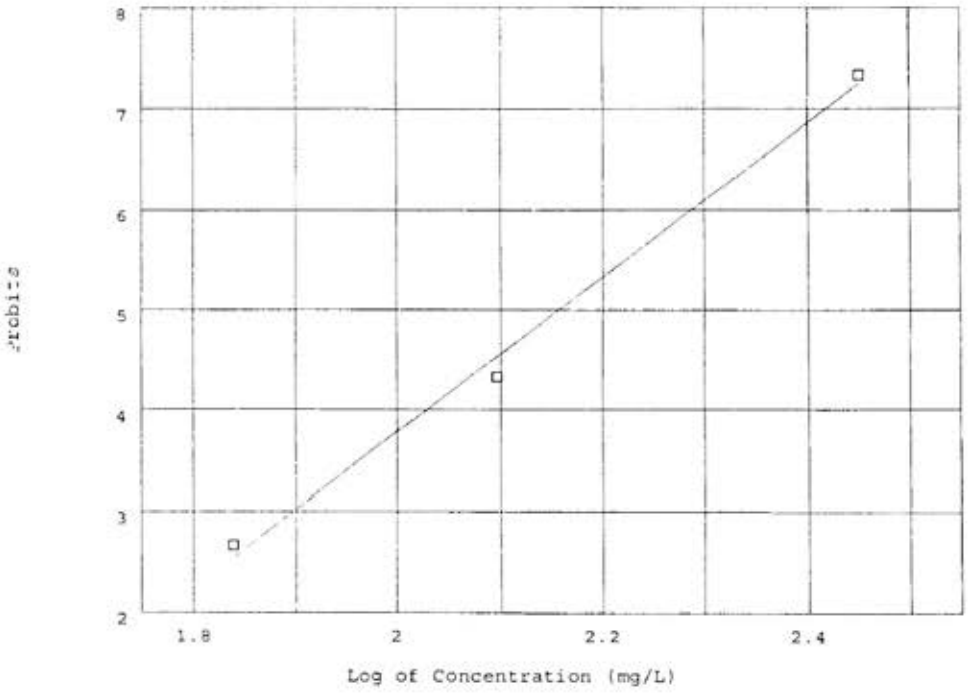
[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.4.1.2.c/01-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	Not fullfilled
Immobilisation of control animals <10%	**yes**	
Control animals not staying at the surface	**yes**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**yes**	

Figure A7.4.1.2.c/01-1: Concentration-Response Curve for *Daphnia magna* exposed to N-(n-octyl) Malonamic acid

Figure 1. Concentration-Response Curve for *Daphnia magna* Exposed to N-(n-octyl) Malonamic Acid, Technical, PMN



The slope of the concentration-response line is 7.7.

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

Section A7.4.1.2.c/02 **Acute toxicity of N-(n-octyl) acetamide to invertebrates- Fresh water, *Daphnia magna***

Annex Point IIA VII.7.2

Official use only

		1 REFERENCE
1.1	**Reference**	Reference type: Study report Year: 2002 Report date: 25 June 2002 [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 850.1010, OECD 202, US EPA TSCA 797.1300, US EPA FIFRA 72-2, and EC Council Directive 91/414/EEC
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% NNOA
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]
3.3.1	Method of analysis for reference	[redacted]

Section A7.4.1.2.c/02 **Acute toxicity of N-(n-octyl) acetamide to invertebrates- Fresh water, *Daphnia magna***

Annex Point IIA VII.7.2

	substance		
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 A7.4.1.2.c/02-5	
3.4.5	Duration of the test	48 h	
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	Nominal (mg a.i./L) 2.5, 5.0, 10, 20, 40	
4.2.2	Actual concentrations of test substance	measured concentration (mg NNOA/L)	
4.2.3	Effect data (Immobilisation)	see table A7.4.1.2.c/02-6); see table A7.4.1.2.c/02-7	x
4.2.4	Concentration / response curve	The slope of the 48-hour dose-response line was 14 as calculated by the regression analysis of percent immobility transformed to probits versus log concentration. See Figure A7.4.1.2.c/02-1 below.	x
4.2.5	Other effects	quiescent	
4.3	**Results of controls**	normal	
4.4	**Test with**	Not performed	

0 hr	48 hr	mean
2.61	2.24	2.4
5.24	5.23	5.2
9.35	9.61	9.5
18.4	18.6	19
44.6	37.1	41

Section A7.4.1.2.c/02 **Acute toxicity of N-(n-octyl) acetamide to invertebrates- Fresh water, *Daphnia magna***

Annex Point IIA VII.7.2

	reference substance		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	US EPA OPPTS 850.1010, OECD 202, US EPA TSCA 797.1300, US EPA FIFRA 72-2, and EC Council Directive 91/414/EEC, Acute static 48h *Daphnia magna* toxicity study with analytical confirmation of test substance concentrations.	
5.2	**Results and discussion**		
5.2.1	EC_0	48 h = 9.5 mg NNOA/L	
5.2.2	EC_{50}	48 h = 28 mg NNOA/L	
5.2.3	EC_{100}	48 h = 41 mg NNOA/L	
5.3	**Conclusion**	see table A7.4.1.2.c/02-8	
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	29 November 2007
Materials and Methods	Agree with Applicant's version
Results and discussion	**Comment (4.2.3 and 4.2.4):** At the highest concentration there was 100% mortality, while in the next lower concentration 0% mortality was observed. In this case the LC50 value can be calculated as the geometric mean value of these two concentrations as it has been done in this study summary. However, no clear dose-response-relationship can be established with this approach. The result can nevertheless be used, as it shows that the metabolite NNOA is far less toxic to invertebrates than the parent DCOIT.
Conclusion	Agree with Applicant's version
Reliability	**Comment (5.3.1 and 5.3.2):** Due to the lack of a dose-response-relationship, the reliability is changed from 1 to 2, reliable with restrictions.
Acceptability	Acceptable with the restrictions noted above.
Remarks	-

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

Section A7.4.1.2.c/02	Acute toxicity of N-(n-octyl) acetamide to invertebrates-Fresh water, *Daphnia magna* – **TABLES AND FIGURES**

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Table A7.4.1.2.c/02-5: Test conditions

Criteria	Details
Test temperature	20.2 to 20.8 °C
Dissolved oxygen	6.6 to 10 mg/L
pH	7.9 to 8.4
Adjustment of pH	not described
Aeration of dilution water	not described
Quality/Intensity of irradiation	707 lux
Photoperiod	16 hr light, 8 hr dark, 30 minute simulated dawn and dusk