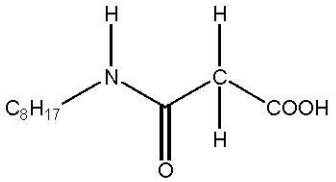
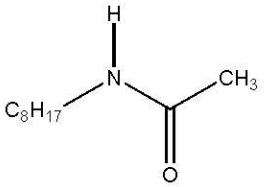
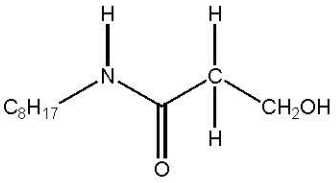
Table A7.1.2.2.2.c-10: Quantitation of DCOIT in Sediment Stored at Room Temperature and Frozen a nd Subsequently Soxhlet Extracted

Day/Sample	Percent in DCM:MeOH	Percent of ^{14}C-Applied	Percent of ^{14}C-Recovered
Day 0			
Frozen	95.83	90.56	86.50
Day 35			
Frozen	88.59	90.67	87.37
Room Temp.	82.60	92.73	81.08
Day 63			
Frozen	83.42	91.11	82.50
Day 98			
Frozen	94.67	87.76	93.52
Day 136			
Frozen	99.14	90.10	96.92
Room Temp.	86.23	108.86	85.52
Day 189			
Frozen	100.00	110.73	99.59
Room Temp.	98.92	105.25	98.07
Day 224			
Frozen	98.29	104.48	96.97
Room Temp.	96.66	100.38	95.36
Average	93.12 ± 6.14	97.51 ± 8.14	91.22 ± 6.42

Table A7.1.2.2.2.c-11: Quantitation of Control Sediment Spiked With ^{14}C-DCOIT, Stored at Room Temperature, and Subsequently Soxhlet Extracted

Day	Quantitation of DCOIT		
	Percent in DCM:MeOH Soxhlet	Percent of ^{14}C-Applied	Percent of ^{14}C-Recovered
98	100.00	81.16	99.58
189	100.00	117.39	99.82
224	98.73	108.88	98.55
Average	99.58 ± 0.60	102.48 ± 15.47	99.32 ± 0.55

Table A7.1.2.2.2.c-12: Quantitation and Identification of Metabolites

Compound[1]	Percent of Applied Activity	Structure
N-(n-octyl) malonamic acid (B and C-2)	16.0	
N-(n-octyl) acetamide (A and D-3)	12.4	
N-(n-octyl)-β-hydroxypropionamide (D-1 and D-2)	4.1	
Unidentified metabolites[2]	11.8	
Residue: 1N HCl soluble	2.9	
Residue: 1N NaOH soluble	10.6	
Methanol wash	3.8	
Bound Residue	19.2	

[1] Chromatographic bands from Table A7.1.2.2.2.c-8 are in parenthesis
[2] Unidentified metabolites are the 10 minor HPLC peaks from Table A7.1.2.2.2.c-8. The largest peak comprising 2.8% of the applied radioactivity

Table A7.1.2.2.2.c-13: Extent and Nature of Bound Residues

Day	Percent of Applied in Sediment after Soxhlet extraction	Percent of Applied ^{14}C-Activity (% bound residue)				Percent Recovery of Bound Residues
		0.25N HCl Reflux	Humin	Humic Acid	Fulvic Acid	
0	53.4	0.1 (0.2)	53.4 (100)	6.5 (12.2)	0.7 (1.3)	113.6
30	60.3	0.1 (0.2)	45.3 (75.1)	5.1 (8.5)	1.2 (2.0)	85.7

[redacted]

[redacted]

Section A7.1.2.2.2.d Sea Water/Sediment degradation study - Anaerobic

Annex Point IIIA XII 2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	**Kinetics and metabolite characterization:** Reference type: Study report Year: 1991 Report date: 21 March 1991 [redacted] **Nature of bound residues:** Reference type: Study report Year: 1992 Report date: 19 May 1992 [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Rohm and Haas Company	
1.2.2			
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline Study**	Yes. U.S. Environmental Protection Agency, 40 CFR § 158, Subdivision N, Chemistry, Environmental Fate 162-3	
2.2	**GLP**		
2.3	**Deviations**	GLP deviations were minor. • Sediment and seawater physiochemical characterization was not performed under GLP guidelines. • The ^{14}C DCOIT test material was synthesized prior to the initiation of GLP regulations (October 16, 1989). The material has been characterized subsequently following GLP guidelines. • The ^{13}C DCOIT used in this study was not in compliance with GLP. Purity analysis was scientifically valid but it was not done according to GLP guidelines.	x

- The ^{12}C chromatography standards while completely characterized, were done so prior to the implementations of GLP.

3 MATERIAL AND METHODS

3.1 Test Material

^{14}C-DCOIT (RH-5287) (^{14}C), [redacted]

3.1.1 Lot/Batch number [redacted]

3.1.2 Purity [redacted]

3.1.3 Further relevant properties [redacted]

3.2 Reference substances [redacted]

3.2.1 Nature of reference substances [redacted]

3.3 Sediment and Water Characterization [redacted]

3.4 Test procedures

3.4.1 Test system [redacted] x

Document III-A / Section A7.1.2

Section A7.1.2.2.2.d **Sea Water/Sediment degradation study - Anaerobic**
Annex Point IIIA XII 2.1

		[redacted]	
3.4.2	Preparation of test solution	[redacted]	
3.4.3	Initial Test substance concentration	[redacted]	
3.4.4	Duration of test	[redacted]	
3.4.5	Sampling details	[redacted]	x
3.4.6	Replicates	[redacted]	
3.4.7	Extraction procedures	[redacted]	
3.4.8	Bound residues-extent and nature	[redacted]	

Section A7.1.2.2.2.d **Sea Water/Sediment degradation study - Anaerobic**
Annex Point IIIA XII 2.1

3.4.9 Analytical methods

3.4.10 Degradation products

4 RESULTS

4.1 Distribution and recovery of radioactivity

The distribution of ^{14}C-activity between the water phase, the Soxhlet extractions, volatiles, and bound residues for the 0.05 ppm and 1 ppm dosing levels is presented in Tables A7.1.2.2.2.d-3 and A7.1.2.2.2.d-4, respectively. Over the 12 sampling intervals, the water phase averaged 6.6 ± 2.6% and 5.5 ± 1.9% of the applied ^{14}C-activity for the 0.05 ppm and 1 ppm dosing levels, respectively. The remaining activity was primarily associated with the sediment with less than 9% being detected as volatiles (predominately $^{14}CO_2$ as the amount of activity in the ethylene glycol trap was minimal). Soxhlet extractable residue generally decreased with time ranging from approximately 10-38% and 20-40% for the 0.05 ppm and 1 ppm dosing levels, respectively. The percent of applied activity in the bound residue ranged from about 40-67% (mean ~50%) for the 0.05 dosing level. For the 1 ppm dosing level it ranged from about 25-51% (mean ~41%). Recovery of applied ^{14}C-activity was 86.8 ± 10.0% and 83.5 ± 9.9% for the 0.05 ppm and 1 ppm dosing rates, respectively.

The results from chromatography of the extractable ^{14}C-activity for both dosing levels appear in Table A7.1.2.2.2.d-5. On Day 0, parent was present at less than 3% for both dosing levels. It took approximately 1 hour to process (separate phases, add sodium sulfate and precipitated silica) and biologically inactivate the sediment, which contained over 90% of the ^{14}C-activity. Thus Day 0 is really Hour 1 (or Day 0.04). Most of the ^{14}C-activity was chromatographically polar with two major peaks having retention times of approximately 4 and 7 minutes. Based on retention time of standards and chemistry, these two peaks correspond to N-(n-octyl) malonamic acid and N-(n-octyl) oxamic acid for the peak at 4 minutes and N-(n-octyl) acetamide for the peak at 7 minutes.

4.2 Half-life

The results in Table A7.1.2.2.2.d-5 show that on Day 0 less than 3% of the applied activity was DCOIT. Except for an anomaly on Day 14 at 0.05 ppm dosing level essentially no parent compound was subsequently detected. Immediately after dosing and mixing the ^{14}C- DCOIT into the system, the water and sediment phases were separated. The water phase was immediately partitioned with dichloromethane and then chromatographed. The sediment phase, which contained over 90% of

x

	the applied radioactivity, was immediately suspended in sodium sulfate/precipitated silica matrix and placed into a freezer. Thus the Day 0 sample is actually Day 0.04 (1 hour) since it took this long to process and bio-inactivate the sample. Due to this rapid biodegradation, it was impossible to accurately calculate the half-life. Based on the available data, the half-life of DCOIT in this anaerobic seawater:sediment system is less than 1 hour.	
4.3 Identification of metabolites	$^{14}CO_2$ was a major metabolite and its presence can only occur if the isothiazolone ring is cleaved and undergoes subsequent oxidation. Due to insufficient quantities of metabolites, mass spectral identification was unsuccessful. However, the chromatographically polar metabolites observed cochromatographed with the standards N-(n-octyl) malonamic acid, N-(n-octyl) oxamic acid, and N-(n-octyl) acetamide. Additionally, the polar metabolites observed in this anaerobic study were chromatographically similar to those seen in the aerobic aquatic study. In the aerobic study, a supplemental study showed by mass spectroscopy that the major metabolites were N-(n-octyl) malonamic and acid, N-(n-octyl) acetamide and N-(n-octyl)-β-hydroxypropionamide was identified as a minor constituent.	
4.4 Extent and nature of bound residues	Reference 2 describes the extent and nature of the bound residues. Extracted sediments were exhaustively extracted with 0.25 N HCl followed by 1N NaOH. The results for samples from Day 0 and 365 are presented in Table A7.1.2.2.2.d-6. Over 70% of the bound residue is associated with the insoluble fraction of humin. The n-octyl chain probably acts similar to surfactants and polymers whose nonpolar tails have been shown to intercalate within the lattice framework of minerals such as clay and montmorillonites	x
4.5 Metabolic pathway	A proposed metabolic pathway is presented in Figure A7.1.2.2.2.d-1.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The test guidelines followed were the U.S. Environmental Protection Agency, 40 CFR § 158, Subdivision N, Chemistry, Environmental Fate 162-3. Between the original report and the supplemental report there are no deficiencies of the test guidelines. Erlenmeyer flasks containing 54.1 g wet sediment (20 g dry weight) and 66 ml of seawater obtained from the York River, Virginia, USA plus glucose were flushed with nitrogen and placed into an incubator to establish anaerobic conditions. After 30 days the flasks were dosed with either 0.05 ppm or 1 ppm of ^{14}C DCOIT. On Day 0, 1, 5, 7, 14, 29, 61, 90, 120, 180, 270 and 365, volatiles were trapped, the sediment and water phases separated, and these three phases quantitated by radioassay. The sediment phase was extracted with methylene chloride:methanol followed by methanol alone. Parent was quantitated in the extracts by reversed phase HPLC. Extracts were chromatographed by reversed phase HPLC and characterized by comparison to standards. The bound residues from the extracted sediments were exhaustively extracted using 0.25 N HCl and 1 N NaOH. The basic extract was further separated into humic acid, fulvic acid, and humin fractions.	
5.2 Results and	In the anaerobic seawater:sediment microcosm studied, the half-life of	

discussion		RH-5287 was less than 1 hour. This was derived from the Day 0 samples which approximately took 1 hour to bio-inactivate. In these samples less than 3% of the applied radioactivity was parent. At all sampling intervals over 85% of the ^{14}C-activity was detected in the sediment phase. About 30-60% of the radioactivity was detected in the bound residues. At the study termination, $^{14}CO_2$ comprised about 7-8% of the applied radioactivity. Over 70% of the activity in the bound residue was present in the insoluble humin fraction and indicates that the octyl chain is intercalating within the soil crystal lattice. The presence of $^{14}CO_2$ demonstrates that the isothiazolone ring has been cleaved and undergone additional oxidation. The metabolites have been characterized by comparison of HPLC retention times of unknowns and standards as being N-(n-octyl) malonamic acid and N-(n-octyl) acetamide. Based on results from the aerobic study, the reduced metabolite N-(n-octyl)-β-hydroxypropionamide is probably also present.	
5.3	**Conclusion**	DCOIT rapidly biodegrades in anaerobic seawater:sediment microcosm with a half-life of less than 1 hour. The half-life in several other biologically active matrices has also been shown to be rapid. Metabolism involves cleavage of the isothiazolone ring and subsequent oxidation to metabolites such as N-(n-octyl) malonamic acid and N-(n-octyl) acetamide. Both of these compounds have been shown to be readily biodegradable (OECD 301B; see section A7.1..2.3).	x
5.3.1	Reliability	2-valid with restrictions	
5.3.2	Deficiencies	None per guidelines followed. Due to low dosing rates and insufficient quantities of metabolites isolated, mass spectral identification was not possible. However, the isolated metabolites are chromatographically similar to those identified in the aerobic study.	x

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	29 June 2007, revised 7 August 2009, revised 3 June 2010
Materials and Methods	**Comment (2.3)**: US EPA Guideline study. There is only one sediment type tested with relatively high organic matter content. According to newer guidelines, at least two different sediment types and their associated waters are required for the kinetic experiment. A coarse textured sediment low in organic matter is lacking in this study. However, the EPA Guidelines 40 CFR 158 or OPPTS 835.3180 do not require at least two sediments and the presence of microbes capable of DCOIT degradation in sandy sediments has been demonstrated. **Comment (3.4.1)**: Application method of test compound was not stated. Glucose was added to sediment 30 days before DCOIT was introduced, so the application method seems to be different from the aerobic study. However, no further information on the application method in this study could be made available.

	Comment (3.4.5): The seawater and sediment were separated by quantitatively transferring the contents to a bottle and centrifuging the sample. For getting realistic DT_{50} values for the water phase this is not considered a suitable separation method because ideally the surface water should be carefully removed with minimum disturbance of the sediment. Measurements show traces of oxygen; however, the system can be regarded as anaerob and sulphate reducing bacteria may be active.
Results and discussion	**Comment 4.2**: Disappearance of DCOIT in the seawater system was very rapid compared to the freshwater system. This my partly be explained by differences in application and sampling methods, but most probably due to the production of nucleophilic sulphur compounds produced by e.g sulphate reducing bacteria. The absence of abiotic degradation in this test system has not been unambiguously demonstrated, due to the absence of abiotic control. At the study termination (365 days, $^{14}CO_2$ comprised about 7-8% of the applied radioactivity. It may be almost impossible to distinguish quatitatively between abiotic and biotic degradation of DCOIT in anaerobic seawater-sediment systems because high concentrations of nucleophiles will always be present in biologically active marine systems. Ideally, the regression analysis should have been done with individual measured values of both replicates; however, for DCOIT regression analysis was performed with the average of measured values for both replicates. However, the difference for the resulting half-life is likely to be small. In the aerobic soil biodegradation study (IIIA 7.2.1) the same approach was followed and when performing the regression analysis with individual measured values of both replicates half-lives are identical with the half-lives from calculations with the average of the measured values. **Comment (4.4)**: After 365 days, 44 % of applied radioactivity was contained as bound residues. ^{14}C-label is rapidly incorporated into bound residues (PES or bound radioactivity). It can not be clarified whether this comprises of DCOIT or metabolites. However, DCOIT is not likely to be contained in this fraction. In a sterile soil incubated at 6°C, DCOIT was completely extracted after 30 days, and only 4.6% of applied ^{14}C-label was recovered in the PES fraction (Doc IIIA7.2.1; Table A7.2.1-9 and 11). **Comment (4.4 and 5.2)**: The strong, irreversible binding of DCOIT to sediment might also be due to nucleophilic reaction of organic matter with reactive intermediate degradation products of DCOIT. This is substantiated by the irreversibility of the adsorption in the determination of adsorption isotherms, the fast formation of non-extractable residues in the soil degradation study and by the complete extractability of un-metabolised DCOIT from sterile samples or samples stored at low temperature.
Conclusion	**Comment (5.3):** Agree with applicant's version. However, this half-life is not considered valid for the aquatic marine environment as no DT_{50} for the water phase could be established in this study. DCOIT was only detected in sediment, probably due to the application and sampling method used.
Reliability	2, valid with restrictions
Acceptability	Acceptable with the restrictions noted above.
Remarks	**Comment (5.3.2):** Due to low dosing rates and insufficient quantities of metabolites isolated, mass spectral identification was not possible. However, the isolated metabolites are chromatographically similar to those identified in the aerobic study. It is therefore reasonable to assume that metabolism involves cleavage of the isothiazolone ring and subsequent oxidation to metabolites such as N-(n-octyl) malonamic acid and N-(n-octyl) acetamide. Both of these compounds have been shown to be readily biodegradable (see section IIIA7.1.2.3).

Section A7.1.2.2.2.d	Sea Water/Sediment degradation study – Anaerobic
	TABLES AND FIGURES

Table A7.1.2.2.2.d-1: Physiochemical Characterization of the Sediment

Parameter	York River Sediment
pH	6.6
Percent Sand	13
Percent Silt	65
Percent Clay	22
Texture Class	Silt Loam
Organic Matter (% dry)	5.3
Cation Exchange Capacity (meq/100g)	29
Field Capacity (%)	53
Percent Moisture at 1/3 bar	
Bulk Density (gm/cc)	
Sulfur (Pyritic; %)	0.5
Sulfur (Sulfate; %)	0.13
Sulfur (Total; %)	1.02

Table A7.1. 2.2.2.d -2: Physicochemical Characteristics of Seawater

Alkalinity (Total)	84 mg/L $CaCO_3$
Carbon, Total Organic	3.2 mg/L
pH	7.4
Salinity	19.62 g/kg
Solids, Total Suspended	54 mg/L
Specific Conductance	32,100 µmhos/cm
Sulfate	2,513 mg/L
Total Aluminum	0.8 mg/L
Total Barium	<0.5 mg/L
Total Cadmium	0.08 mg/L
Total Calcium	104 mg/L
Total Chromium	<0.05 mg/L
Total Copper	<0.02 mg/L
Total Iron	1.01 mg/L
Total Lead	0.36 mg/L
Total Magnesium	636 mg/L

Total Manganese	0.1 mg/L
Total Nickel	0.35 mg/L
Total Potassium	266 mg/L
Total Silver	<0.03 mg/L
Total Sodium	6,315 mg/L
Total Zinc	0.12 mg/L

Table A7.1.2.2.2.d -3: Distribution of Radioactivity Following Treatment at 0.05 ppm ^{14}C DCOIT

Day/Replicate	Percent of Applied ^{14}C-Activity[1]						
	H_2O Phase	Soxhlet 1[2]	Soxhlet 2[3]	Ethylene Glycol Trap	NaOH Trap	Bound Residue	Recovery of ^{14}C-Activity[4]
0	3.6	29.7	5.6	NA[5]	NA	47.1	86.0
1	6.3	19.8	8.6	ND[6]	ND	53.8	89.4
5	7.7	24.1	10.0	ND	1.3	49.3	92.4
7	9.3	15.7	11.5	ND	0.3	60.4	97.2
14	11.7	21.8	10.6	ND	1.1	41.5	86.7
29	7.6	13.0	11.5	ND	4.0	41.4	77.5
61	5.5	16.1	10.3	0.4	8.4	40.1	80.6
90	7.5	10.8	10.2	0.2	7.6	58.5	97.9
120	6.3	13.7	0.5	0.8	11.1	47.6	80.0
180	5.6	9.9	4.8	ND	8.5	48.9	77.7
270	3.8	3.9	5.8	0.4	8.5	66.7	89.0
365	5.7	5.3	[7]	ND	6.7	44.0	

[1] Average of duplicates except Day 120
[2] Soxhlet 1 was performed with methylene chloride:methanol
[3] Soxhlet 2 was performed with methanol
[4] Erlenmeyer flasks were rinsed with methanol after sediment and water phases removed. The methanol rinse was quantitated but since the amount was negligible, it is not reproduced in the table but is included as part of the recovery.
[5] NA= not applicable
[6] ND = not detectable
[7] Sample lost

Table A7.1.2.2.2.d -4: Distribution of Radioactivity Following Treatment at 1 ppm ^{14}C DCOIT

Day/Replicate	Percent of Applied ^{14}C-Activity[1]						
	H_2O Phase	Soxhlet 1[2]	Soxhlet 2[3]	Ethylene Glycol Trap	NaOH Trap	Bound Residue	Recovery of ^{14}C-Activity[4]
0	3.7	28.8	9.2	NA[5]	NA	25.0	67.3
1	6.4	26.8	11.6	ND[6]	0.2	50.8	95.8
5	6.8	26.6	13.8	ND	0.8	47.0	95.8
7	8.3	25.1	13.5	ND	0.4	44.9	92.6
14	8.6	24.3	15.7	ND	1.4	37.2	87.6
29	5.6	14.7	13.6	<0.1	3.2	45.0	82.3
61	5.5	15.1	12.0	<0.1	5.3	35.5	73.6
90	4.8	14.0	14.0	<0.1	6.6	46.1	85.6
120	4.7	15.4	13.2	0.1	6.4	33.7	73.8
180	5.2	12.5	10.5	0.2	7.3	41.2	77.0
270	4.2	12.0	10.6	0.1	7.8	48.1	87.8
365	3.3	13.7	[7]	0.2	8.2	40.4	

[1] Average of duplicates
[2] Soxhlet 1 was performed with methylene chloride:methanol
[3] Soxhlet 2 was performed with methanol
[4] Erlenmeyer flasks were rinsed with methanol after sediment and water phases removed. The methanol rinse was quantitated but since the amount was negligible, it is not reproduced in the table but is included as part of the recovery.
[5] NA= not applicable
[6] ND = not detectable
[7] Sample lost

Table A7.1.2.2.2.d -5: Quantitative Characterization of extractable ^{14}C-Activity by HPLC

Day	Percent of Applied ^{14}C-Activity[1]					
	0.05 ppm			1 ppm		
	DCOIT	Other <[2]	Other >[3]	DCOIT	Other <[2]	Other >[3]
0	2.0	13.3	0.9	2.2	9.3	11.8
1	ND[4]	20.7	6.4	< 0.1	19.2	18.7
5	ND	25.1	9.1	ND	20.1	20.0
7	ND	20.7	1.9	ND	17.0	18.1
14	4.8	25.3	2.1	ND	18.7	21.6
29	ND	23.0	1.5	ND	17.4	10.9
61	1.3	18.7	3.8	ND	13.8	13.3
90	ND	18.6	2.5	ND	12.3	11.3
120	ND	12.6	1.3	ND	14.5	14.0
180	ND	12.6	2.4	ND	3.0	20.0
270	ND	8.7	1.0	ND	1.7	25.0
365	---[5]NA	---[5] NA	---[5] NA	ND	---[5] NA	---[5] NA

[1] Average of duplicate samples except on Days 7, 14, and 120
[2] Metabolites chromatographically more polar than DCOIT
[3] Metabolites chromatographically less polar than DCOIT
[4] ND = not detectable at 2 times background
[5] NA = not analyzed

Table A7.1.2.2.2.d -6: Extent and Nature of Bound Residues

Day	Percent of Applied radioactivity in Sediment after Soxhlet extraction	Percent of Applied ^{14}C-Activity (% bound residue)				Percent Recovery of Bound Residues
		0.25 N HCl Reflux	Humin	Humic Acid	Fulvic Acid	
0	21.0	0.1 (0.5)	15.2 (72.4)	3.4 (16.2)	0.2 (1.0)	90.0
365	42.6	0.1 (0.2)	30.3 (71.1)	3.6 (8.5)	0.2 (0.5)	81.2

Table A7.1.2.2.2.d -7: Physiochemical measurements taken throughout study perios

Sampling day	pH	Dissolved oxygen mg/L	Eh
Day 0:			
1 ppm			
Flask 1A	5.7	1.1	-40
Flask 2A	5.3	1.1	-188
0.05 ppm			
Flask 2	5.3	1.2	-215
Flask 3	5.3	1.2	-245
Day 1:			
1 ppm			
Flask 27	6.2	1.4	-74
Flask 228	6.2	1.4	-162
0.05 ppm			
Flask 1	5.5	1.3	-134
Flask 4	5.5	1.3	-158
Day 5:			
1 ppm			
Flask 29	5.7	1.3	-178
Flask 30	6.5	1.3	-145
0.05 ppm			
Flask 5	5.7	1.3	-110
Flask 6	7.0	1.3	-273

Document III-A / Section A7.1.2

[redacted]

Document III-A / Section A7.1.2

Section A7.1.2.3/01 Annex Point IIA7.6.1.1 — Ready Biodegradability of metabolites – N-(n-octyl) Malonamic Acid

Official use only

1 REFERENCE

1.1	**Reference**	Reference type: Study report Year: 2003 Report date: 5 November 2003 [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2		
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes. OECD No. 301B (Modified Sturm Test); EU Commission Directive 92/69 EEC, Part C.4-C, Carbon Dioxide (CO_2) Evolution (Modified Sturm Test), 1992.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	N-(n-octyl) malonamic acid (a metabolite of DCOIT)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Purity	[redacted]
3.1.3	Further relevant properties	[redacted]
3.1.4	TS inhibitory to microorganisms	[redacted]
3.2	**Reference substance**	[redacted]
3.2.1	Initial concentration of reference substance	[redacted]
3.3	**Testing procedure**	

Document III-A / Section A7.1.2

Section A7.1.2.3/01
Annex Point IIA7.6.1.1

Ready Biodegradability of metabolites – N-(n-octyl) Malonamic Acid

3.3.1	Inoculum	[redacted]
3.3.2	Test system	[redacted]
3.3.3	Test conditions	[redacted]
3.3.4	Initial Test Substance concentration	[redacted]
3.3.5	Duration of test	[redacted]
3.3.6	Analytical parameters	[redacted]
3.3.7	Sampling	[redacted]
3.3.8	Intermediates/ degradation products	[redacted]
3.3.9	Nitrate/nitrite measurement	[redacted]
3.3.10	Controls	[redacted]
3.3.11	Calculations/Statistics	[redacted]

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Graph	An overview of biodegradation appears in Figure A7.1.2.3/01-1. In Figure A7.1.2.3/01-2 the 10 day window is emphasized.
4.1.2	Degradation	The CO_2 production resulting from the biodegradation of N-(n-octyl) malonamic acid significantly increased from Day 2 until Day 12. At Day 12 the mean extent of biodegradation of N-(n-octyl) malonamic acid amounted to 81%. After Day 12 the extent of CO_2 production slowed down and at Day 28 the mean extent of biodegradation of N-(n-octyl) malonamic acid was 89%.
4.1.3	Degradation of Test substance in Abiotic Control	From Figure A7.1.2.3/01-1, there was no biodegradation of N-(n-octyl) malonamic acid in the abiotic control (NNOMA + $HgCl_2$).
4.1.4	Degradation of Reference substance	The CO_2 production resulting from the biodegradation of the reference substance, sodium benzoate, significantly increased from before Day 2 sampling until Day 14. At Day 14 the mean extent of biodegradation of sodium benzoate amounted to 81% thus confirming the suitability of the activated sludge (> 60% by Day 14). At the end of the test, Day 28, sodium benzoate was completely degraded.
4.1.5	Biodegradation in Toxicity Control	The extent of biodegradation in the toxicity controls (NNOMA + Na-Benzoate) showed a similar course over the 28 day exposure period as the reference controls (Na-Benzoate only). The CO_2 production significantly increased to 84% until Day 12. At the end of the test, Day 28, the extent of biodegradation in the toxicity control amounted to 88%. Thus, according to the guidelines, the N-(n-octyl) malonamic acid had no inhibitory effect on activated sludge microorganisms
4.1.6	Other observations	Only minimal amounts of residual CO_2 were present in the test solution at the end of the study. A maximum of 1.6 mg of inorganic carbon was detected in the absorber flask after acidification and aeration.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	This study employed OECD 301 B to quantitate the oxidation of organic carbon in N-(n-octyl) malonamic acid to CO_2 Nine flasks containing 2400 to 3000 ml mineral salts solution (KH_2PO_4, K_2HPO_4, Na_2HPO_4, NH_4Cl, $MgSO_4$, $CaCl_2$, and $FeCl_3$) plus 90 ml of activated sludge inoculum were aerated overnight with CO_2-free air. The morning after purging, N-(n-octyl) malonamic acid was added to four flasks. To one of these flask, 10 mg/L of $HgCl_2$ was added (abiotic control) while to another flask 25.7 mg/L of the reference item, sodium benzoate, was added (toxicity control). To 2 procedure control flasks, only sodium benzoate (25.7 mg/L) was added while to 2 additional flasks neither the test substance nor the reference substance was added (inoculum control). The final flask contained only $HgCl_2$ (10 mg/L) (abiotic blank). Evolved CO_2 was trapped in 0.05 M NaOH absorbers connected in series to the exit air line of each test flask. The flasks were

Document III-A / Section A7.1.2

Section A7.1.2.3/01
Annex Point IIA7.6.1.1

Ready Biodegradability of metabolites – N-(n-octyl) Malonamic Acid

		incubated in the dark at 22-23°C. On Days 0, 2, 5, 7, 9, 12, 14, 19, 23, 27, 28, and 29 aliquots were withdrawn from each of the NaOH absorbers and total inorganic carbon was quantitated by a TOC analyzer.
5.2	**Results and discussion**	Per OECD 301B guidelines, N-(n-octyl) malonamic acid is ready biodegradable. Over 60% of the organic carbon was oxidized to CO_2 within the 10 day window. From Day 2 to Day 12, the biodegradation exceeded 75% and at the end of the 28 day study period, the mean extent of biodegradation was 89%. The presence of $HgCl_2$ essentially halted the oxidation of the test material. In controls containing sodium benzoate, the average extent of biodegradation on Day 14 was 81% confirming the suitability of the system. The presence of N-(n-octyl) malonamic acid had essentially no effect on the oxidation of sodium benzoate .
5.3	**Conclusion**	This study fulfills the requirements and demonstrates that N-(n-octyl) malonamic acid, a metabolite of DCOIT, is ready biodegradable.
5.3.1	Reliability	1-valid without restrictions.
5.3.2	Deficiencies	None.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	10 October 2006
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1, reliable without restrictions
Acceptability	Acceptable
Remarks	-

Section A7.1.2.3/01	Ready Biodegradability of metabolites – N-(n-octyl) Malonamic Acid **TABLES AND FIGURES**

Document III-A / Section A7.1.2

[redacted]

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

Figure A7.1.2.3/01-1: Overview of Biodegradation of N-(n-Octyl) Malonamic Acid (NNOMA) and Sodium Benzoate

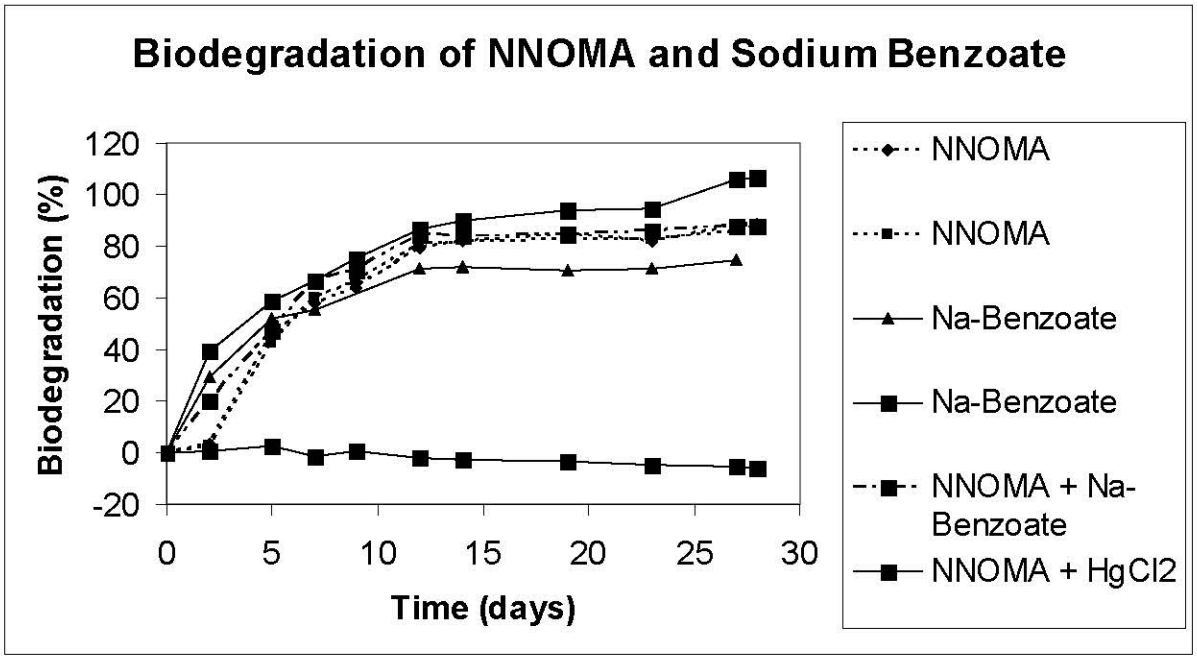
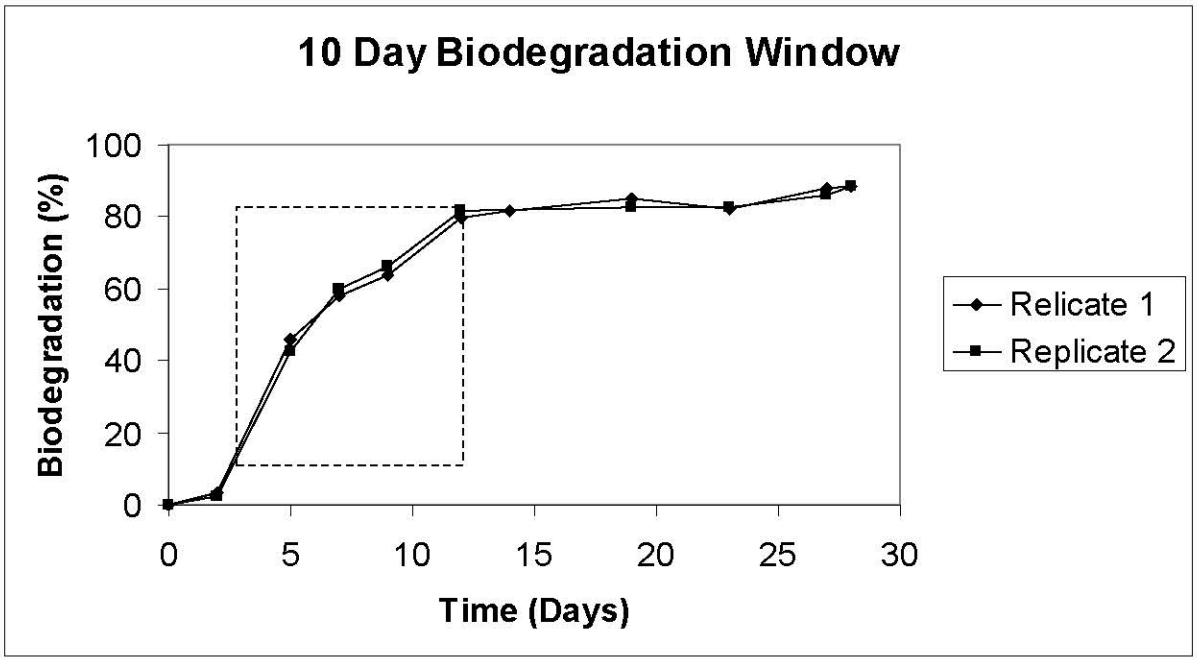


Figure A7.1.2.3/01-2: 10 Day Window for Biodegradation of N-(N-Octyl) Malonamic Acid (NNOMA)



Section A7.1.2.3/02 Annex Point IIA7.6.1.1 — Ready Biodegradability of metabolites – N-(n-octyl) Acetamide

Official use only

1 REFERENCE

1.1	**Reference**	Reference type: Study report Year: 2003 Report date: 5 November 2003 [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2		
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes. OECD No. 301B (Modified Sturm Test); EU Commission Directive 92/69 EEC, Part C.4-C, Carbon Dioxide (CO_2) Evolution (Modified Sturm Test), 1992.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	N-(n-octyl) Acetamide (a metabolite of DCOIT)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Purity	[redacted]
3.1.3	Further relevant properties	[redacted]
3.1.4	TS inhibitory to microorganisms	[redacted]
3.2	**Reference substance**	[redacted]
3.2.1	Initial concentration of reference substance	[redacted]
3.3	**Test ing procedure**	

Section A7.1.2.3/02
Annex Point IIA7.6.1.1

Ready Biodegradability of metabolites – N-(n-octyl) Acetamide

3.3.1 Inoculum

3.3.2 Test system

3.3.3 Test conditions

3.3.4 Initial Test Substance concentration

3.3.5 Duration of test

3.3.6 Analytical parameters

3.3.7 Sampling

3.3.8 Intermediates/ degradation products

3.3.9 Nitrate/nitrite measurement

3.3.10 Controls

3.3.11 Calculations/Statistics

4 RESULTS

Section A7.1.2.3/02
Annex Point IIA7.6.1.1

Ready Biodegradability of metabolites – N-(n-octyl) Acetamide

4.1	**Degradation of test substance**	
4.1.1	Graph	An overview of biodegradation appears in Figure A7.1.2.3/02-1. In Figure A7.1.2.3/02-2 the 10 day window is emphasized.
4.1.2	Degradation	The CO_2 production resulting from the biodegradation of N-(n-octyl) acetamide significantly increased from Day 2 until Day 12. At Day 12 the mean extent of biodegradation of N-(n-octyl) acetamide amounted to 80%. After Day 12 the extent of CO_2 production slowed down and on Day 28 the mean extent of biodegradation of N-(n-octyl) acetamide was 89%.
4.1.3	Degradation of Test substance in Abiotic Control	From Figure A7.1.2.3/02-1, there was no biodegradation of N-(n-octyl) acetamide in the abiotic control (NNOA + $HgCl_2$).
4.1.4	Degradation of Reference substance	The CO_2 production resulting from the biodegradation of the reference substance, sodium benzoate, significantly increased from before Day 2 until Day 14. At Day 14 the mean extent of biodegradation of sodium benzoate amounted to 81% thus confirming the suitability of the activated sludge (> 60% by Day 14). At the end of the test, Day 28, sodium benzoate was completely degraded.
4.1.5	Biodegradation in Toxicity Control	The extent of biodegradation in the toxicity controls (NNOA + Na-Benzoate) showed a similar course over the 28 day exposure period as the reference controls (Na-Benzoate only). The CO_2 production significantly increased to 82% until Day 12. At the end of the test, Day 28, the extent of biodegradation in the toxicity control amounted to 83%. Thus, according to the guidelines, the N-(n-octyl) acetamide at the dosing concentration had no inhibitory effect on activated sludge microorganisms.
4.1.6	Other observations	Only minimal amounts of residual CO_2 were present in the test solution at the end of the study. A maximum of 5.4 mg of inorganic carbon was detected in the absorber flask after acidification and aeration.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	This study employed OECD 301 B to quantitate the oxidation of organic carbon in N-(n-octyl) acetamide to CO_2. Nine flasks containing 2400 to 3000 ml of mineral salt (KH_2PO_4, K_2HPO_4, Na_2HPO_4, NH_4Cl, $MgSO_4$, $CaCl_2$, and $FeCl_3$) solution plus 90 ml of activated sludge inoculum were aerated overnight with CO_2-free air. The morning after purging, N-(n-octyl) acetamide (21 mg/L) was added to four flasks. To one of these flask, 10mg/L of $HgCl_2$ was added (abiotic control) while to another flask 25.7 mg/L of the reference item, sodium benzoate, was added (toxicity control). To 2 procedure control flasks, only sodium benzoate (25.7 mg/L) was added while to 2 additional flasks neither the test substance nor the reference substance was added (inoculum control). The final flask contained only $HgCl_2$ (10 mg/L) (abiotic blank). Evolved CO_2 was trapped in 0.05 M NaOH absorbers connected in series to the exit air line of each test flask. The flasks were incubated in the dark at 22-23°C. On Days 0, 2, 5, 7, 9, 12, 14, 19, 23, 27, 28, and 29 aliquots were withdrawn from each of the NaOH absorbers and total inorganic carbon was quantitated by a TOC

Section A7.1.2.3/02
Annex Point IIA7.6.1.1

Ready Biodegradability of metabolites – N-(n-octyl) Acetamide

		analyzer.
5.2	**Results and discussion**	Per OECD 301B guidelines, N-(n-octyl) acetamide is ready biodegradable. Over 60% of the organic carbon was oxidized to CO_2 within the 10 day window. From Day 2 to Day 12, the biodegradation exceeded 75% and at the end of the 28 day study period, the mean extent of biodegradation was 89%. The presence of $HgCl_2$ essentially halted the oxidation of the test material. In controls containing sodium benzoate, the average extent of biodegradation on Day 14 was 81% confirming the suitability of the system. The presence of sodium benzoate had essentially no effect on the oxidation of N-(n-octyl) acetamide.
5.3	**Conclusion**	This study fulfills the requirements and demonstrates that N-(n-octyl) acetamide, a metabolite of DCOIT, is ready biodegradable.
5.3.1	Reliability	1-valid without restrictions.
5.3.2	Deficiencies	None.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	10 October 2006
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1, reliable without restrictions
Acceptability	Acceptable
Remarks	-

Section A7.1.2.3/02 Annex Point IIA7.6.1.1	Ready Biodegradability of metabolites – N-(n-octyl) Acetamide **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]

Document III-A / Section A7.1.2

[redacted]

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

Figure A7.1.2.3/02-1: Overview of Biodegradation of N-(n-Octyl) Acetamide (NNOA) and Sodium Benzoate

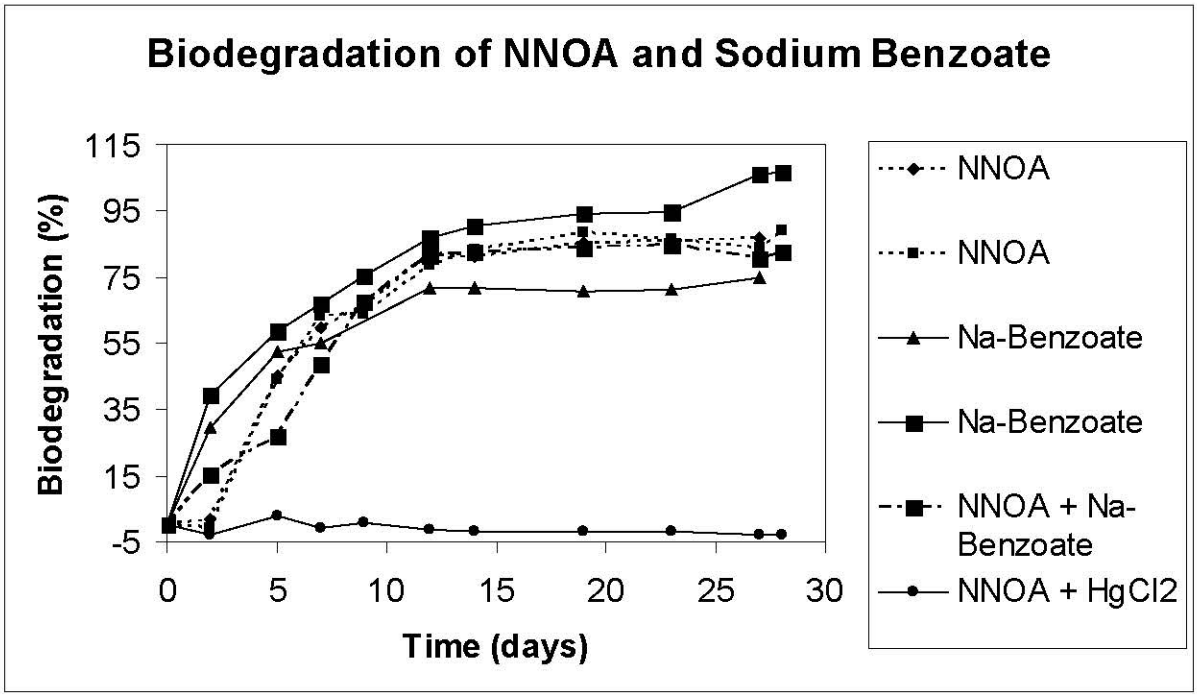
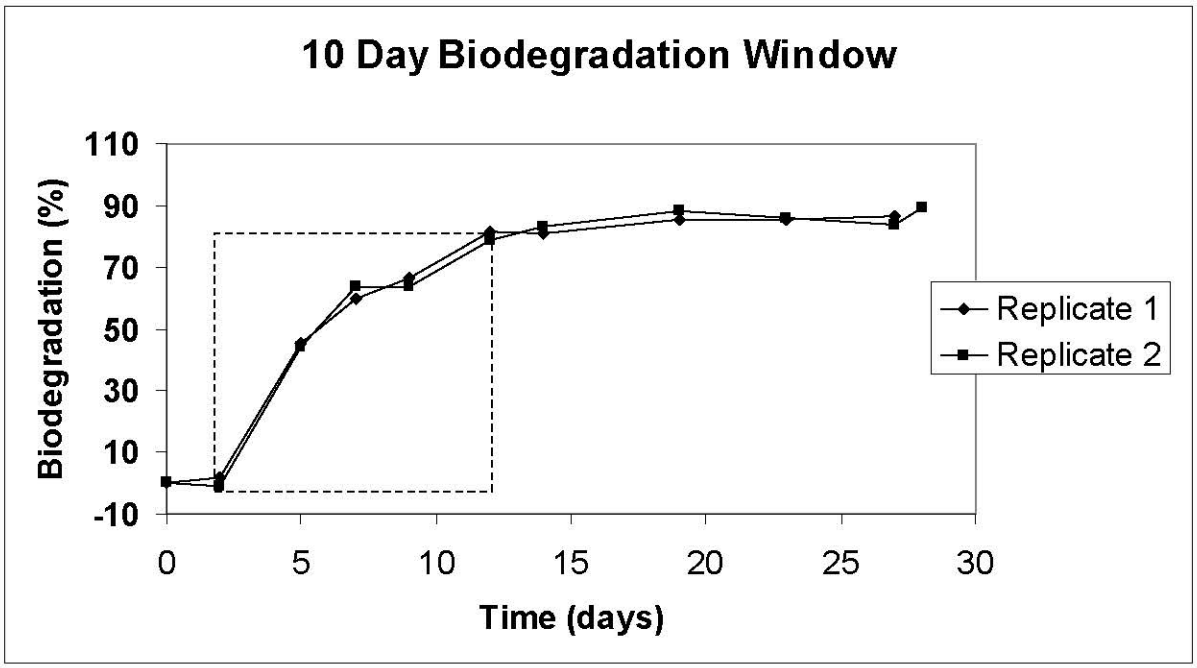


Figure A7.1.2.3/02-2: 10-Day Window for Biodegradation of N-(N-Octyl) Acetamide (NNOA)



Section A7.1.2.3/03 Annex Point IIA7.6.1.1	Ready Biodegradability of metabolites – 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid

Official use only

1 REFERENCE

1.1	**Reference**	Reference type: Study report Year: 2006 Report date: 15 June 2006 [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 No. 301B (Modified Sturm Test); EU Commission Directive 92/69 EEC, Part C.4-C, Carbon Dioxide (CO_2) Evolution (Modified Sturm Test), 1992.
2.2	**GLP**	Yes
2.3	**Deviations**	Determination of carbon content of the test material comprised of 83% 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid not performed under GLP.

3 MATERIALS AND METHODS

3.1	**Test material**	2-chloro-2-(n-octylcarbamoyl)-1-1ethene sulfonic acid (a metabolite of DCOIT)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Purity	[redacted]
3.1.3	Further relevant properties	
3.1.4	TS inhibitory to microorganisms	[redacted]
3.2	**Reference substance**	[redacted]
3.2.1	Initial concentration of reference	[redacted]

Section A7.1.2.3/03
Annex Point IIA7.6.1.1

Ready Biodegradability of metabolites – 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid

	substance		
3.3	**Testing procedure**		
3.3.1	Inoculum	[redacted]	
3.3.2	Test system	[redacted]	
3.3.3	Test conditions	[redacted]	
3.3.4	Initial Test Substance concentration	[redacted]	x
3.3.5	Duration of test	[redacted]	
3.3.6	Analytical parameters	[redacted]	
3.3.7	Sampling	[redacted]	
3.3.8	Intermediates/ degradation products	[redacted]	
3.3.9	Nitrate/nitrite measurement	[redacted]	
3.3.10	Controls	[redacted]	
3.3.11	Calculations/ Statistics	[redacted]	

Section A7.1.2.3/03
Annex Point IIA7.6.1.1

Ready Biodegradability of metabolites – 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid

[redacted]

[redacted]

[redacted]

4 RESULTS

4.1 Degradation of test substance

4.1.1	Graph	A summary of the biodegradation results corrected for inoculum controls and abiotic blank appears in Table A7.1.2.3/03-4 and is graphically represented in Figure A7.1.2.3/03-1.
4.1.2	Degradation	The CO_2 production resulting from the biodegradation of 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid increased throughout the study period (Day 2 tol Day 28). At Day 14 the mean extent of biodegradation of 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid amounted to 36.4% while by Day 28, 52.0%. The stringent guideline pass level of at least 60% oxidation of carbon to CO_2 in a 10 day window within a 28 day period was not satisfied. Since the purity of 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid was only 83% it is possible that the impurities could have affected the oxidation to CO_2.
4.1.3	Degradation of Test substance in Abiotic Control	From Table A7.1.2.3/03-4 and Figure A7.1.2.3/03-1, there was no biodegradation of 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid in the abiotic control (2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid + $HgCl_2$).
4.1.4	Degradation of Reference substance	The CO_2 production resulting from the biodegradation of the reference substance, sodium benzoate, significantly increased from before Day 2 sampling until Day 9. At Day 9 the mean extent of biodegradation of sodium benzoate amounted to 65.3% and by Day 14, 72.5%, thus confirming the suitability of the activated sludge (> 60% by Day 14). At the end of the test, Day 28, biodegradation of sodium benzoate reached a mean of 88.8%.
4.1.5	Biodegradation in Toxicity Control	The extent of biodegradation in the toxicity controls (2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid + Na-Benzoate) proceeded rapidly over the 28 day exposure period (Table A7.1.2.3/03-4 and Figure A7.1.2.3/03-1). By Day 14 the CO_2 production was 57.2% and by Day 28, 60.8%. According to the test guidelines, if biodegradation in the toxicity control exceeds 25% within 14 days, the test item is deemed to have no inhibitory effect on the activated sludge. Thus, 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid was not inhibitory to the activated sludge at the test concentration of of 26 mg/L.
4.1.6	Other observations	Only negligible amounts of residual CO_2 were present in test solution at the end of the study. The pH did not change over the 28 day study period. At Day 0 the pH in the study flasks was 7.5 and at termination

7.4-7.6 (Table A7.1.2.3/03-3).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

This study employed OECD 301 B to quantitate the oxidation of organic carbon in 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid to CO_2

Nine flasks containing 2400 to 3000 ml mineral salts solution (KH_2PO_4, K_2HPO_4, Na_2HPO_4, NH_4Cl, $MgSO_4$, $CaCl_2$, and $FeCl_3$) plus 90 ml of activated sludge inoculum were aerated overnight with CO_2-free air. The morning after purging, 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid was added to four flasks. To one of these 4 flask, 10 mg/L of $HgCl_2$ was added (abiotic control) while to another flask 25.7 mg/L of the reference item, sodium benzoate, was added (toxicity control). To 2 procedure control flasks, only sodium benzoate (25.7 mg/L) was added while to 2 additional flasks neither the test substance nor the reference substance was added (inoculum control). The final flask contained only 10 mg/L $HgCl_2$ (abiotic blank). Evolved CO_2 was trapped in 0.05M NaOH absorbers connected in series to the exit air line of each test flask. The flasks were incubated in the dark at 20-21°C. On Days 0, 2, 5, 7, 9, 12, 14, 20, 23, 27, 28, and 29 aliquots were withdrawn from the NaOH absorbers and total inorganic carbon was quantitated by a TOC analyzer.

5.2 Results and discussion

Per OECD 301B guidelines, 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid cannot be classified as ready biodegradable. The oxidation of organic carbon to CO_2 did not exceed 60% in a 10 day window within a 28 day test period. On Day 14 biodegradation was 36.4% and on Day 28, 52.0%. The presence of $HgCl_2$ essentially halted the oxidation of the test material. In controls containing sodium benzoate, the average extent of biodegradation on Day 14 was 72.5% confirming the suitability of the system. Per the test guidelines, the presence of 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid had no effect on the oxidation of sodium benzoate and therefore was not inhibitory to the activated sludge microorganisms.

The test material was extremely difficult to synthesize and purify (purity of 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid used in this test was about 83%). It is possible the impurities affected the oxidation of organic carbon to CO_2. This can only be examined when a synthetic method is developed that will provide a higher purity test material.

5.3 Conclusion

This study fulfils the requirements and demonstrates that 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid, a metabolite of DCOIT, is not ready biodegradable under the current test conditions (including the test material being only 83% pure). 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid did undergo extensive biodegradation in this test with 52% of the organic carbon oxidizing to CO_2 in 28 days. While the compound is not ready biodegradable, it will rapidly biodegrade and thus not be persistent in the environment. x

5.3.1 Reliability

1-valid without restrictions.

Section A7.1.2.3/03 **Annex Point IIA7.6.1.1**	**Ready Biodegradability of metabolites – 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid**

5.3.2 Deficiencies None.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	10 January 2007
Materials and Methods	**Comment (3.3.4):** The test concentration is slightly below the QSAR predicted water solubility of this metabolite of 25 mg/l.
Results and discussion	Agree with applicant's version
Conclusion	**Comment (5.3):** We agree with the applicant's version with respect to the result of the study that 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid is not readily biodegradable under the current test conditions. However, it is not accurate to state that the compound will rapidly biodegrade in the environment. 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic is not persistent and will certainly biodegrade in the environment to a certain extent, but biodegradation will not be rapid.
Reliability	1, reliable without restrictions
Acceptability	Acceptable
Remarks	-

Section A7.1.2.3/03 Ready Biodegradability of metabolites – 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid

TABLES AND FIGURES

[redacted]

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

Document III-A / Section A7.1.2

[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 A7.1.2

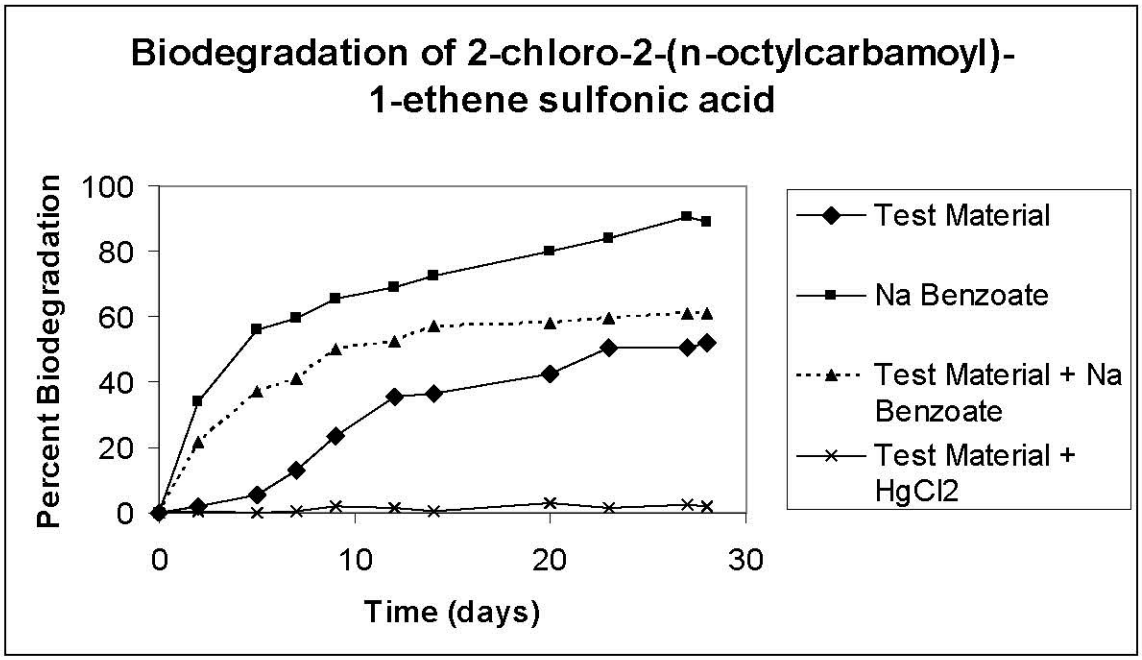
Table A7.1.2.3/03-4: Biodegradation of 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid (Test Compound) and Sodium Benzoate (Reference Compound)

Time (days)	Percent Biodegradation[a]							
	Test Compound[b]			Na-Benzoate (ref. cmpound)			Toxicity Control	Abiotic Control
	1	2	Mean	1	2	Mean		
2	2.4	1.3	1.9	35.1	32.7	33.9	21.5	0.4
5	5.8	5.3	5.5	58.3	54.0	56.1	37.2	0.2
7	14.3	11.3	12.8	61.9	56.8	59.4	40.8	0.6
9	23.7	23.7	23.7	68.2	62.3	65.3	50.0	1.8
12	36.1	34.9	35.6	70.4	67.5	68.9	52.5	1.5
14	40.2	32.7	36.4	75.0	70.1	72.5	57.2	0.3
20	45.8	38.8	42.3	82.0	78.3	80.2	57.9	3.0
23	51.0	50.0	50.5	86.0	82.1	84.1	59.3	1.3
27	55.7	45.3	50.5	91.1	89.8	90.4	60.9	2.4
28	57.6	46.4	52.0	91.1	86.4	88.8	60.8	1.8

[a] Values corrected for inoculum control or abiotic blank as appropriate

[b] Test Compound: 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid.

Figure A7.1.2.3/03-1: Overview of Biodegradation of 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid and Sodium Benzoate



Note: The results from test compound and Na-Benzoate are replicate means

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

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

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

Product type 21: Antifouling products

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

Part III

Fate and behaviour in the environment

Section A7.1.3: Studies on adsorption and desorption

Section A7.1.4: Field studies on accumulation in sediments

Section A7.2: Fate and behaviour in soil

Section A7.3: Fate and behaviour in air

TABLE OF CONTENTS

Section A7.1.3.a Adsorption and Desorption test - Sludge

Annex Point IIA VII.7.7

Official use only

		1 REFERENCE
1.1	**Reference**	Reference type: Study report Year: 2002 Report date: 23 December 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. U.S. Environmental Protection Agency, Fate, Transport, and Transformation Test Guideline 835.1110 Activated Sludge Sorption Isotherm.
2.2	**GLP**	Yes
2.3	**Deviations**	No GLP deviations or deviations from the US EPA guidelines.
		3 MATERIALS AND METHODS
3.1	**Test material**	^{14}C-DCOIT (RH-5287) (^{14}C) [redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Purity	[redacted]
3.1.3	Further relevant properties	[redacted]
3.1.4	Method of analysis	[redacted]
3.2	**Degradation products**	[redacted]

Section A7.1.3.a Adsorption and Desorption test - Sludge

Annex Point IIA VII.7.7

[redacted]

3.2.1 Method of analysis for degradation products [redacted]

3.3 Reference substance [redacted]

3.3.1 Method of analysis for reference substance [redacted]

3.4 Sludge

Return activated sludge was obtained from Return Sludge Riser, Burley-Menston Sewage Treatment Works, West Yorkshire, UK. This treatment works serves a population of about 12,600 and is primarily for domestic waste (trade effluent is < 0.003%). The sludge had the following characteristics:

Percent Organic Carbon:	37.2
Cation Exchange Capacity meq/100g:	33.9

The sludge was separated from the waste water by decanting and rinsing 3 times with water. The resulting sludge solids were frozen and then freeze-dried. The freeze-dried sludge was passed through a 2mm mesh screen. Before use, the powdered sludge was deactivated by desiccation at about 103°C for two days. This desiccation ended on the day of use.

3.5 Testing procedure

3.5.1 Test system-solubility test [redacted]

3.5.2 Test system-adsorption to containers [redacted]

3.5.3 Test system-equilibration time determination [redacted]

3.5.4 Test-Analytical verification [redacted]

3.5.5 Test system-Analytical [redacted]

Document III-A / Section A7.1.3, A7.1.4, A7.2 and A7.3

Section A7.1.3.a Adsorption and Desorption test - Sludge

Annex Point IIA VII.7.7

[redacted]

3.5.6 Test system-Definitive test

The sorption isotherm test was performed using six concentrations of sludge:0.01M $CaCl_2$ solution (0.1 g/L, 0.5 g/L, 1.0 g/L, 3.0 g/L, 6.0 g/L and 9.0 g/L). The dry weight of deactivated sludge and volume of 0.01M $CaCl_2$ added to Teflon centrifuge tubes are shown in Table A7.1.3a-1. Duplicate tubes containing 0.01M $CaCl_2$ only were prepared as controls.

Stock solution of ^{14}C DCOIT was prepared at 1.06 mg/ml in ethanol. The test was initiated by the addition of 29 µl (30.83 µg) of ^{14}C DCOIT under sterile conditions giving a nominal concentration of 1 mg/L.

Centrifuge tubes were shaken continuously for 1 hr on an end-over-end shaker. They were then centrifuged at 2000 g, the aqueous phase decanted, the weight of the supernatant determined and radioassayed. Sludge samples were initially extracted with 5 ml of acetone and then air dried. The acetone extracts were radioassayed and the air dried sludge combusted prior to radioassaying.

4 RESULTS

4.1 Equilibration time

Figure A7.1.3.a-1 presents the results from the equilibrium determination. Equilibrium was reached within 1 hour of the addition of ^{14}C DCOIT. HPLC analysis of the supernatant showed that parent was stable over a 2 hr test period for the 3g/L sludge:solution samples and 1.5 hr for the 9 g/L sludge:solution samples. By 4 hours of equilibration, ^{14}C DCOIT was observed to have biodegraded.

Consequently, an equilibration time of 1 hour was selected for the sorption isotherm test.

4.2 Distribution of ^{14}C DCOIT between sludge and $CaCl_2$ solution

Table A7.1.3.a-2 provides the quantity of ^{14}C DCOIT that was adsorbed to the sludge after a 1 hour equilibration. At 0.01g of sludge per liter of $CaCl_2$ solution about 25% of the applied ^{14}C DCOIT was adsorbed to sludge whereas at 9 g of sludge per liter of $CaCl_2$ solution, about 94% was adsorbed.The concentration of ^{14}C DCOIT in solution (Ce, µg ^{14}C DCOIT/ml of $CaCl_2$ solution) and associated with the sludge (X/m, µg of ^{14}C DCOIT/g of sludge) after 1 hour equilibration as well as the adsorption coefficient (K_d) are presented in Table 7.1.3a-3.

4.3 K_d

Table A7.1.3.a-3 provides the K_d for the different sludge to $CaCl_2$ solution ratios. The values of K_d ranged from 1636 to 3262 ml/g.

4.4 K_f and 1/n

The Freundlich sorption coefficient (K_f) and 1/n, calculated by linear regression (Figure A7.1.3a-2) were 2466 and 1.089, respectively. The high value for the Freundlich sorption coefficient indicates that DCOIT is strongly adsorbed to activated sludge.

Section A7.1.3.a Adsorption and Desorption test - Sludge

Annex Point IIA VII.7.7

4.5	**pH**	The pH of each supernatant was recorded at the end of the study and the values ranged from 5.89 – 6.09. Thus all values were within a pH range of 6.0 ± 0.2.
4.6	**Recovery of applied ^{14}C-activity**	Recovery of applied ^{14}C-activity is presented in Table A7.1.3.a-4. Recoveries ranged from 90.9% – 99.0% with the exception of replicate 2 for the 1 g/L concentration which had 78.5% recovery. This was probably due to incomplete sample combustion. The combustion could not be replicated because there was an insufficient quantity of dry sludge. Average recovery was 94.4± 3.1%.
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test guideline followed was U.S. Environmental Protection Agency, Fate, Transport, and Transformation Test Guideline 835.1110 Activated Sludge Sorption Isotherm. There were no deviations from this test guideline. The sorption isotherm test was performed with six concentration ratios of sludge:0.01M $CaCl_2$ solution: 0.1 g/L, 0.5 g/L, 1.0 g/L, 3.0 g/L, 6.0 g/L and 9.0 g/L. Duplicate vessels for each concentration were shaken for 1 hour, centrifuged, and the supernatant and solids radioassayed.
5.2	**Results and discussion**	DCOIT adsorbs tightly and readily to activated sludge. Equilibrium between the aqueous and solid phase was established in less than 1 hour.
5.2.1	Adsorption of active substance	The adsorption of ^{14}C DCOIT to the sludge ranged from about 25% of the applied activity in a mixture of 0.1 g of sludge per liter of $CaCl_2$ solution to about 94% in a mixture of 9 g of sludge per liter of $CaCl_2$ solution. Complete results appear in Table 7.1.3a-2
5.2.2	K_d	The adsorption coefficients (K_d) from the six sludge to $CaCl_2$ solution ratios ranged from 1636 to 3262 ml/g. Complete results appear in Table A7.1.3.a-3.
5.2.3	K_f	The Freundlich sorption coefficient (K_f), determined from the adsorption isotherm (Figure A7.1.3.a-2) was 2466.
5.3	**Conclusion**	The Freundlich sorption constant (K_f) was 2466. The high value for the Freundlich sorption constant indicates that DCOIT is sorbed to the activated sludge and is unlikely to remain in the aqueous phase for the typical concentrations of sludge (3-9 g of sludge/L of solution) expected in a waste treatment plant.
5.3.1	Reliability	1- Valid without restrictions
5.3.2	Deficiencies	None.

Document III-A / Section A7.1.3, A7.1.4, A7.2 and A7.3

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	19. October 2006
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version. DCOIT adsorbs tightly and readily to activated sludge.
Conclusion	Agree with applicant's conclusion. The Freundlich sorption constant (K_f) was 2466. The high value for the Freundlich sorption constant indicates that DCOIT is sorbed to the activated sludge and is unlikely to remain in the aqueous phase for the typical concentrations of sludge (3-9 g of sludge/L of solution) expected in a waste treatment plant.
Reliability	1,valid without restrictions
Acceptability	Acceptable
Remarks	-