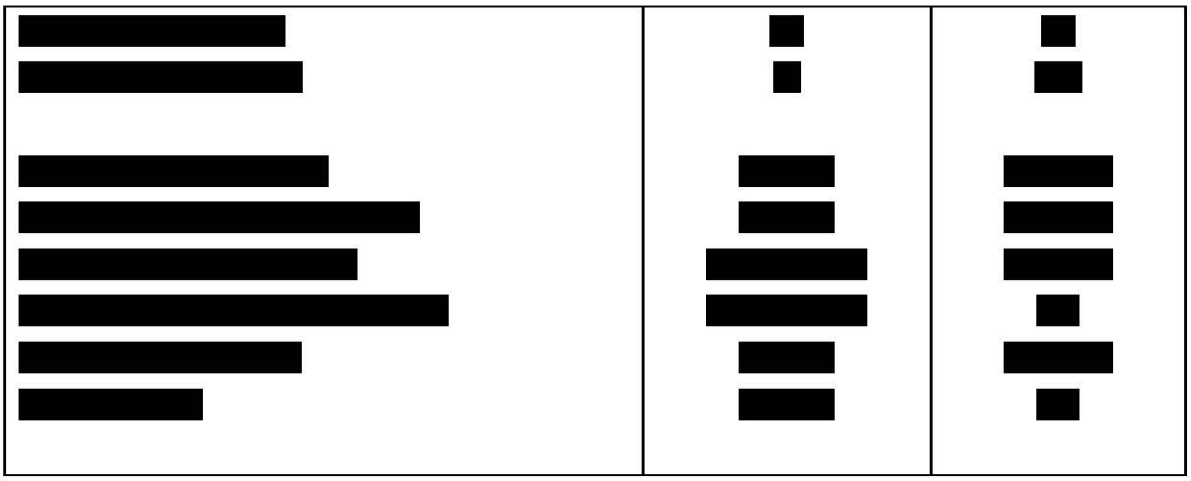
	such as the amino acid cysteine, are quite reactive at sulphur. Cysteine has been shown to be a very effective decontamination agent causing rapid cleavage of the isothiazolone ring. This is generally the case for all thiazoles. In the non-sterile samples, nucleophiles may also have played an important role. According to Eh measurements, the non-sterile sediment has been anaerobic for a period of time. Anaerobic metabolites as sulfides, mercaptans etc., may also catalyse cleavage of the isothiazolone ring, but can be considered biologically mediated. Rapid biological degradation has been demonstrated for both estuarine water (A7.1.2.2.1) and soil (A7.2.1) and biological degradation is certainly also important in the present study. Although the sterile control was contaminated only 0.3 % was recovered as $^{14}CO_2$ after 30 days, compared to 1.1 % after 13 days and 10.7 % after 101 days in the non-sterile samples. Thus, it is concluded that DCOIT biodegrades in fresh water:sediment microcosm with a half-life of 2 days at 9°C, even though abiotic/chemical processes certainly also contributed to this half-life. DCOIT was applied to the water surface and only detected in the water phase. Presumably most of it was degraded before reaching the sediment. Dissipation from water phase followed first order kinetics. When recalculated to 12°C according to the TGD, the DT_{50} = 1.6 days for the water phase (k=0.4336 $days^{-1}$). A DT_{50} for the sediment cannot be derived from this study. However, this half-life can be considered valid for the freshwater-sediment system as a whole. **Comment (5.3)**: "QSAR calculations show that the metabolites 3,3'dithiobis-(n-octyl)-3-chloropropenamide and 2-chloro-3-(formyldithio)-N-octylpropenamide are not expected to be readily biodegradable. However, these two compounds were present at less than 5% of applied radioactivity each and their ecotoxicological impact can therefore be assumed to be minimal.
Conclusion	Agree with applicant's version
Reliability	**Comment (5.3.1):** Due to the restrictions described the reliability is changed from 1 to 2 - reliable with restrictions
Acceptability	Acceptable with the restrictions noted above
Remarks	**Comment (5.3.2)**: According to the OECD Guideline degradation should be tested in two different sediment types. In principle, omitting the sandy sediment is a serious deficiency. However, degradation rates and metabolic pathways have been investigated in a range of sediments and soils from different environmental compartments (including sandy soils), which all show rapid dissipation. Except from the surface water study, several metabolites seem common to most environments and soil types. Therefore this deficiency is acceptable and no further testing is considered necessary.

Section A7.1.2.2.2.a Fresh Water/Sediment Degradation study – Aerobic

TABLES AND FIGURES

[redacted]



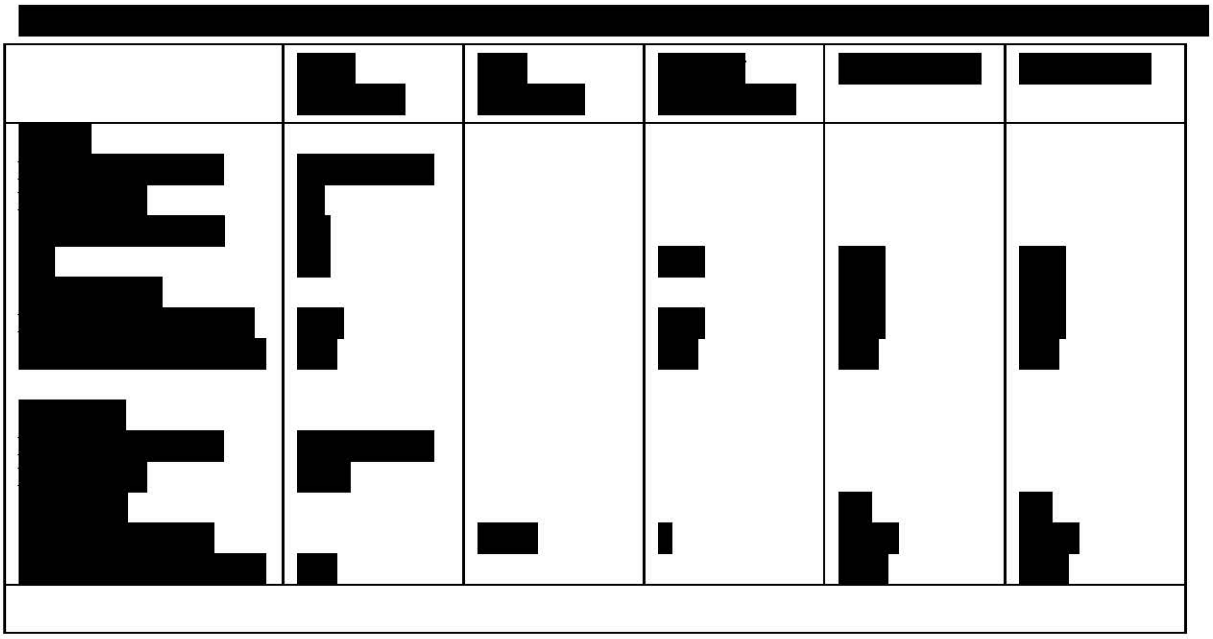


Table A7.1.2.2.2.a-3: Distribution of Radioactivity in Preliminary Studies Following Dosing With ^{14}C DCOIT in in % applied radioactivity

Sample	Water	Sediment		Volatiles	Recovery
		Extractable	Non-extractable		
0.1 mg/L					
Day 0	87.4	2.1	6.0	NA[1]	95.5
Day1	23.6	12.7	32.5	< LOQ	68.6
1 mg/L					
Day 0	96.7	2.6	5.4	NA	104.7
Day 1	30.2	13.3	28.6	< LOQ	72.2
Day 14	9.8	31.0	57.0	< LOQ	99.4
5 mg/L					
Day 0	85.1	6.8	11.8	NA	103.5
Day 1	23.4	17.3	29.1	< LOQ	69.4

[1] NA = Not applicable

Table A7.1.2.2.2.a-4: Quantitation of DCOIT in Water and Sediment during Preliminary Studies in % applied radioactivity

Sample	Aerobic	
	Water	Sediment
0.1 mg/L		
Day 0	82.1	<LOD
Day1	20.6	<LOD
1 mg/L		
Day 0	75.7	0.2
Day 1	29.7	0.8
Day 14	1.6	3.1
5 mg/L		
Day 0	81.8	0.6
Day 1	21.3	1.1

Table A7.1.2.2.2.a-5: Distribution of Radioactivity in Definitive Study Following Dosing at 1 mg/L ^{14}C DCOIT; Non-Sterile System.

Day/Replicate	Percent of Applied ^{14}C-Activity[1]					
	Water Phase	Sediment-Extractable	Ethyl Digol Trap	KOH Traps	Bound Residue	Recovery of ^{14}C-Activity
0	79.0	3.7	NA[2]	NA	17.3	100.0
0.042	90.5	1.6	ND[3]	ND	11.2	103.2
0.125	75.8	4.9	ND	ND	14.1	94.7
0.25	72.5	7.5	ND	ND	20.0	100.0
1	62.8	10.9	ND	ND	25.5	99.2
3	29.4	24.6	ND	0.1	39.7	93.7
7	17.1	30.4	0.1	0.3	45.0	92.8
13	5.1	39.5	ND	1.1	46.0	91.6
101	1.0	25.0	ND	10.7	61.7	98.3
					Average Recovery:	97.01 ± 4.93%

[1] Average of duplicate
[2] NA= not applicable
[3] ND = not detectable

Table A7.1.2.2.2.a-6: Quantitation of ^{14}C DCOIT in the Water Phase of the Definitive Study in Non-Sterile and Sterile Systems[1]

Day	**Percent of Applied ^{14}C-Activity[2]**	
	Non-Sterile	**Sterile**
0	**78.4**	
0.042	**89.1**	
0.125	**74.3**	
0.25	**70.7**	
1	**57.0**	**27.7**
3	**26.3**	
7	**13.7**	**2.1**
13	**1.0**	
30		**1.4**

[1] DCOIT only detected in water. None was detected in the sediment phase.
[2] Average of duplicates.

Table A7.1.2.2.2.a-7: Distribution of Radioactivity in Definitive Study Following Dosing at 1 mg/L ^{14}C DCOIT; Sterile System.

Day/Replicate	Percent of Applied ^{14}C-Activity[1]					
	Water Phase	Sediment-Extractable	Ethyl Digol Trap	KOH Traps	Bound Residue	Recovery of ^{14}C-Activity
1	30.0	22.6	ND[2]	ND	44.6	97.1
8	3.2	37.2	ND[3]	ND	52.8	93.2
30	3.1	21.5	ND	0.3	60.9	85.8
					Average Recovery:	92.00 ± 5.54%

[1] Average of duplicate
[2] ND = not detectable

Table A7.1.2.2.2.a-8: Average Percent of DCOIT and Metabolites in the Water Phase from the Non-Sterile Aerobic Experiments

Compound	Percent of Applied ^{14}C-Activity Detected as Parent or Metabolite on Indicated Sampling Day							
	0	0.042	0.125	0.250	1	3	7	13
DCOIT	78.4	89.1	74.3	70.7	57.0	26.3	13.7	1.0
Others	0.6	1.4	1.5	1.8	5.8	3.1	3.4	4.1

Table A7.1.2.2.2.a-9: Average Percent of DCOIT and Metabolites Extracted with Solvent from the Sediment Phase of the Non-Sterile Aerobic Experiments.

Compound	Percent of Applied ^{14}C-Activity Detected as Parent or Metabolite on Indicated Sampling Day								
	0	0.042	0.125	0.250	1	3	7	13	101
Zone M	0.1	0.1	0.1	0.2	0.4	0.7	1.0	1.0	0.5
Zone N	0.6	0.3	0.5	0.8	1.3	3.2	4.5	4.0	1.4
Zone O	ND[2]	ND	0.1	0.3	0.4	0.9	1.6	1.9	1.5
NNOMA[1]	0.3	0.1	0.4	0.5	0.7	2.9	1.7	2.0	1.0
Peak Q	0.4	0.2	0.4	0.4	0.6	1.3	1.7	2.0	1.4
NNOA[1]	0.5	0.2	0.6	0.9	1.0	1.8	1.9	3.8	2.3
Peak S	0.1	0.1	ND	0.2	0.4	0.9	1.1	1.6	1.7
Peak T	0.6	0.3	0.7	1.1	0.9	2.3	2.4	2.1	2.0
Peak U	1.3	0.4	1.4	1.7	0.8	2.0	2.1	2.8	5.9
Peak V					2.2	4.9	6.5	9.4	5.3
Peak W				0.4	0.6	2.0	1.8	5.7	ND
Others	ND	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1

[1] NNOMA = N-(n-octyl) malonamic acid; NNOA = N-(n-octyl) acetamide
[2] ND = Not Detected.

Table A7.1.2.2.2.a-10: Average Percent of Metabolites Detected in Pooled Fraction of Peaks U, V, and W from the Sediment Phase (see Table 7.1.2.2.2-9)

Compound	Percent of Applied ^{14}C-Activity Detected as Parent or Metabolite on Indicated Sampling Day								
	0	0.042	0.125	0.250	1	3	7	13	101
Minor Metabolites	0.4	0.1	0.4	0.6	1.1	2.7	3.1	5.4	3.4
Major Metabolites	0.9	0.3	1.0	1.5	2.5	6.2	7.3	12.5	7.8
MZ496[1]	0.4	0.1	0.5	0.7	1.2	2.9	3.4	5.9	3.7
MZ308[1]	0.4	0.1	0.4	0.6	1.0	2.4	2.8	4.9	3.0
Other	0.1	<0.1	0.1	0.2	0.3	0.9	1.0	1.7	1.1

[1]MZ 496 = 3,3'dithiobis-(n-octyl)-3-chloropropenamide and MZ 308 = 2-chloro-3-(formyldithio)-N-octylpropenamide

Table A7.1.2.2.2.a-11: Characterization of Non-Extractable Bound Radioactivity in % applied radioactivity

Day	Percent of Applied ^{14}C-Activity					
	Non-Sterile System			Sterile System		
	Humin	Fulvic Acid	Humic Acid	Humin	Humic Acid	Fulvic Acid
0	9.4	5.2	2.7			
0.042	7.1	4.0	2.3			
0.125	7.8	3.5	1.2			
0.25	14.2	5.7	2.0			
1	18.2	3.8	1.1	36.1	8.5	1.2
3	27.6	9.0	3.2			
7	32.6	11.9	4.4	34.8	10.5	3.3
13	30.9	10.1	2.7			
30				41.1	13.7	5.1
101	39.9	14.6	3.5			

Table A7.1.2.2.2.a-12: Characterization of Non-Extractable Bound Radioactivity in % of bound residue

Day	Percent of Bound Residue					
	Non-Sterile System			Sterile System		
	Humin	Fulvic Acid	Humic Acid	Humin	Humic Acid	Fulvic Acid
0	54	30	16			
0.042	53	30	17			
0.125	62	28	10			
0.25	65	26	9			
1	79	16	5	79	19	2
3	69	23	8			
7	67	24	9	72	22	6
13	71	23	6			
30				69	23	8
101	69	25	6			

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[redacted]

Section A7.1.2.2.2.b Fresh Water/Sediment Degradation study - Anaerobic
Annex Point IIIA XII 2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Reference type: Study report Year: 2005 Report date: 14 July 2005 [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Rohm and Haas Company	
1.2.2			
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline Study**	Yes. OECD Guideline No. 308, Aerobic and Anaerobic Transformations in Aquatic Sediment Systems. April 2002.	
2.2	**GLP**	Yes	
2.3	**Deviations**	There was one GLP deviation : the modeling of the degradation kinetics was performed using a software package, ModelMaker, that has not been formally validated but is considered a standard method of analyzing data.	x
		3 MATERIAL AND METHODS	
3.1	**Test Material**	^{14}C –DCOIT (RH-5287) , [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	[redacted]	

Section A7.1.2.2.2.b **Fresh Water/Sediment Degradation study - Anaerobic**
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	substances	[redacted]
3.3	**Sediment and Water Characterization**	[redacted] x
3.4	**Test procedures**	
3.4.1	Test system	[redacted]
		[redacted]
		[redacted]
		[redacted] x
3.4.2	Preparation of test solution	[redacted]
3.4.3	Initial Test substance	[redacted]

Section A7.1.2.2.2.b **Fresh Water/Sediment Degradation study - Anaerobic**
Annex Point IIIA XII 2.1

concentration

3.4.4 Duration of test

3.4.5 Sampling details

3.4.6 Replicates

3.4.7 Extraction procedures

3.4.8 Bound residues- extent and nature

Section A7.1.2.2.2.b **Fresh Water/Sediment Degradation study - Anaerobic**
Annex Point IIIA XII 2.1

3.4.9 Analytical methods

[redacted]

3.4.10 Degradation products

[redacted]

4. RESULTS

4.1 Preliminary studies

Preliminary studies using water and sediment from Bury Pond were conducted to establish the dosing concentration, the extraction procedures and the sampling interval for the anaerobic definitive study. Dosing concentration in the preliminary studies was 0.1 mg/L, 1 mg/L and 5 mg/L. From the results of the preliminary study (Tables A7.1.2.2.2.b-3 and A7.1.2.2.2.b-4) it was determined to dose the anaerobic systems at 1 mg/L (1 ppm).

4.2 Definitive study

4.2.1 Distribution and recovery of radioactivity

Non-sterile system :

The distribution of ^{14}C-activity between the water phase, the solvent extractable residues, volatiles, and bound residues for an anaerobic non-sterile system dosed at 1 ppm is presented in Table A7.1.2.2.2.b-5. The total radioactivity in the water phase decreased rapidly from 85.3% (Day 0) to 2% of applied radioactivity at termination of the study (Day 100). Solvent extractable residues increased with time from 2.1% of the applied radioactivity on Day 0 to 36.9% on Day 7 (and 34.4% on Day 100). ^{14}C-activity detected in volatile traps was generally minimal. There was essentially no activity detected in the organic trap (ethyl digol). In the CO_2 traps (KOH) there was little activity detected until Day 100 when it comprised 5.2% of the applied radioactivity. Nonextractable (bound) residues comprised 9.0% of the applied on Day 0 and increased to 51.7% on Day 7 (and 49.9% on Day 100). Recovery of applied ^{14}C-activity ranged from 83.7% (Day 1) to 99.1% (Day 7) and averaged 93.71 ± 5.89%.

		Table A7.1.2.2.2.b-6 provides the quantitation of parent as a percent of applied radioactivity in the water phase for both the non-sterile and sterile systems. Parent was only detected in the water phase; none was detected in the sediment phase. In the non-sterile system, parent declined rapidly so that by Day 7 it comprised less than 1% of the applied radioactivity. From the results in Table A7.1.2.2.2.b-5 and A7.1.2.2.2.b-6 the percent parent in the water phase can be calculated. On Day 0, DCOIT comprises 89.1% (76/85.3) of the water phase and this decreases to 42.7% (9.1/21.3) on Day 1 and 8.6% (0.7/8.1) on Day 14. <u>Sterile system :</u> Table A7.1.2.2.2.b-7 provides the distribution of ^{14}C-activity between the different phases for the sterile system dosed with 1 ppm ^{14}C DCOIT. An examination showed that there was no microbial activity at Day 0 or 30 (termination). Similar to the non-sterile system, ^{14}C-activity in the water phase decreased with time and that in the sediment phase increased. Quantitation of parent compound (as percent of applied) is presented in Table A7.1.2.2.2.b-6. Similar to the non-sterile system there is a rapid decline in parent.	
4.2.2	Half-life	As no DCOIT was detected in the sediment, it was only possible to calculate a rate of dissipation of DCOIT from water phase. The concentration of parent (as percent applied) in the non-sterile system is presented in Table A7.1.2.2.2.b-6. The half-life was determined by best-fit linear regression analysis using ModelMaker software. The degradation constant; **k (days^{-1}): 3.28** and correlation coefficient **r^2: 0.89** The dissipation kinetics are expressed below **DT_{50} (days): 0.21** **DT_{75} (days): 0.42** **DT_{90} (days) 0.70**	x
4.2.3	Identification of metabolites	The initial metabolite profile in water and sediment for the non-sterile system is presented in Tables A7.1.2.2.2.b-8 and A7.1.2.2.2.b-9, respectively. In water (Table A7.1.2.2.2.b-8), eight bands of ^{14}C-activity were detected with parent being the primary compound especially during the early part of the experiment. Parent decreased from 76% of the applied radioactivity on Day 0 to less than 1% by Day 7. Also identified were N-(n-octyl) malonamic acid and N-(n-octyl) acetamide. Band V ranged from a replicate average of 3.5% (Day 0.125) of the applied activity to 8.3% (Day 1). No parent was detected in the sediment at any sample interval. In the sediment, there were initially about 11 metabolites identified (Table A7.1.2.2.2.b-9). All but region U/V/W was present at less than 5% (replicate average; percent of applied dose). Two of these regions were	x

		identified by cochromatography with standards as N-(n-octyl) malonamic acid and N-(n-octyl) acetamide. Band U/V/W replicate average ranged from 0.5% of the applied activity on Day 0 to 17.3% on Day 100. A pool of the U/V/W band was purified by additional TLC, and identified by mass spectroscopy. The results appear in Table A7.1.2.2.2.b-10. With the additional TLC, two bands of metabolites were initially observed; major band (accounting for approximately 70% of the activity) and a minor band (ca. 30%). Analysis of the major band resulted in the identification of 2 major metabolites, 3,3'dithiobis-(n-octyl)-3-chloropropenamide and 2-chloro-3-(formyldithio)-N-octylpropenamide. Both of these metabolites were present at less than 10% of the applied activity with 3,3'dithiobis-(n-octyl)-3-chloropropenamide, the larger of the two, being present at a maximum of 8.5% of the applied activity. CO_2 was identified as a metabolite comprising 5.2% of the applied activity by Day 100.	
4.2.5	Extent and nature of bound residues	After successive extractions of the sediment with various solvents, the remaining residue was exhaustively extracted for 18-24 hours with 0.5 N NaOH. The results for the non-sterile and sterile samples are presented in Tables A7.1.2.2.2.b-11 and A7.1.2.2.2.b-12. The insoluble fraction of humin contains about 5% – 38% of the applied radioactivity (Table A7.1.2.2.2.b-11). This correlates to 60% to 75% of the total bound residue (Table A7.1.2.2.2.b-12). 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. Of the remaining residue, about 20-25% of total bound activity is in the fulvic acid fraction and 5-9% in the humic acid fraction.	x
4.5.6	Metabolic pathway	A metabolic pathway is presented in Figure A7.1.2.2.2.b-1.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods		The test guideline employed was OECD Guideline No. 308, Aerobic and Anaerobic Transformations in Aquatic Sediment Systems (April 2002). There were no deficiencies and only one GLP deviation: use of an unvalidated software package (ModelMaker) to calculate degradation kinetics. For the definitive studies, 100 g of sediment (dry weight basis) and 400 g of water were added to bottles. For the sterile studies, water and sediment were sterilized prior to addition. Nitrogen (passed thru a sterile filter for the sterile samples) was drawn through the system and volatiles trapped in either ethyl digol or KOH. The systems were allowed to acclimatize for about 4 weeks prior to dosing at 1 ppm ^{14}C DCOIT. Duplicate bottles were removed from the non-sterile system on Days 0, 0.042, 0.125, 0.250, 1, 3, 7, 4 and 100 and from the sterile system on Days 1, 7, and 30. The water and sediment phase were separated by decanting. The water phase was partitioned with an organic solvent, and the organic phase chromatographed (TLC). The sediment was extracted with acetonitrile:HCl and KOH:methanol and chromatographed using TLC. Metabolites were isolated by TLC and identified by either cochromatography with standards or by LC-MS. The bound residues from the extracted sediments were exhaustively extracted using 0.5 NaOH. The basic extract was further separated into humic acid, fulvic acid, and humin fractions.	

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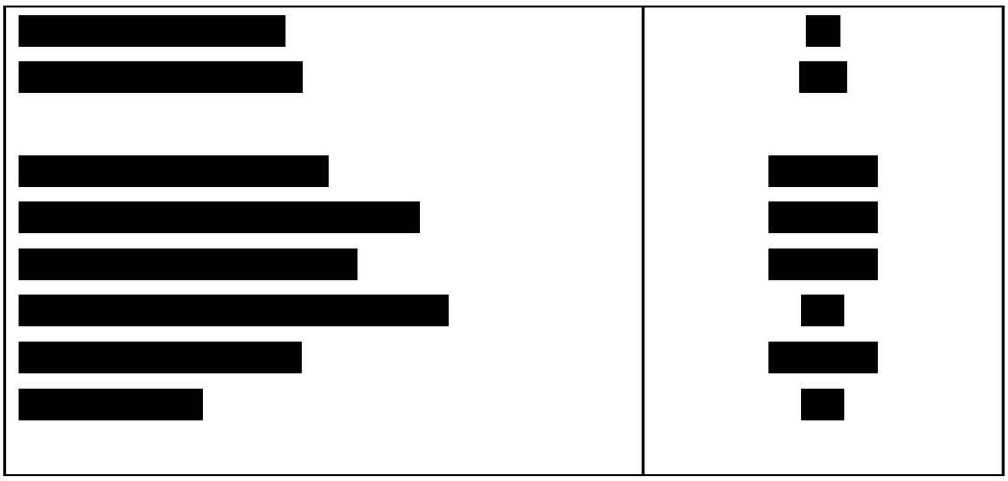
		Physical and chemical characterization of the system such as pH, temperature, Eh and TOC were determined periodically throughout the in-life study. Volatiles traps were periodically replaced and the removed trap quantitted by LSC.	
5.2	**Results and Discussion**	In the anaerobic fresh water:sediment microcosm studied, the half-life of DCOIT was 0.21days. There was a steady decrease of ^{14}C activity in the water phase which correlated with a steady increase in the sediment. On Day 0, 85.3% of the applied radioactivity was in the water phase but by Day 100 that had decreased to 2%. ^{14}C-activity in the solvent extractable sediment residue increased from 2.1% of the applied activity on Day 0 to 36.9% on Day 7. Activity in the bound residue increased to 51.7% of applied radioactivity on Day 7 and remained fairly constant thereafter. The humin fraction of the bound residues comprised over 60% of the bound residues. 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. By day 100, 5.2 % of the applied activity was $^{14}CO_2$. The distribution of metabolites is presented in Table A7.1.2.2.2.b-7, 8, and 9 and a metabolic pathway is presented in Figure A7.1.2.2.2.b-1. No metabolites was detected at greater than 10%. Parent was only detected in the water phase, not in the sediment phase. N-(n-octyl) malonamic acid and N-(n-octyl) acetamide were detected in both the water and sediment phase at less than 5% of the applied radioactivity. In the sediment phase, the major metabolites were 3,3'dithiobis-(n-octyl)-3-chloropropenamide and 2-chloro-3-(formyldithio)-N-octylpropenamide. They were present at a maximum of 8.5% and 3.6% (applied radioactivity), respecitively. Similar results were obtained for the sterile system. This abiotic degradation is probably the result of a nucleophile being released from the sediment by the autoclaving process.	x
5.3	**Conclusion**	DCOIT rapidly biodegrades in anaerobic fresh water:sediment microcosm with a half-life of 0.21 days. The half-life in other biologically active matrices has also been shown to be rapid. Metabolism involves cleavage of the isothiazolone ring and either subsequent oxidation to metabolites such as N-(n-octyl) malonamic acid and N-(n-octyl) acetamide or condensation to form 3,3'dithiobis-(n-octyl)-3-chloropropenamide and 2-chloro-3-(formyldithio)-N-octylpropenamide. N-(octyl) malonamic acid and N-(n-octyl) acetamide have been shown to be readily biodegradable (OECD 301B; see section A7.1.2.3).	x
5.3.1	Reliability	1-valid without restrictions.	x
5.3.2	Deficiencies	None	x

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	29 June 2007, revised 22 January 2009, revised 3 June 2010
Materials and Methods	**Comment (2.3):** There is one Guideline deficiency: At least two different sediment types and their associated waters are required for this study. A coarse textured sediment low in organic matter is lacking. **Comment (3.3):** In the sediment anaerobic conditions were derived. Regarding the water phase the redox potential was not lower than -100 mV as stated in the guideline; however, slightly reducing conditions were established (iron reduction may occur at +100 mV and lower) and the water was definitely not oxic. **Comment (3.4.1)**: Sterile experiment. Method of sterilization has not been stated in the study summary. According to additional information provided by the applicant, the vessels were autoclaved three times at 121°C, for 45 minutes each time. All manipulations with sterile flasks were performed in a laminar flow hood using aseptic techniques.
Results and discussion	**Comment (4.2.2)**: 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 (4.2.3):** Eleven metabolites (besides CO_2) were detected, each present at less than 10% of the applied activity, and four metabolites were identified. Two metabolites, 3,3'-dithiobis-(n-octyl)-3-chloroprpenamide and 2-chloro-3-(formyldithio) –N-cotylpropenamide, were identified by mass spectroscopy and two others, N-(n-octyl) malonamic acid and N-(n-octyl) acetamide, by cochromatogrpahy with a standard. **Comment (4.2.5**): After 100 days, 49.9 % of applied radioactivity was contained in the bound residues fraction. ^{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). 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. **Comment (5.2):** Parent DCOIT initially disappeared comparably fast in the sterile control as in the biotic samples, possibly due to nucleophiles produced during the autoclaving process. Nucleophiles such as SH^- and CN^- cleave the isothiazolone ring, with sulphur the most common point of attack, while OH^- and serine attack at C-5 with a displacement of Cl^-. Organic thiols, such as the amino acid cysteine, are quite reactive at sulphur. Cysteine has been shown to be a very effective decontamination agent causing rapid cleavage of the isothiazolone ring.

	This is generally the case for all thiazoles. In the non-sterile samples, nucleophiles may also have played an important role. Anaerobic metabolites as sulfides, mercaptans etc., may also catalyse cleavage of the isothiazolone ring, but can be considered biologically mediated. This may also explain the fact that anaerobic degradation is faster than aerobic degradation. Rapid biological degradation has been demonstrated for both estuarine water (A7.1.2.2.1) and soil (A7.2.1), and biological degradation is certainly also important in the present study. 0.2 % of applied activity was recovered as $^{14}CO_2$ in the sterile control after 30 days, compared to 0.6 % after 14 days and 5.6 % after 100 days in the non-sterile samples. Thus, it is concluded that DCOIT rapidly biodegrades in anaerobic fresh water:sediment microcosm with a half-life of 0.21 days at 9°C, even though abiotic/chemical processes certainly also contributed to this half-life.
Conclusion	**Comment (5.3)**: DCOIT was applied to the water surface and only detected in the water phase. Presumably most of it was degraded before reaching the sediment. Dissipation from water phase followed first order kinetics. When recalculated to 12°C according to the TGD, the DT_{50} = 0.17 days for the water phase (k = 4.1 $days^{-1}$). DCOIT was not detected in the sediment, but degradation is so rapid that the same rate can be considered valid for the whole freshwater-sediment system, making it applicable to both water and sediment. QSAR calculations show that the metabolites 3,3'dithiobis-(n-octyl)-3-chloropropenamide and 2-chloro-3-(formyldithio)-N-octylpropenamide are not expected to be readily biodegradable. However, these two compounds were present at less than 10% of applied radioactivity (2-chloro-3-(formyldithio)-N-octylpropenamide < 5% and 3,3'dithiobis-(n-octyl)-3-chloropropenamide <10%) and their ecotoxicological impact can therefore be assumed to be minor.
Reliability	**Comment (5.3.1)**: Due to the restrictions the reliability is changed from 1 to 2 - valid with restrictions
Acceptability	Acceptable with the restrictions noted above
Remarks	**Comment (5.3.2)**: According to the OECD Guideline degradation should be tested in two different sediment types. In principle, omitting the sandy sediment is a serious deficiency. However, degradation rates and metabolic pathways have been investigated in a range of sediments and soils from different environmental compartments (including sandy soils), which all show rapid dissipation. Except from the surface water study, several metabolites seem common to most environments and soil types. Therefore this deficiency is acceptable and no further testing is considered necessary.

Section A7.1.2.2.2.b	Fresh Water/Sediment Degradation study - Anaerobic **TABLES AND FIGURES**

[redacted]



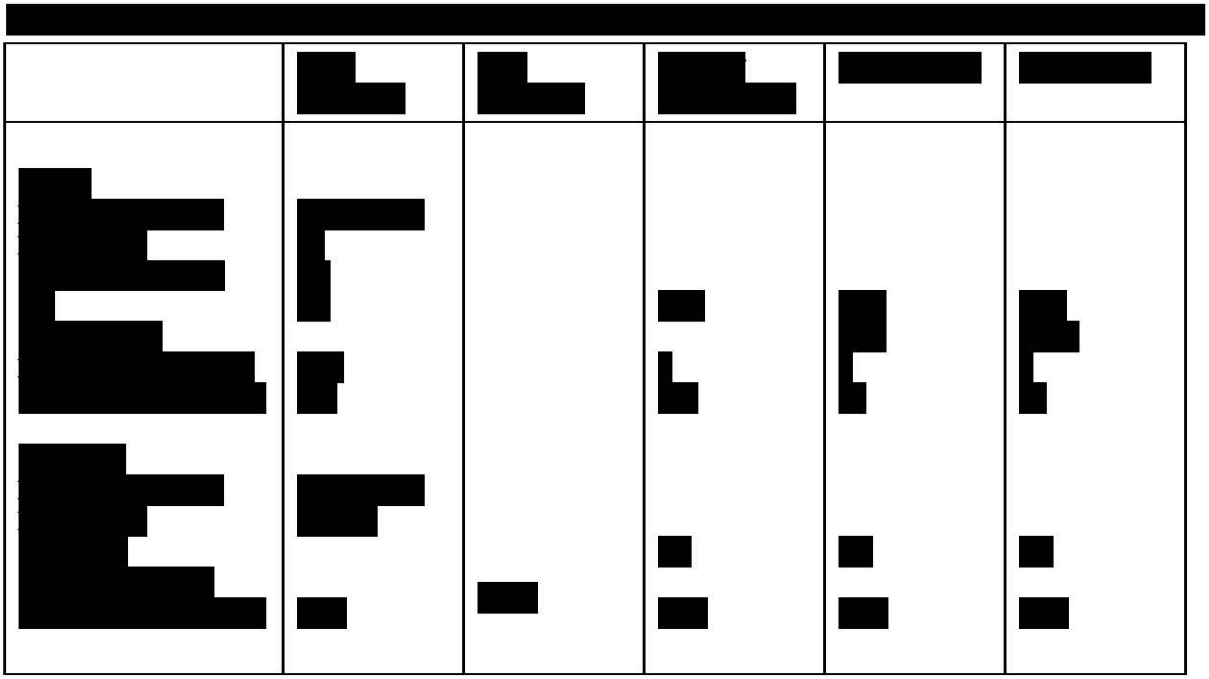


Table A7.1.2.2.2.b-3: Distribution of Radioactivity in Preliminary Study Following Dosing With ^{14}C RH-5287 in Preliminary Studies in % applied radioactivity

Sample	Water	Sediment		Volatiles	Recovery
		Extractable	Non-extractable		
0.1 mg/L					
Day 0	85.0	3.8	5.5	NA[1]	94.2
Day1	46.2	16.9	18.2	< LOQ	81.3
1 mg/L					
Day 0	82.1	5.3	7.2	NA	94.5
Day 1	39.4	25.0	25.8	< LOQ	90.6
Day 14	16.5	40.5	38.3	0.6	95.8
5 mg/L					
Day 0	83.1	7.5	7.6	NA	98.2
Day 1	33.8	28.7	28.5	< LOQ	91.0

[1] NA = Not applicable

Table A7.1.2.2.2.b-4: Quantitation of RH-5287 in Water and Sediment during Preliminary Studies in % applied radioactivity

Sample	Aerobic	
	Water	Sediment
0.1 mg/L		
Day 0	70.1	<LOD
Day1	8.7	<LOD
1 mg/L		
Day 0	79.7	1.0
Day 1	14.7	4.4
Day 14	2.0	7.8
5 mg/L		
Day 0	81.7	1.4
Day 1	30.8	3.4

Table A7.1.2.2.2.b-5: Distribution of Radioactivity in Definitive Study Following Dosing at 1 mg/L ^{14}C RH-5287; Non-Sterile System.

Day/Replicate	Percent of Applied ^{14}C-Activity[1]					
	Water Phase	Sediment-Extractable	Ethyl Digol Trap	KOH Traps	Bound Residue	Recovery of ^{14}C-Activity
0	85.3	2.1	NA[2]	NA	9.0	96.3
0.042	79.2	3.0	ND[3]	ND	14.4	96.5
0.125	55.6	11.9	ND	ND	27.9	95.4
0.25	38.9	20.1	ND	ND	33.5	92.4
1	21.3	28.9	ND	ND	33.6	83.7
3	12.4	35.3	0.1	ND	49.8	97.5
7	10.3	36.9	ND	0.2	51.7	99.1
14	8.1	33.8	0.1	0.6	48.8	91.4
100	2.0	34.4	ND	5.2	49.9	91.5
					Average Recovery:	93.71 ± 5.89%

[1] Average of duplicate
[2] NA= not applicable
[3] ND = not detectable

Table A7.1.2.2.2.b-6: Quantitation of ^{14}C DCOIT in the Water Phase of the Definitive Study in Non-Sterile and Sterile Systems[1]

Day	**Percent of Applied ^{14}C-Activity[2]**	
	Non-Sterile	**Sterile**
0	**76.0**	
0.042	**64.2**	
0.125	**48.6**	
0.25	**30.3**	
1	**9.1**	**21.6**
3	**1.5**	
7	**0.7**	**0.6**
14	**0.7**	
30		**0.3**

[1] DCOIT only detected in water. None was detected in the sediment phase.
[2] Average of duplicates.

Table A7.1.2.2.2.b-7: Distribution of Radioactivity in Definitive Study Following Dosing at 1 mg/L ^{14}C DCOIT; Sterile System

Day/Replicate	Percent of Applied ^{14}C-Activity[1]					
	Water Phase	Sediment-Extractable	Ethyl Digol Trap	KOH Traps	Bound Residue	Recovery of ^{14}C-Activity
1	29.7	25.6	ND[2]	ND	40.1	95.4
8	8.7	29.1	ND	ND	63.3	101.0
30	7.8	35.8	ND	0.2	51.8	95.5
					Average Recovery:	97.28 ± 3.75%

[1] Average of duplicate
[2] ND = not detectable

Table A7.1.2.2.2.b-8: Average Percent of DCOIT and Metabolites in the Water Phase from the Non-Sterile Anaerobic Experiments

Compound	**Percent of Applied ^{14}C-Activity Detected as Parent or Metabolite on Indicated Sampling Day**							
	0	**0.042**	**0.125**	**0.250**	**1**	**3**	**7**	**14**
Baseline	**0.3**	**0.8**	**0.3**	**0.4**	**0.3**	**0.8**	**0.5**	**0.6**
Polars	**ND[2]**	**ND**	**ND**	**0.1**	**0.2**	**0.6**	**0.8**	**0.2**
NNOMA[1]	**ND**	**ND**	**ND**	**ND**	**0.5**	**0.4**	**0.5**	**0.4**
NNOA[1]	**0.2**	**0.6**	**0.6**	**0.6**	**0.6**	**0.9**	**0.5**	**0.4**
Zone T	**0.4**	**0.5**	**ND**	**0.2**	**0.8**	**0.5**	**0.6**	**0.3**
DCOIT	**76.0**	**64.2**	**48.6**	**30.3**	**9.1**	**1.5**	**0.7**	**0.7**
Zone V	**4.1**	**7.2**	**3.5**	**4.1**	**8.3**	**5.2**	**4.5**	**4.4**
Others	**4.4**	**6.0**	**2.7**	**3.4**	**1.6**	**2.0**	**2.0**	**1.3**

[1] NNOMA = N-(n-octyl) malonamic acid and NNOA = N-(n-octyl) acetamide.
[2] ND = not detected.

Table A7.1.2.2.2.b-9: Average Percent of DCOIT and Metabolites Extracted with Solvent from the Sediment Phase of the Non-Sterile Anaerobic Experiments

Compound	Percent of Applied ^{14}C-Activity Detected as Parent or Metabolite on Indicated Sampling Day								
	0	0.042	0.125	0.250	1	3	7	14	100
Zone M	0.1	0.2	0.3	0.4	1.5	0.2	0.4	1.0	0.2
Zone N	0.4	0.5	2.9	2.3	2.6	4.0	5.1	3.0	2.6
Zone O	0.2	0.2	0.6	1.2	0.8	2.2	2.3	1.6	1.4
NNOMA[1]	0.2	0.3	1.1	0.9	0.9	1.3	1.8	0.9	0.2
Peak Q	0.2	0.3	0.7	1.3	1.0	2.0	3.2	2.3	1.6
NNOA[1]	0.2	0.3	1.1	1.9	3.5	3.4	3.1	2.9	2.7
Peak S	0.3	0.2	0.7	1.1	1.3	2.1	1.9	1.7	1.7
Peak T	0.3	0.5	1.0	2.6	2.9	4.2	3.2	3.4	2.3
Peak U	0.5	0.8	3.1	7.4	12.7	13.5	11.2	12.0	17.3
Peak V									
Peak W							1.9	4.5	
Others	ND[2]	ND	ND	ND	ND	0.1	0.1	ND	0.1

[1] NNOMA = N-(n-octyl) malonamic acid; NNOA = N-(n-octyl) acetamide
[2] ND = Not Detected.

Table A7.1.2.2.2.b-10: Average Percent of Metabolites Detected in Pooled Fraction of Peaks U, V, and W from the Sediment Phase (see Table 7.1.2.2.2-9)

Compound	Percent of Applied ^{14}C-Activity Detected as Parent or Metabolite on Indicated Sampling Day								
	0	0.042	0.125	0.250	1	3	7	14	100
Minor Metaboliters	0.2	0.2	0.9	2.2	3.8	4.1	3.9	5.0	5.2
Major Metabolites	0.4	0.6	2.2	5.2	8.9	9.5	9.2	11.6	12.1
MZ496[1]	0.3	0.4	1.5	3.6	6.2	6.7	6.4	8.1	8.5
MZ308[1]	0.1	0.2	0.7	1.6	2.7	2.9	2.8	3.5	3.6

[1] MZ 496 = 3,3'dithiobis-(n-octyl)-3-chloropropenamide and MZ 308 = 2-chloro-3-(formyldithio)-N-octylpropenamide

Table A7.1.2.2.2.b-11: Characterization of Non-Extractable Bound Radioactivity in % applied radioactivity

Day	Percent of Applied ^{14}C-Activity					
	Non-Sterile System			Sterile System		
	Humin	Fulvic Acid	Humic Acid	Humin	Humic Acid	Fulvic Acid
0	5.2	2.4	2.7			
0.042	8.4	3.5	2.3			
0.125	13.0	4.9	1.2			
0.25	16.7	5.2	2.0			
1	26.6	7.4	1.1	25.8	5.9	1.8
3	32.9	2.7	3.2			
7	37.7	10.4	4.4	43.0	12.6	2.8
14	31.6	12.7	2.7			
30				43.1	7.4	0.7
100	34.2	13.1	3.5			

Table A7.1.2.2.2.b-12: Characterization of Non-Extractable Bound Radioactivity in % of bound residue

Day	Percent of Bound Residue					
	Non-Sterile System			Sterile System		
	Humin	Fulvic Acid	Humic Acid	Humin	Humic Acid	Fulvic Acid
0	60	28	12			
0.042	65	27	11			
0.125	67	25	7			
0.25	71	22	6			
1	74	21	5	77	18	5
3	75	6	19			
7	74	20	6	74	22	5
13	65	26	8			
30				84	14	1
101	68	26	6			

[redacted]

Section A7.1.2.2.2.c Sea Water/Sediment Degradation study - Aerobic
Annex Point IIIA XII 2.1

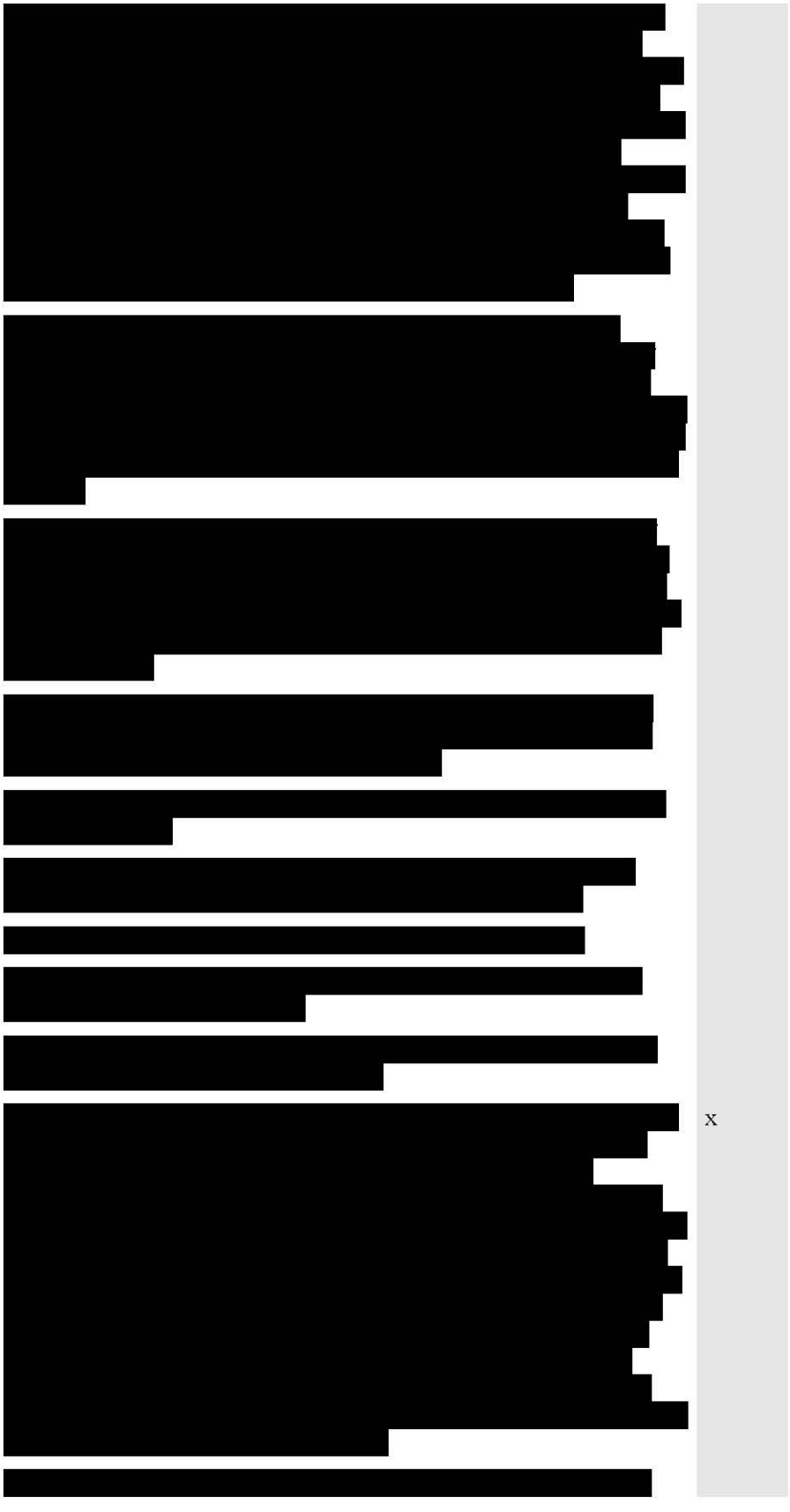
Official use only

		1 REFERENCE
1.1	**Reference**	**Kinetics and some metabolite identification:** Reference type: Study report Year: 1991 Report date: 26 March 1991 [redacted] **Nature of bound residues:** Reference type: Study report Year: 1992 Report date: 1 May 1992 [redacted] **Supplemental metabolites identification:** Reference type: Study report Year: 1993 Report date: 7 May 1993 [redacted] **Extractability and Stability of DCOIT in Sediment:** Reference type: Study report Year: 1995 Report date: 26 June 1995 [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2		
1.2.3	Criteria for data	[redacted]

	protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline Study**	Reference 1, 2 and 3: Yes. U.S. Environmental Protection Agency, 40 CFR § 158, Subdivision N, Chemistry, Environmental Fate 162-4. Reference 4: No applicable guideline	
2.2	**GLP**	Yes	
2.3	**Deviations**	GLP deviations were minor. • Sediment and seawater physiochemical characterization was not performed under GLP guidelines. • No in-life audit for Reference 2 though the raw data and report were audited. • The ^{14}C RH-5287 test material was synthesized prior to the initiation of GLP regulations (October 16, 1989). The material has been subsequently characterized following GLP guidelines. • The ^{13}C RH-5287 used in this study was not in compliance with GLP. Purity analysis was scientifically valid but it was not done according to GLP guidelines. • The ^{12}C chromatography standards while completely characterized, was done so prior to the implementations of GLP. • Microbial cultures used as a matrix for generating metabolites were prepared in a non-GLP laboratory. • Microbial treatment methods were developed during the study and thus not describe in the protocol.	x
		3 MATERIAL AND METHODS	
3.1	**Test Material**	^{14}C –DCOIT (RH-5287)	
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	[redacted]	

substances	[redacted]	
3.3 Sediment and Water Characterization	[redacted]	
3.4 Test procedures		
3.4.1 Test system	[redacted]	x

Section A7.1.2.2.2.c **Sea Water/Sediment Degradation study - Aerobic**
Annex Point IIIA XII 2.1



3.4.2	Preparation of test solution	
3.4.3	Initial Test substance concentration	
3.4.4	Duration of test	
3.4.5	Sampling details	x

Section A7.1.2.2.2.c **Sea Water/Sediment Degradation study - Aerobic**
Annex Point IIIA XII 2.1

[redacted]

3.4.6 Replicates

[redacted]

3.4.7 Extraction procedures

[redacted]

Section A7.1.2.2.2.c **Sea Water/Sediment Degradation study - Aerobic**
Annex Point IIIA XII 2.1

3.4.8 Bound residues- extent and nature

3.4.9 Analytical methods

3.4.10 Degradation products

4 RESULTS

4.1 Preliminary studies

Preliminary studies were conducted to develop an effective method of extracting the ^{14}C-activity from sediment. Sediment was spiked with ^{14}C DCOIT and extracted. Table A7.1.2.2.2.c-3 summaries the results. Soxhlet extraction with methylene chloride:methanol was the superior method.

4.2 Definitive study

x

4.2.1 Distribution and recovery of radioactivity

For Reference 1, 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.c-4 and A7.1.2.2.2.c-5, respectively. The water phase contained less than 8.5% of the applied activity and generally in the 3-4% range. Soxhlet extractable residue slowly decreased with time. The ^{14}C-activity in the ethylene glycol traps was minimal while $^{14}CO_2$ trapped in the NaOH traps increased to almost 22% and 9% of the applied activity in the 0.05 ppm and 1 ppm dosing levels, respectively. This suggests that 1 ppm DCOIT in this system is somewhat inhibitory to microbial activity. It has been shown in the lab that DCOIT exhibits biocidal activity at less than 0.25 ppm. After 1 day the bound residue is about 60-65% and generally remains constant throughout the study. Recovery of applied ^{14}C-activity was 103.6 ± 10.8% and 96.7 ± 13.5% for the 0.05 ppm and 1 ppm dosing rates, respectively.

The results from chromatography of the ^{14}C-activity extracted from sediment samples for both dosing levels appear in Table A7.1.2.2.2.c-6. Parent was only detectable on Day 0, and at less than 6% of the total applied ^{14}C-activity. It took approximately 1 hour to process (separate phases, add sodium sulfate and precipitated silica) and biologically deactivate 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.

For Reference 3, Table A7.1.2.2.2.c-7 shows that the initial KOH:methanol extraction of the sediment did a very good job of extracting the ^{14}C-activity. The recovery from the Sep-Pak® clean-up, 90%, was very good. With the microbial cultures, ethyl acetate extracted 69% to 91% of the applied ^{14}C-activity.

The sediment treated samples were employed for quantitation of metabolites in reference 3. Metabolites were isolated by chromatography (TLC, HPLC). The quantitative results are presented in Table A7.1.2.2.2.c-8. Successive chromatography of the isolated TLC bands showed that the bands often comprised more than one compound. The identification of the compounds isolated by chromatography is discussed below.

For Reference 4, the distribution between the dichlormethane:methanol and methanol Soxhlet extractions and the insoluble residue is presented in Table A7.1.2.2.2.c-9. Practically all the radioactivity was extracted with the initial dichlormethane:methanol Soxhlet extraction. Average recovery of applied ^{14}C-activity in the dichloromethane:methanol

		extract was 105.07 ± 9.80% while recovery of total radioactivity was 107.10 ± 9.25%. Therefore, on average less than 2.5% of the applied activity was present in the methanol Soxhlet extract and the insoluble residue combined. Due to the efficiency of the dichlormethane:methanol solvent to quantitatively extract ^{14}C-activity, after Day 0 only this fraction was chromatographically analyzed. Table A7.1.2.2.2.c-10 presents the quantitation of DCOIT from the Soxhlet extraction of sediment stored either at room temperature or frozen. DCOIT (replicate average) as a percent of the total ^{14}C-applied ranges from 87.76% (Day 98, Frozen) to 110.73% (Day 189, Frozen) with an average value of 97.51 ± 8.14%. Quantitation (replicate average) as a percent of recovered ^{14}C-activity ranges from 81.08% (Day 35, Room Temperature) to 99.59% (Day 189, Frozen) with an average value of 91.22 ± 6.42%. Frozen control samples mixed with precipitated silica and anhydrous sodium sulphate were spiked with ^{14}C-DCOIT and immediately extracted in a manner similar to the stored samples. Quantitation of ^{14}C-DCOIT is presented in Table 7.1.2.2.2.c-11. The average quantitation of ^{14}C-DCOIT as a percent of applied activity is 102.48 ± 15.74% while as a percent of recovered ^{14}C-activity, 99.32 ± 0.55%	
4.2.2	Half-life	A kinetic analysis was only performed in Reference 1. The results in Table A7.1.2.2.2.c-6 show that only at Day 0 was DCOIT detected. By the next sampling interval and subsequent intervals, no parent compound was 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 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 seawater:sediment system is less than 1 hour.	x
4.2.3	Identification of metabolites	The metabolite identification from Reference 3 is presented in Table A7.1.2.2.2.c-12. $^{14}CO_2$ was a major metabolite and its presence can only occur if the isothiazolone ring is cleaved and undergoes subsequent oxidation. Three metabolites, N-(n-octyl) malonamic acid, N-(n-octyl) acetamide, and N-(n-octyl)-β-hydroxypropionamide were isolated and identified as metabolites. Ten additional compounds were isolated ranging from 0.1 to 2.8% of the applied ^{14}C-activity. An attempt was made to identify several of these minor metabolites but the quantity available was insufficient for successful mass spectroscopic identification.	
4.2.5	Extent and nature of bound residues	Reference 2 describes the extent and nature of the bound residues. Soxhlet extracted sediments were exhaustively extracted with 0.25 N HCl followed by 1N NaOH. The results for samples from Day 0 and 30 are presented in Table A7.1.2.2.2.c-13. Over 75% 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

4.5.6	Metabolic pathway	A metabolic pathway is presented in Figure A7.1.2.2.2.c-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 162-4. Between the original report and the two supplemental reports there are no deficiencies. Initially, Erlenmeyer flask containing 55.7 g wet sediment (20 g dry weight) and 64.3 ml of seawater obtained from the York River, Virginia, USA were dosed with either 0.05 ppm or 1 ppm of ^{14}C DCOIT. On Day 0,1, 2, 5, 9, 15, 20, 26, and 30 volatiles were trapped, the sediment and water phases separated, and these three phases quantitated by radioassay. The water phase was partitioned with methylene chloride. The sediment phase was Soxhlet extracted. 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 0.25N HCl and 1N NaOH. The basic extract was further separated into humic acid, fulvic acid and humin fractions. Metabolite identification was performed by dosing only sediment (no seawater) with 0.1 ppm ^{14}C DCOIT overnight. Using TLC and HPLC metabolites were quantitated. Microbial cultures derived from the sediment were dosed at 5 ppm ^{14}C DCOIT ppm to assist with metabolite identification by providing additional quantities of metabolites. Chromatographic behavior of the culture derived metabolites was correlated to those from the sediment and metabolites identified by mass spectroscopy. For quantitation of DCOIT in sterile sediment (Reference 4) stored at room temperature or frozen, jars containing 30 g of sterile sediment obtained from the York River were dosed aseptically with a 1.03 ppm ^{14}C-DCOIT and stored either at room temperature or frozen. Periodically duplicate samples were removed and Soxhlet extracted with dichlormethane:methanol (1:1) and then methanol. After concentration of the extracts, they were chromatographed and DCOIT quantitated by HPLC.	
5.2	**Results and Discussion**	In the aerobic seawater:sediment microcosm studied, the half-life of DCOIT was less than 1 hour. This was derived from the Day 0 samples which actually took 1 hour to bio-inactivate. In these samples less than 6% of the applied radioactivity was parent. At all sampling intervals about 70% to 90% of the ^{14}C-activity was detected in the sediment phase. About 50-60% of the activity was detected in the bound residues. At the study termination, $^{14}CO_2$ comprised about 9-21% of the applied activity. Over 75% of the activity in the bound residue was present in the insoluble humin fraction indicating that the octyl chain is intercalating within the soil crystal lattice. The presence of CO_2 demonstrates that the isothiazolone ring has been cleaved and undergone additional oxidation. Initially the isolated metabolites were characterized by comparing their HPLC retention times to standards. The metabolites were characterized as being primarily N-(n-octyl) malonamic acid/N-(n-octyl) oxamic acid and N-	x

		(n-octyl) acetamide. Subsequently the metabolites were definitively identified by mass spectroscopy as N-(n-octyl) malonamic acid, N-(n-octyl) acetamide and N-(n-octyl)-β-hydroxypropionamide. About 10 additional metabolites were detected ranging from 0.1 to 2.8% of the total applied ^{14}C-activity. Insufficient quantities of these were present for mass spectroscopy. The results from the extractability and storage stability studies demonstrates that DCOIT can be quantitatively extracted from sediment and soil. This implies that any ^{14}C-activity that remains in sediment after extraction is a degradate and not parent.	
5.3	**Conclusion**	DCOIT rapidly biodegrades in seawater:sediment microcosm with a half-life of less than 1 hour. The half-life in other biologically active matrices has also been shown to be rapid. Metabolism involves cleavage of the isothiazolone ring and subsequent oxidation of metabolites such as N-(n-octyl) malonamic acid and N-(n-octyl) acetamide. Both of these compounds have been shown to be ready biodegradable (OECD 301B; see section A7.1.2.3). Parent can be quantitatively extracted from sediment and therefore the ^{14}C-activity after extraction and associated with the bound residue/post extraction solids (PES) is not parent but ring cleaved metabolites.	
5.3.1	Reliability	1-valid without restrictions.	x
5.3.2	Deficiencies	None.	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 EPAGuidelines 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 (supplemental metabolites identification A7.1.2.2.2.c/03). **Comment (3.4.1)**: Application method of test compound was not stated. Additional information provided by the applicant states that the seawater spiked with DCOIT was placed in the flask first and that the sediment was added after that. According to the new OECD Guideline 308 the test compound should be carefully mixed into the water phase, disturbing the sediment as little as possible. This was not done accordingly in this test. **Comment (3.4.5)**: The seawater and sediment were separated by either centrifugation (Days 2-30) or filtration (Days 0-1), but ideally the surface water should be carefully removed with minimum disturbance of the sediment, in order

	to get reliable DT_{50} values for the water phase. Eh, pH and dissolved oxygen has not been measured during the first 9 days of the study. As no acclimatisation took place it is possible that the sediment was not fully anaerobic when it was added. However, Eh was about zero at day 9 when the first measuresments were conducted with increasing tendency in subsequent measurements. It seems therefore probable that Eh was negative at day 0 and sulphide compounds have been present. These could have reacted with DCOIT, which lead to a DT50 less than 1 hour.
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 in the anaerobic parts of the sediment. Due to the absence of abiotic control the absence of abiotic degradation in this test test system has not been unambiguously demonstrated. On the other hand, more than 20 % of applied radioactivity was recovered as CO_2 (at the low concentration), which demonstrates that microbial metabolism is involved in ultimate degradation. It may almost be impossible to distinguish between abiotic and biotic degradation of DCOIT in seawater-sediment systems because high concentrations of nucleophiles will always be present in biologically active marine sediments. Even if it could not unambiguously be demonstrated that the sediment was anaerob, the study simulates the conditions in marine sediment surfaces, which are not completely anoxic. In the freshwater-sediment study it has been shown that biodegradation was much faster under anaerobic than under aerobic conditions. The study shows that in marine sediments, degradation is very fast even if there should be some oxygen present. **Comment (4.2.2)**: 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 (4.2.5)**: After 30 days, 63.5 % of applied radioactivity was contained in the bound residues fraction. ^{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 (4.2.5 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	Agree with applicant's version
Reliability	**Comment (5.3.1 and 5.3.2):** Due to the restrictions described the reliability is changed from 1 to 2 – valid with restrictions
Acceptability	Acceptable with the restrictions noted above.

Remarks	According to information provided buy the applicant, nucleophiles such as SH^- and CN^- cleave the isothiazolone ring, with sulphur being the most common point of attack, while OH^- and serine attack at C-5 with a displacement of Cl^-. Organic thiols, such as the amino acid cysteine, are quite reactive at sulphur. Anaerobic metabolites as sulfides, mercaptans etc., may also catalyse cleavage of the isothiazolone ring, but can be considered biologically mediated. Rapid biological degradation has been demonstrated for both estuarine water (A7.1.2.2.1) and soil (A7.2.1) and biological degradation is certainly also important in the present study.

Section A7.1.2.2.2.c	Sea Water/Sediment Degradation study - Aerobic
	TABLES AND FIGURES

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

Parameter	York River Sediment	Bethany Creek Sediment	Carter Creek Sediment
pH	6.6	7.3	7.8
Percent Sand	20	79	6
Percent Silt	60	13	39
Percent Clay	20	8	55
Texture Class	Silt Loam	Loamy Sand	Clay
Organic Matter (% dry)	8.1	2.8	6.9
Cation Exchange Capacity (meq/100g)	35	29.8	33.0
Field Capacity (%)	58		
Percent Moisture at 1/3 bar		16.5	44.2
Bulk Density (gm/cc)		0.93	0.91
Sulfur (Pyritic; %)	0.72		
Sulfur (Sulfate; %)	0.12		
Sulfur (Total; %)	1.18		

Table A7.1.2.2.2.c-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.c-3: Preliminary Study Examining Extraction Efficiency of ^{14}C-Activity from Sediment

Extraction Method	Extraction Efficiency (%)
Ether:Methanol (1:1); Stirring	34
0.1 M $Ca(OH)_2$; Shaker	7.1
30% Aqueous Ethanol; Reflux	15.0
70% Aqueous Ethanol; Reflux	25.5
Acetic Acid/Zinc, Shaker	8.0
Acetic Acid/Zinc, Stirring	8.0
Acetone; Stirring	4.4
Acetone:Hexane (1:1); Stirring	3.8
20% Aqueous Methanol; Soxhlet	32.0
Water; Soxhlet	19.5
Methylene Chloride:Methanol (9:1); Soxhlet	>70

Table A7.1.2.2.2.c-4: 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
0	3.2	30.9	16.6	NA[4]	NA	62.0	116.0
1	7.8	15.3	14.8	0.6	0.6	62.2	101.1
2	8.2	15.1	14.0	2.5	2.5	55.3	98.2
5	7.5	14.6	12.7	ND	8.1	66.8	109.6
9	3.8	12.6	12.0	1.6	8.4	59.0	95.1
15	3.9	13.5	8.8	ND	8.2	56.5	90.8
20	2.3	13.6	11.0	0.5	9.1	77.9	114.4
26	ND	9.4	10.9	1.2	14.2	67.0	102.6
30	1.3	7.4	8.8	1.7	21.9	63.5	104.6

[1] Average of duplicate
[2] Soxhlet 1 was performed with methylene chloride:methanol
[3] Soxhlet 2 was performed with methanol
[4] NA= not applicable
[5] ND = not detectable

Table A7.1.2.2.2.c-5: 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
0	5.1	28.3	15.4	NA[4]	NA	65.6	102.1
1	4.8	24.9	15.7	1.0	0.1	57.3	103.7
2	5.2	18.1	17.0	0.0	1.6	49.8	91.5
5	5.1	17.6	10.9	0.2	2.1	53.8	89.5
9	3.7	14.5	10.4	1.5	2.3	61.4	93.6
15	4.3	15.0	12.5	0.2	5.9	55.6	93.5
20	3.0	16.7	10.8	0.6	7.0	66.8	104.8
26	0.8	10.7	11.4	2.0	7.2	68.4	101.1
30	0.7	9.6	15.2	0.7	8.7	54.5	89.4

[1] Average of duplicate except day 15 (portion of one of the Soxhlet 2 replicates lost)
[2] Soxhlet 1 was performed with methylene chloride:methanol
[3] Soxhlet 2 was performed with methanol
[4] NA= not applicable
[5] ND = not detectable

Table A7.1.2.2.2.c-6: Quantitative Characterization of ^{14}C-Activity by HPLC extracted from sediment samples

Day	Percent of Applied ^{14}C-Activity[1]						
	0.05 ppm			1 ppm			
	DCOIT	Other <[2]	Other >[3]	DCOIT	RH-893	Other <[2]	Other >[3]
0	5.1	41.1	1.2	4.4	5.1	31.7	4.8
1	ND[4]	44.7	0.7	NA[5]	NA	NA	NA
2	ND	27.6	1.3	ND	ND	35.1	ND
5	ND	27.0	0.3	ND	1.0	25.8	1.7
9	ND	23.9	0.7	ND	ND	19.6	5.2
15	ND	22.3	ND	ND	ND	27.5	ND
20	ND	24.8	ND	ND	ND	27.4	ND
26	ND	23.0	ND	ND	ND	22.8	ND
30	ND	13.1	ND	ND	ND	24.8	ND

[1] Average of duplicate samples
[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.c-7: Summary of Recoveries from Application of ^{14}C DCOIT to Sediment

Extraction Step	Average Percent of Total Applied Activity
0.15 M KOH:Methanol	68.4
1N HCL	2.9
1N NaOH	10.6
Methanol Wash	3.8
Bound Residue	19.2
Clean-up and Isolation	**Percent Recovery**
Sep-Pak® Clean-up	90.2
Methylation with BF_3/Methanol	136.2
TLC and Elution	78.0

Table A7.1.2.2.2.c-8: Summary of Chromatographic Quantitation of Metabolites

TLC-1[1] Bands	TLC-2[2] Bands	HPLC Peaks	Percent of Applied ^{14}C-Activtiy
A		Major Minor-1 Minor-2 Minor-3	9.6 2.8 0.7 1.0
B		Major Minor	13.5 3.4
C	1 2 3 4	Major Minor Major Major Minor-1 Minor-2 	1.9 0.3 2.5 0.5 0.1 0.1 1.0
D	1 2 3	Major Major Major	2.1 2.0 2.8

[1] Initial TLC
[2] Subsequent TLC—Bands from initial TLC were isolated and rechromatographed in a different solvent

Table A7.1.2.2.2.c-9: Recovery of ^{14}C-Activity from Sediment Stored at Room Temperature or Frozen and Subsequently Soxhlet Extracted.

Day/Sample	^{14}C-Activity Recovery as a Percent of Applied Dose[1]			
	DCM/Methanol	Methanol	Insoluble Residue	Recovery (%)
Day 0 Frozen	95.41	1.86	8.37	104.74
Day 35 Frozen Room Temp.	102.36 112.36	0.23 0.21	1.17 1.86	103.75 114.43
Day 63 Frozen	108.89	0.27	0.94	110.09
Day 98 Frozen	92.72	0.36	0.78	93.87
Day 136 Frozen Room Temp.	90.87 126.96	1.13 0.24	0.94 0.82	91.94 128.02
Day 189 Frozen Room Temp.	110.73 106.24	0 0.46	0.47 0.39	111.20 107.08
Day 224 Frozen Room Temp.	106.32 103.76	0.26 0.27	1.18 1.15	107.76 105.17
Average	105.07 ± 9.80	0.48 ± 0.51	1.64 ± 2.16	107.10 ± 9.25

[1] Average of duplicate samples