Document III-A / Sections A6.8 to A6.17

Section A6.12.6 Annex Point IIA6.9.6/01	Medical Data – sensitization/allergenicity observations – reference A6.12.6/08	
	4 RESULTS	
4.1 Clinical Signs	Approximately one-half of the subjects dosed with 350 ppm DCOIT showed responses that ranged between barely perceptible to moderate in degree.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Duplicate occlusive human patch tests were conducted using ethanol as the vehicle. One set of patches was removed after 24 hours and the second set of patches was removed after 48 hours.	
5.2 Results and discussion	There were no distinct differences in irritation between the four concentrations of DCOIT tested. The number and degree of responses observed at the lowest concentration (350 ppm DCOIT) were not markedly different from the responses observed at the highest concentration (1000 ppm DCOIT). Two subjects reacted adversely to all test concentrations of DCOIT and still exhibited reactions 10 to 15 days after patch removal.	
5.3 Conclusion	Studies of RH-287 Technical in ethanol demonstrated that 350 ppm DCOIT is at or near the threshold concentration for irritation and sensitization.	X

Evaluation by Competent Authorities

Evaluation by Rapporteur Member State

Date	26 September 2006
Materials and Methods	Agree with applicant's summary and conclusion. **Comment (3.1):** Personal communication with Rohm and Haas, January 2008: Kathon™ 930, (30% DCOIT in xylene) diluted in ethanol DCOIT test concentrations: 350 ppm, 500 ppm, 750 ppm, 1000 ppm in ethanol **Comments (3.3.2):** The quantity of test substance applied (0.01 ml on 8mm circular chamber discs) should have been stated.
Results and discussion	Agree with applicant's version
Conclusion	Studies of RH-287 Technical in ethanol elicited about the same degree of skin irritation in test subjects when applied in various concentrations from 350 to 1000 ppm. Two subjects experienced reactions that appeared to be of allergic nature probably from exposure to the test substance
Remarks	

Section A6.12.6/09 Medical Data – sensitization/allergenicity observations

Annex Point IIA6.12.6/09 Reference A6.12.6/09

			Official use only
		1 REFERENCE	
1.1	**Reference**	A6.12.6/09.Kawai K, Nakagawa M, Sasaki Y, Kawai K: **Occupational contact dermatitis from Kathon™ 930.** Contact Dermatitis 1993 Feb;28(2):117-8.	
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
		3 MATERIALS AND METHODS	
3.1	**Substance**	Kathon™930 biocide (30 % DCOIT in xylene)	
3.2	**Persons exposed**		
3.2.1	Sex	Males and females	
3.2.2	Age/weight	Only indicated for the 8 workers with dermatitis; i.e. 20-63 years old	
3.2.3	Known Diseases	Not specified	
3.2.4	Number of persons	19 workers	
3.2.5	Other information		
3.3	**Exposure**	Dermal	
3.3.1	Reason of exposure	Occupational exposure in a textile finishing factory in Japan	
3.3.2	Frequency of exposure	Repeated exposure	
3.3.3	Overall time period of exposure	A new type of biocide (Kathon™930) was added to the finishing agent about 3 weeks prior to the occurrence of dermatitis.	
3.3.4	Duration of single exposure	Not specified	
3.3.5	Exposure concentration/dose	30% active ingredient (DCOIT) solution in xylene was used as a biocide added to the finishing agent of textiles.	
3.3.6	Other information	The textile finishing unit: Protective gloves used when handling the finishing agent. Short-sleeved shirts worn and no protective equipment on upper arms or forearms. The drying and inspection unit: The finished textiles were dried at room temperature for about 24 hours, and then checked for quality. The finished textiles were handled directly. Because of the length of the textiles, the workers carried them by using their forearms.	

3.4	**Examinations**	Open patch test were performed on 6 patients with the finishing agents, with and without 0.2% biocide. Dermal examination of treated areas was carried out. Closed patch test with Kathon™CG [5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one in the ratio of 3:1] (50 ppm aq.) in the same patients.
3.5	**Treatment**	Not reported
3.6	**Remarks**	None
		4 RESULTS
4.1	**Clinical Signs**	Eight out of 19 workers (2 males and 6 females) developed itchy reddish eruptions on exposed areas of skin 23-38 days after the new biocide was introduced. The 8 workers worked in the textile finishing unit (2 persons) and the drying and inspection unit (6 persons). In addition to dermatitis on the forearm and/or upperarm four of the patients working in the drying and inspection unit also developed erythema on their faces/necks. Airborne contact dermatitis may have occurred in these patients.
4.2	**Results of examinations**	Five out of 6 patients tested with open patch test showed strong positive reactions to the finishing agents with 0.2% biocide, and none showed any reaction to the finishing agents without biocide. The patient who showed no positive reaction to the finishing agents with 0.2% biocide at D2 and D3, did show pigmentation on the same tested skin at D17. She had taken corticosteroids orally 2 days prior to the tests because her dermatitis was severe. As a result she may have shown a false negative reaction. No cross reaction to Kathon CG observed.
4.3	**Effectivity of medical treatment**	Not reported
4.4	**Outcome**	Not reported
4.5	**Other**	
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	An outbreak of occupational contact dermatitis due to Kathon ™-930 added to the finishing agent was described. Eight out of 19 persons employed in a Japanese textile finishing factory developed oedematous reddish eruptions on their forearms, upper arms, face or neck approximately three weeks after the biocide was introduced. Patch test was carried out on 6 of the patients
5.2	**Results and discussion**	Five out of 6 patients showed strong positive reactions in the open patch test to the finishing agents with 0.2% biocide

Document III-A / Sections A6.8 to A6.17

Section A6.12.6/09	**Medical Data – sensitization/allergenicity observations**
Annex Point IIA6.12.6/09	**Reference A6.12.6/09**

5.3 Conclusion	DCOIT showed a strong sensitizing effect among workers after skin exposure in a textile finishing factory.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 March 2007
Materials and Methods	The applicant's version is acceptable
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Remarks	The concentration of DCOIT in the finishing solution causing the outbreak of occupational contact dermatitis, is not clearly stated in the article.

Section A6.12.7
Annex Point IIA6.9.7

Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment
Reference A6.12.7

Official use only

1 REFERENCE

1.1 **Reference**

A6.12.5/01 Wooder M. (2006) 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one – DCOIT (CAS 64359-81-5), Diagnosis of poisoning including specific signs of poisoning and clinical tests, Treatment in case of accidental exposure or poisoning Rohm and Haas Company memo, Unpublished.

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1 **Substance**

The information given below refers to DCOIT/RH-287.

3.2 **Persons exposed**

Potentially industrial of professional workers handling DCOIT concentrated formulations, in case of accident.

3.3 **Exposure**

Ingestion, Dermal, Inhalation: In case of accident.

4 RESULTS

4.1 **Clinical Signs**

See section A6.12.5.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 **Results and discussion**

There is no antidote for the effects of DCOIT. In all cases the patient should be treated symptomatically.

In case of accidental exposure the following actions are recommended:

Inhalation: Move the patient to fresh air. Give artificial respiration if breathing has stopped. If symptoms persist, seek medical attention.

Skin contact: Immediately wash the contaminated skin site with water, under a shower if available. Remove contaminated clothing and seek medical attention.

Eye contact: Rinse the eye(s) immediately with running water for at least 15 minutes. Seek medical attention.

Ingestion: Drink 1 or 2 glasses of water. Immediately consult a physician.

Note to physician: DCOIT is corrosive. It may not be advisable to induce vomiting. Possible mucosal damage may contraindicate the use of gastric lavage. It may be necessary to employ measures against circulatory shock and convulsions.

5.2 **Conclusion**

The treatment in case of accident or poisoning is the general treatment recommended for corrosive substances.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	22 March 2007
Materials and Methods	The applicant's version is acceptable
Results and discussion	Agree with aplicant's version
Conclusion	Agree with applicant's version
Remarks	

Section A6.12.8 Prognosis following poisoning
Annex Point IIA6.9.8 **Reference A6.12.8**

Official use only

		1 REFERENCE
1.1	**Reference**	**A6.12.5/01** Wooder M. (2006) 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one – DCOIT (CAS 64359-81-5), Diagnosis of poisoning including specific signs of poisoning and clinical tests, Treatment in case of accidental exposure or poisoning Rohm and Haas Company memo, Unpublished.
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)
		3 MATERIALS AND METHODS
3.1	**Substance**	The information given below refers to DCOIT/RH-287.
3.2	**Persons exposed**	Potentially industrial of professional workers handling DCOIT concentrated formulations, in case of accident.
3.3	**Exposure**	Ingestion, Dermal, Inhalation: In case of accident.
		4 RESULTS
4.1	**Clinical Signs**	See section A.12.5
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Results and discussion**	
5.2	**Conclusion**	The prognosis following accidental exposure or poisoning will depend upon the extent of the exposure and the speed of obtaining appropriate medical treatment.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	22 March 2007
Materials and Methods	The applicant's version is acceptable
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Remarks	

Section A6.13 Annex Point IIIA6.2	Toxic effects on livestock and pets	
	Justification for non-submission of data	Official use only
Other existing data [ ]	Technically not feasible [ ]	Scientifically unjustified [ ]
Limited exposure [X]	Other justification []	
Detailed justification:	[redacted]	

Document III-A / Sections A6.8 to A6.17

Section A6.13 **Annex Point IIIA6.2**	**Toxic effects on livestock and pets**
	[redacted]
Undertaking of intended data submission [ ]	No
	Evaluation by Competent Authorities
	Evaluation by Rapporteur Member State
Date	22 October 2007
Evaluation of applicant's justification	Acceptable
Conclusion	Acceptable
Remarks	

Section A6.14 Annex Point IIIA11.2	Other tests related to the exposure of humans	
	Justification for non-submission of data	Official use only
Other existing data [ ] Limited exposure [X]	Technically not feasible [ ] Scientifically unjustified [X] Other justification [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	22 October 2007	
Evaluation of applicant's justification	Acceptable	
Conclusion	Acceptable	
Remarks		

Section A6.15 Annex Point IIIA6.4	Food and feedingstuffs	
	Justification for non-submission of data	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	The use of DCOIT based wood preservatives on wood which is likely to come into prolonged direct contact with foodstuffs or feedstuffs are not expected. It is therefore justified not to submit data on residue in food and feedstuffs.	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	22 October 2007	
Evaluation of applicant's justification	Acceptable	
Conclusion	Acceptable	
Remarks		

Section A6.16 **Annex Point IIIA6.3.5-IIIA11.2**	**Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary**	
	Justification for non-submission of data	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	No further studies are considered necessary.	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	22 October 2007	
Evaluation of applicant's justification	Acceptable	
Conclusion	Acceptable	
Remarks		

Section A6.17 **Annex Point IIIA6.6**	**Toxicity test on metabolites from treated plants**	
	Justification for non-submission of data	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	DCOIT is not used in products for action against plants. Therefore toxic effects of metabolites from treated plants do not need to be assessed and could be waived.	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	22 October 2007	
Evaluation of applicant's justification	Acceptable	
Conclusion	Acceptable	
Remarks		

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

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

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

Product type 8: Wood preservatives

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

Part I

Fate and behaviour in the environment

Section A7.1.1: Fate and behaviour in water

Degradation, initial studies

TABLE OF CONTENTS

Section A7.1.1.1.1/01 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

Official use only

1 REFERENCE

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

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes. U.S. Environmental Protection Agency, 40 CFR § 158, Subdivision N, Chemistry, Environmental Fate 161-1 and OECD 111.
2.2	**GLP**	Yes
2.3	**Deviations**	To satisfy the US EPA guidelines, the OECD screening test was not performed and instead the definitive test was directly initiated. The degradate standards, N-(n-octyl) malonamic acid, N-(n-octyl) oxamic acid, and N-(n-octyl) acetamide were not GLP characterized at the time. However, standards were used only for qualitative and not quantitative purposes and none of these compounds were subsequently identified as hydrolytic degradation products. Sterile agar plates used for examining solution sterility were not prepared under GLP procedures. The FT-ICR-MS instrument was not GLP validated. However, it was used to confirm the LC-MS/MS data and this instrument was GLP validated.

3 MATERIALS AND METHODS

3.1	**Test material**	^{14}C-DCOIT [redacted]
3.1.1	Lot/Batch number	[redacted]

Document III-A / Section A7.1.1

Section A7.1.1.1.1/01 **Hydrolysis as a function of pH and identification of breakdown products**

Annex Point IIA7.6.2.1

3.1.2	Specification	[redacted]
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	[redacted]
3.2	**Reference substance**	[redacted]
3.2.1	Initial concentration of reference substance	[redacted]
3.3	**Test solution**	[redacted]

Document III-A / Section A7.1.1

Section A7.1.1.1.1/01 Annex Point IIA7.6.2.1	Hydrolysis as a function of pH and identification of breakdown products

3.4 Testing procedure

3.4.1 Test system

[redacted]

3.4.2 Temperature x

3.4.3 pH

Document III-A / Section A7.1.1

Section A7.1.1.1.1/01 **Hydrolysis as a function of pH and identification of breakdown products**

Annex Point IIA7.6.2.1

[redacted]

3.4.4 Duration of the test [redacted]

3.4.5 Number of replicates [redacted]

3.4.6 Sampling [redacted]

3.4.7 Analytical methods [redacted]

Document III-A / Section A7.1.1

Section A7.1.1.1.1/01 **Hydrolysis as a function of pH and identification of breakdown products**

Annex Point IIA7.6.2.1

[redacted]

3.5 Preliminary test

[redacted]

4 RESULTS

4.1 Concentration and hydrolysis values

[redacted] Recoveries ranged from 96.6% to 108.9% with an average of 100.9 ± 2.3%.

Table A7.1.1.1.1/01-4 contains the replicate average data for the quantitation of parent compound at three pH's and two temperatures. These results show that parent compound is very stable in acidic solutions. The higher the pH and or temperature the more rapid the degradation.

Tables A7.1.1.1.1/01-5, A7.1.1.1.1/01-6, and A7.1.1.1.1/01-7 contain the replicate average percentage of ^{14}C hydrolytic products detected at pH 7/40°C, pH 9/25°C, and pH 9/40°C, respectively. The structure of the degradates is presented in Table A7.1.1.1.1/01-9. In the report the major metabolite is identified as isomers of 2-(n-octyl)carbamoyl-2-chloro-1-oxoethane sulfinic acid. Subsequent analysis using NMR (see Report N° TR-04-017 summarized in next section A7.1.1.1.1/02 below) has identified the compound instead as isomers of 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid. x

4.2 Hydrolysis rate constant (k_h)

The rate constants for degradation of parent compound at pH 4, 7, and 9 as well as 25°C and 40°C is presented in Table A7.1.1.1.1/01-8. The rate constant was calculated from the equation $k = \ln 2/t_{½}$. Correlating with the quantitation data in Table A7.1.1.1.1/01-4, the higher the pH and temperature, the larger the degradation rate constant and the more rapid the degradation.

The correlation coefficient (r^2) for parent degradation kinetics is also presented in Table A7.1.1.1.1/01-8. Except for pH 4 and 25°C the correlation coefficient is good exceeding 0.98 at the faster reaction rates. The low r^2 (and thus low linearity) value observed for pH4/25°C is most likely due to the very limited degradation that occurred.

4.3 Dissipation time

Table A7.1.1.1.1/01-8 also contains the calculated DT_{50} and DT_{90} for degradation of parent at the three pH's and two temperatures examined in this study. The higher the pH and temperature, the shorter the DT_{50} and DT_{90} for DCOIT and thus the more rapid its hydrolytic degradation.

4.4 Concentration – time data

Figure A7.1.1.1.1/01-1 presents a graphical presentation of the hydrolysis of DCOIT for pH 9/40°C, pH 9/25°C, and pH 7/40°C.

Document III-A / Section A7.1.1

Section A7.1.1.1.1/01 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

4.5 Specification of the transformation products

Table A7.1.1.1.1/01-9 provides the structure and nomenclature of the major (>10%) and a number of the minor (<10%) hydrolytic products. Figure A7.1.1.1.1/01-2 presents a proposed hydrolytic pathway.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

[redacted]

5.2 Results and discussion

Sample	K (hrs^{-1})	DT_{50} (days)	r_2
pH 4/25°C	0.0001	>60	0.575
pH 4/40°C	0.0003	>60	0.926
pH 7/25°C	0.0004	>60	0.864
pH 7/40°C	0.002	18.7	0.987
pH 9/25°C	0.008	3.5	0.989
pH 9/40°C	0.048	0.6	0.997

x

^{14}C-material balance (Table A7.1.1.1.1/01-3) and recovery from HPLC quantitation (Table A7.1.1.1.1/01-2) was very good through the experiment.

5.2.1 k_H See Table above.

5.2.2 DT_{50} See Table above.

5.2.3 r^2 See Table above.

5.3 Conclusion

This study fulfils the requirement for determining the effect of aqueous hydrolysis on the fate of DCOIT in the environment. As discussed further in Document IIIA sections A7.1.2, A7.2.1 and Document IIA the rate of biodegradation is much more rapid than abiotic degradation. Therefore, biodegradation will determine the kinetic fate of DCOIT. Hydrolysis will have minimal, if any influence on the fate of DCOIT and on its risk assessment. The major degradation products are presented in Table A7.1.1.1.1/01-9. The major degradative pathway involves cleavage of the isothiazolone ring (Figure A7.1.1.1.1/01-2). Various classes of these ring cleaved products have been tested and

x

Document III-A / Section A7.1.1

Section A7.1.1.1.1/01 Annex Point IIA7.6.2.1	Hydrolysis as a function of pH and identification of breakdown products

		found to be readily biodegradable (section A7.1.2.3).
5.3.1	Reliability	1, valid without restrictions.
5.3.2	Deficiencies	No significant deficiencies that will affect the results and conclusions

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	5 October 2006, revised 7 January 2009
Materials and Methods	Comment **(3.1.4):** The water solubility of DCOIT at pH 7 is 3.47 mg/l (20°C).
	Comment **(3.4.2):** The temperature of the test system varied more than 2 °C during the test. OECD 111 requires that the temperature is kept constant within a range of ± 0.1 °C. However, the observed temperature variations are not expected to seriously affect the outcome of the study.
Results and discussion	Comment **(4.1):** In tables A7.1.1.1.1/01-5, -6, -7 and -9 the correct metabolite 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid, identified by NMR in study A7.1.1.1.1/02, is stated, instead of 2-(n-octyl)carbamoyl-2-chloro-1-oxoethane sulfinic acid, which is mentioned in the study report to this study summary.
	Comment **(5.2):** The DT50 at pH7 (25°C), which represents best environmental conditions, is 71.4 days
Conclusion	Agree with applicant's version
	Comment **(5.3):** The metabolite 2-chloro-2-(n-octylcarbamoyl)-1-1ethene sulfonic acid is found not to be readily biodegradable.
Reliability	1, valid without restrictions
Acceptability	Acceptable
Remarks	-

Document III-A / Section A7.1.1

Section A7.1.1.1.1/01	Hydrolysis as a function of pH and identification of breakdown products **TABLES AND FIGURES**

[redacted]

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

[redacted]

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

Document III-A / Section A7.1.1

[redacted]

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

[redacted]

Document III-A / Section A7.1.1

Table A7.1.1.1.1/01-4: Percent of Parent Compound Quantitated by HPLC

Day	Parent Compound as a Percent of ^{14}C Applied (average of replicates)					
	pH 4/25°C	pH 4/40°C	pH 7/25°C	pH 7/40°C	pH 9/25°C	pH 9/40°C
0	100	100	100	100	100	100
0.08						93.3
0.17						87.2
0.25						78.6
0.33						70.6
1					83.5	26.0
1.3						19.2
2					69.1	8.3
3	98.2	96.9	93.8	88.9	53.4	0
4					44.0	
7	96.1	93.0	86.2	70.8	22.9	
11					13.5	
15	89.8	84.3	80.2	54.1		
21	92.0	86.0	84.0	46.1		
30	93.5	80.0	71.8	35.1		

Table A7.1.1.1.1/01-5: Percent of Hydrolysis Products of DCOIT at pH 7 and 40°C

Hydrolysis Product[1]	Percent ^{14}C-activity				
	Day 3	Day 7	Day 15	Day 21	Day 30
1					2.33
2			1.39	12.64	11.05
3		2.57	2.38	6.29	15.57
4					3.39
5				0.45	0.9
6				3.70	1.92
7			3.67	11.76	6.92

[1] Product 1 is unknown

Product 2 is 2-chloro-2-(n-octylcarbamoyl)-l-ethene sulfonic acid

Product 3 is 1-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid

Product 4 is 4-chloro-5-hydroxy-2-(n-octyl)-4-isothiazolin-3-one

Product 5 is N-(n-octyl) propiolic acid amide

Product 6 is 4-chloro-2-(n-octyl)-4-isothiazolin-3-one

Product 7 is 4-chloro-5-methoxy-2-(n-octyl)-4-isothiazolin-3-one.

Table A7.1.1.1.1/01-6: Percent of Hydrolysis Products of DCOIT at pH 9 and 25°C

Hydrolysate Product[1]	Percent ^{14}C-activity					
	Day 0	Day 0.21	Day 0.29	Day 4	Day 7	Day 8
1				19.46	21.74	19.60
2		1.48	1.66	40.74	36.41	39.62
3				12.12	16.25	3.41
4						13.35

1 Product 1 is 2-chloro-2-(n-octycarbamoyl)-1-ethene sulfonic acid
Product 2 is 1-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid
Product 3 is unknown
Product 4 is N-(n-octyl) propiolic acid amide.

Table A7.1.1.1.1/01-7: Percent of Hydrolysis Products of RH-5287 at pH 9 and 40°C

Sample Day	Percent ^{14}C-Activity	
	Product 1	Product 2
0		
0.08	5.00	
0.17	9.61	
0.25	16.03	
0.33	19.81	2.25
1	46.6	8.88
1.3	51.69	8.90
2	56.64	12.12
3	59.06	15.92

1 Product 1 is a 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid and 1-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid
Product 2 is N-(n-octyl) propiolic acid amide.

Table A7.1.1.1.1/01-8: Kinetics for Parent Compound

Day	Parent Compound as a Percent of ^{14}C Applied					
	pH 4/25°C	pH 4/40°C	pH 7/25°C	pH 7/40°C	pH 9/25°C	pH 9/40°C
k[a] (hrs^{-1})	0.0001	0.0003	0.0004	0.002	0.008	0.048
R^2	0.575	0.926	0.864	0.987	0.989	0.997
DT_{50}[b] (days)	259.8	93	71.4	18.7	3.5	0.6
DT_{90}[c] (days)	>700	>300	>250	62.2	11.6	2.0

[a] Rate constant calculated from k = ln2/half-life.
[b] Half-life (DT_{50}) calculated from regression analysis (bivariate fit) performed by JMP Statistical Software Package (SAS Institute).
[c] Time for 90% reduction calculated from DT_{90} = ln10/k.

Table A7.1.1.1.1-9: Structure of metabolites

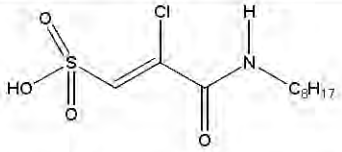
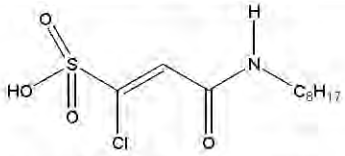
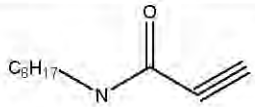
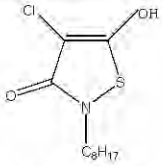
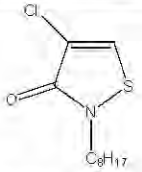
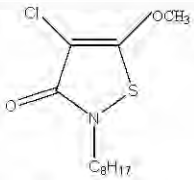
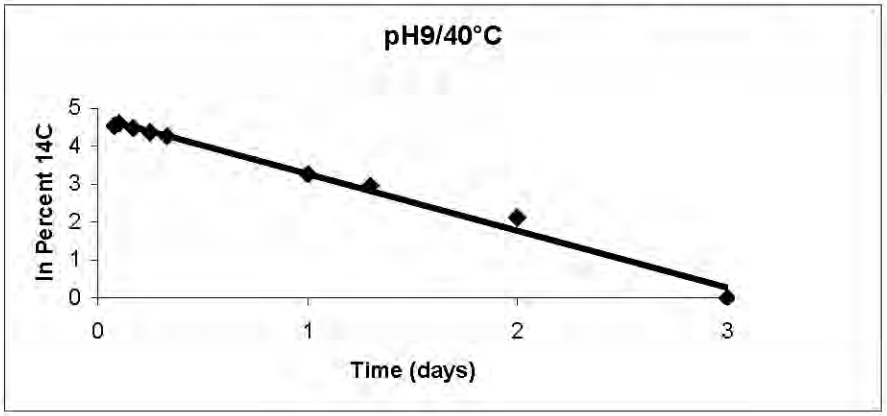
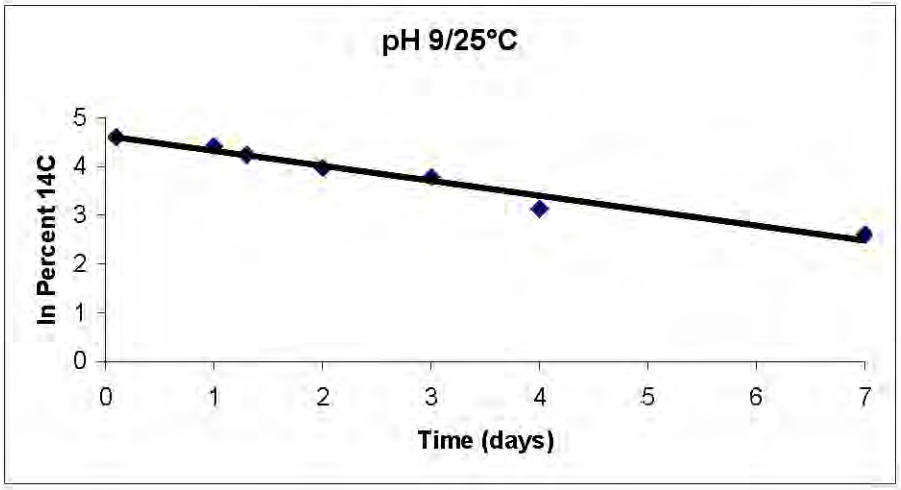
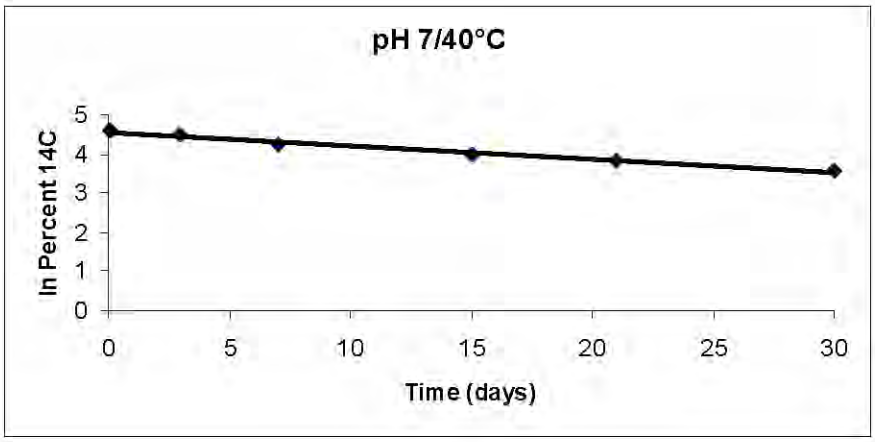
Hydrolysis Prod #		Compound/Structure	pH	Max %
PH 7	pH 9			
2	1	 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid Mol. Wt = 297	pH 7 pH 9	>10%
3	2	 1-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid Mol. Wt = 297	pH 7 pH 9	>10%
5	4	 N-(n-octyl) propiolic acid amide, Mol. Wt. = 181	pH 7 pH 9	>10% at pH 9
4		 4-chloro-5-hydroxy-2-(n-octyl)-3(2H) isothiazolone, Mol. Wt. = 263	pH 7	<10%
6		 4-chloro-2-(n-octyl)-3(2H) isothiazolone, Mol Wt = 247	pH 7	<10%
7		 4-chloro-5-methoxy-2-(n-octyl)-3(2H) isothiazolone, Mol. Wt = 277	pH 7	>10%

Figure A7.1.1.1.1/01-1: Natural log (ln) of the Percent ^{14}C DCOIT Versus Time







Document III-A / Section A7.1.1

[redacted]

Document III-A / Section A7.1.1

Section A7.1.1.1.1/02	**Hydrolysis as a function of pH and identification of breakdown products - Supplemental study**
Annex Point IIA7.6.2.1	**Structural confirmation of the major hydrolysis product**

Official use only

1 REFERENCE

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

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No applicable guideline followed.
2.2	**GLP**	No
2.3	**Deviations**	This study is a supplemental study to the hydrolysis study summarized in section A7.1.1.1.1/01, providing structural confirmation of the major metabolite. The main study was guideline compliant.

3 MATERIALS AND METHODS

3.1	**Test material**	^{14}C DCOIT [redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	[redacted]
[redacted]	[redacted]	[redacted]
3.1.4	Further relevant properties	-

Document III-A / Section A7.1.1

Section A7.1.1.1.1/02	**Hydrolysis as a function of pH and identification of breakdown products - Supplemental study**
Annex Point IIA7.6.2.1	**Structural confirmation of the major hydrolysis product**

3.2 Reference substance

[redacted]

3.2.1 Initial concentration of reference substance

[redacted]

3.3 Test solution

[redacted]

3.4 Testing procedure

[redacted]

3.5 Preliminary test

[redacted]

4 RESULTS

[redacted]

Document III-A / Section A7.1.1

Section A7.1.1.1.1/02	**Hydrolysis as a function of pH and identification of breakdown products - Supplemental study**
Annex Point IIA7.6.2.1	**Structural confirmation of the major hydrolysis product**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Results discussions, and conclusions**	A major metabolite(s) observed in the aqueous hydrolysis and other experiments has been definitively identified by NMR as a sulfonic acid and not a sulfinic acid. The major isomer is C_8H_{17}-NH-C(O)-C(Cl)=CH-SO_3H and the secondary isomer, C_8H_{17}-NH-C(O)-CH=C(Cl)-SO_3H.
5.1.1	Reliability	2, valid with restriction
5.1.2	Deficiencies	Supplemental study to the hydrolysis key study. Non GLP.

Document III-A / Section A7.1.1

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	5 October 2006
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	**Comment (5.2)**: No version supplied by the applicant. It is suggested to adopt the results and discussion as the conclusion. A major metabolite observed in the aqueous hydrolysis of DCOIT has been definitively identified by NMR as a sulfonic acid and not a sulfinic acid. The major isomer is C_8H_{17}-NH-C(O)-C(Cl)=CH-SO_3H and the secondary isomer, C_8H_{17}-NH-C(O)-CH=C(Cl)-SO_3H.
Reliability	2, valid with restrictions
Acceptability	Study was not performed under GLP. Acceptable as supplementary data which provides further structural information on the metabolites of DCOIT hydrolysis.
Remarks	-

Document III-A / Section A7.1.1

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Official use only

1 REFERENCE

1.1 Reference

[redacted]

1.2 Data protection Yes

1.2.1 Data owner Rohm and Haas Company

1.2.2

1.2.3 Criteria for data protection

[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes. U.S. Environmental Protection Agency, 40 CFR § 158, Environmental Fate Assessment Guidelines, Subdivision N, Chemistry, Environmental Fate 161-2

2.2 GLP Yes

2.3 Deviations

- The ^{14}C test materials used in this study was synthesized prior to the enactment of U.S. EPA GLP guidelines. However, as part of the study a GLP radiopurity determination was performed.
- No actinometer study employing p-nitroacetophenone-pyridine, which measures the sunlight's intensity incident on the sample, was performed.

3 MATERIALS AND METHODS

Document III-A / Section A7.1.1

Section A7.1.1.1.2 **Annex Point IIA7.6.2.2**	**Phototransformation in water including identity of transformation products**

3.1	**Test material**	For both studies referenced above, ^{14}C DCOIT (RH-5287) was employed	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	[redacted]	
3.1.3	Purity	[redacted]	
3.1.4	Radiolabelling	[redacted]	
3.1.5	UV/VIS absorption spectra and absorbance value	[redacted]	
3.1.6	Further relevant properties	[redacted]	x
3.2	**Reference substances**	[redacted]	

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

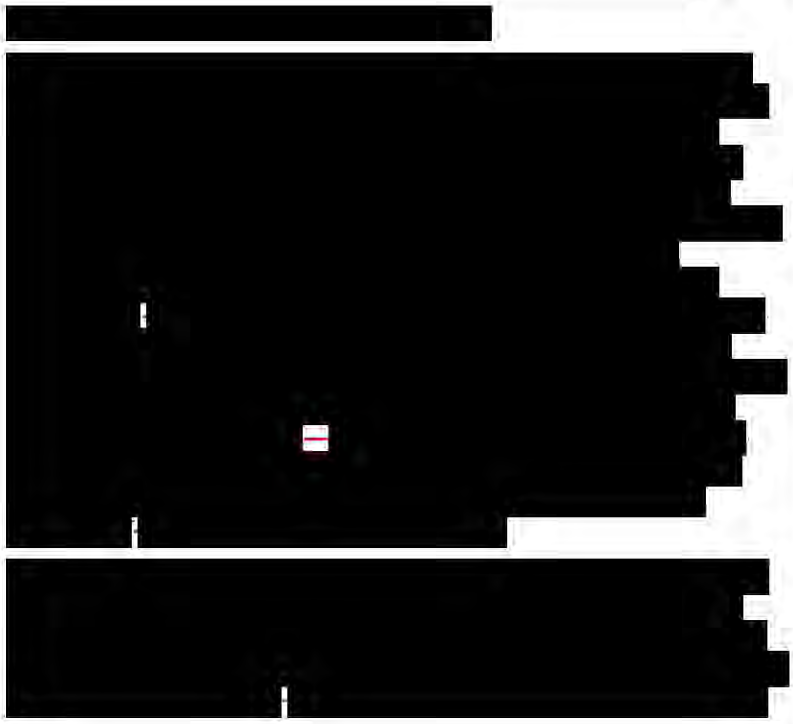
Annex Point IIA7.6.2.2

3.3 Test solution

3.4 Testing procedure

3.4.1 Test system

3.4.2 Properties of light source



3.4.3 Determination of irradiance

3.4.4 Temperature

Document III-A / Section A7.1.1

[redacted]

3.4.5 pH

[redacted]

3.4.6 Duration of the test

[redacted]

3.4.7 Number of replicates

[redacted]

3.4.8 Sampling

[redacted]

3.4.9 Analytical methods

[redacted]

[redacted]

3.5 Transformation products

[redacted]

3.5.1 Method of analysis for transformation products

[redacted]

4 RESULTS

4.1 Screening test

The screening test was not performed. However, preliminary tests were performed to establish the sampling interval and validate the methods for the definitive test.

4.2 Actinometer data

No actinometer study was performed. At the time this study was completed, the existing guidelines did not contain a reference to an actinometer study.

4.3 Controls

Not applicable.

4.4 Photolysis data

4.4.1 Concentration values

Figure A7.1.1.1.2-1 presents a graphical display of the natural log of the parent concentration at Days 0, 15, 20, and 30 for the irradiated and dark control samples. The graph also demonstrates the photolytic dissipation of parent compound. Values from Days 5 and 9 are not included because there was a loss of activity apparently due to adsorption of ^{14}C activity to the glass walls of the containers. This was subsequently corrected by adding an equal volume of acetonitrile to the container prior to storage.

4.4.2 Mass balance

The recovery of applied ^{14}C-activity is tabulated below.

Day	Percent ^{14}C Recovery			
	Reference 1[1]		Reference 2[2]	
	Irradiated	Dark	1.0 ppm	10.0 ppm
0	98.6	101.5		
5	99.6	97.9		
9	96.9[3]	93.8		
15	95.9	100.8	85.3	86.0
20	95.1	74.4		
30	83.5	38.5	79.9	76.5[4]

[1] Average of two replicates
[2] 1 ppm is the average of two replicates and 10 ppm is the average of 4 replicates.

[3] Sample lost during analysis.
[4] Sample lost prior to analysis.

The average recoveries were: Reference 1 irradiated samples, 94.7 ± 6.1; Reference 1 dark controls, 84.5 ± 23.9 (without Day 30, 93.7 ± 11.0); and Reference 2, 82.2 ± 4.8.

4.4.3	k^e_p	The half-life was 13.4 days and 79.7 days in the sunlight and dark control samples, respectively. The rate constants were 0.052 day^{-1} and 0.0087 day^{-1} in the sunlight and dark control samples, respectively.
4.4.4	Kinetic order	Pseudo first order.
4.4.5	k^e_p / k^a_p	Actinometer studies not performed and thus the constant were not calculated.
4.4.6	Reaction quantum yield (ϕ^e_E)	Not determined.
4.4.7	k_{pE}	Since exposure was to natural sunlight and not a xenon lamp, the direct photolysis sunlight rate constant is the same as k_p; 0.052 day^{-1} and 0.0087 day^{-1} in the sunlight and dark control samples, respectively.
4.4.8	Half-life ($t_{1/2E}$)	Not calculated since natural sunlight was used as the light source.

4.5 Specification of the transformation products

Reference 1: The only metabolite definitively identified was $^{14}CO_2$ which in the irradiated samples was present at 2.2% on Day 5 and 14.9% on Day 30. Practically all the ^{14}C-photodegradates were very polar in nature eluting from the HPLC column near the void volume. On Days 15, 20, and 30 this polar fraction accounted for 54.5%, 56.8%, and 67.6% of the ^{14}C activity, respectively. The presence of significant quantities of $^{14}CO_2$ indicates that photodegradation involves the cleavage of the isothiazolone ring and subsequent oxidation of N-(n-octyl) malonamic acid. The chromatographic nature of the metabolites was similar to that of N-(n-octyl) malonamic acid and N-(n-octyl) oxamic acid.

Reference 2: Table A7.1.1.2-1 provides a comprehensive description of the ^{14}C photodegradates. The two major metabolites are N-(n-octyl) oxamic acid (31.4%) and CO_2 (18.5%). The photodegradation pathway for DCOIT is presented in Figure A7.1.1.1.2-2. Degradation involves cleavage of the isothiazolone ring and subsequent oxidation of the alkyl degradates as demonstrated by N-(n-octyl) oxamic acid being the major degradate. A minor pathway involves cleavage of the N-S ring bond and photoisomerization to form 4,5-dichloro-3-(n-octyl) thiazole. This compound subsequent degrades to N-(n-octyl) carbamic acid.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Sterile pH 7 phosphate buffer was prepared and dosed at nominal 1 ppm ^{14}C DCOIT. Ten mls were aseptically transferred to quartz photolysis tubes and placed on a roof top in Lexington, KY, USA in a water bath maintained at 24.7 ± 0.4°C for 30 days. Air was passed through the tubes and evolved $^{14}CO_2$ trapped using NaOH. Replicate tubes were removed at Days 0, 5, 9, 15, 20, and 30 and analyzed by radioassay and HPLC. Test guidelines were U.S. Environmental Protection Agency, 40 CFR § 158, Environmental Fate Assessment Guidelines, Subdivision N, Chemistry, Environmental Fate 161-2. No actinometer study was

		performed since this was not a requirement of the guidelines at the time the study was performed. A second study was conducted to enhance degradate identification using sterile pH 7 phosphate buffer and dosed at nominal 1 ppm and 10 ppm ^{14}C DCOIT. The water bath was maintained at 26.0 ± 0.2°C. Replicate tubes were removed on Days 15 and 30 and analyzed by radioassay and HPLC. Degradate identification relied on mass spectroscopy. The study complied with U.S. Environmental Protection Agency, 40 CFR § 158, Environmental Fate Assessment Guidelines, Subdivision N, Chemistry, Environmental Fate 161-2 guidelines.	
5.2	**Results and discussion**	DCOIT photodegrades in water and natural sunlight at a moderate rate. During the course of the study it was observed that there was some adsorption to the glass vials but this was rectified by adding additional acetonitrile to the aqueous solutions after sampling and removal from the rooftop. The solubility of DCOIT is quite low (ca. 5 ppm). The parent compound was not volatile.	x
5.2.1	k^c_p	The rate constants were 0.052 day^{-1} and 0.0087 day^{-1} in the sunlight and dark control samples, respectively.	
5.2.2	K_{pE}	Not determined.	
5.2.3	ϕ^c_E	Not determined.	
5.2.4	$t_{1/2E}$	Not determined.	
5.3	**Conclusion**	This study fulfils the requirement for determining the effect of aqueous photolysis on the fate of DCOIT in the environment. As discussed in Document IIIA sections A7.1.2, A7.2.1, and in Document IIA the rate of biodegradation is much more rapid than abiotic degradation. Therefore, in a natural environment, biodegradation will determine the kinetic fate of DCOIT. Photolysis will have minimal, if any influence on the fate of DCOIT and on its risk assessment. The major degradation products are N-(n-octyl) oxamic acid, CO_2, and N-(n-octyl) carbamic acid. Structures and other identified products are presented in Table A7.1.1.1.2-1. The major degradative pathway involves cleavage of the isothiazolone ring (Figure A7.1.1.1.2-2). Various classes of these ring cleaved products have been tested and found to be readily biodegradable (see section A7.1.2.3). Similar kinetic and degradate results were obtained by a group at the University of Ioannina, Greece: Sakkas V.A., Kopnstantinov I.K., Albanis T.A., 2002, Aquatic phototransformation study of the antifouling agent Sea-Nine 211: identification of byproducts and the reaction pathway by gas chromatography-mass spectroscopy, Journal of Chromatography A 959:215-227. This published reference is included in the literature search part but full text is not provided as part of this dossier.	
5.3.1	Reliability	2- valid with restrictions	
5.3.2	Deficiencies	This study was conducted in natural sunlight. Deficiencies concern the extrapolation of the photolysis kinetic results to 40-65 degrees North latitude.	
		[redacted]	

Document III-A / Section A7.1.1

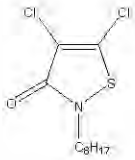
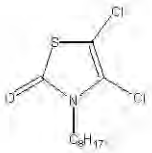
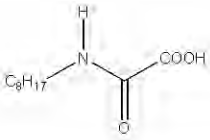
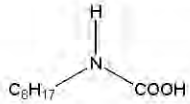
Document III-A / Section A7.1.1

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	5 October 2006
Materials and Methods	**Comment (3.1.6):** The water solubility of DCOIT at pH 7 is 3.47 mg/l (20°C). The halv-life of DCOIT in aquatic systems in the presence of microbes is generally short, but not necessarily less than 24 hours (see section A7.1.2)
Results and discussion	**Comment (5.2):** Agree with applicant's version. DCOIT photodegrades in water and natural sunlight at a moderate rate (Half-life= 13.4 days). During the course of the study it was observed that there was some adsorption to the glass vials but this was rectified by adding additional acetonitrile to the aqueous solutions after sampling and removal from the rooftop.
Conclusion	Agree with applicant's version
Reliability	2, valid with restrictions
Acceptability	Acceptable with the restrictions described above. The study is considered acceptable in spite of several deficiencies. Natural light was used and an actinometer study was absent. From the provided data it is apparent that photolysis is not the major degradation mechanism for DCOIT released into the aquatic environment and any further testing cannot be warranted.
Remarks	-

Document III-A / Section A7.1.1

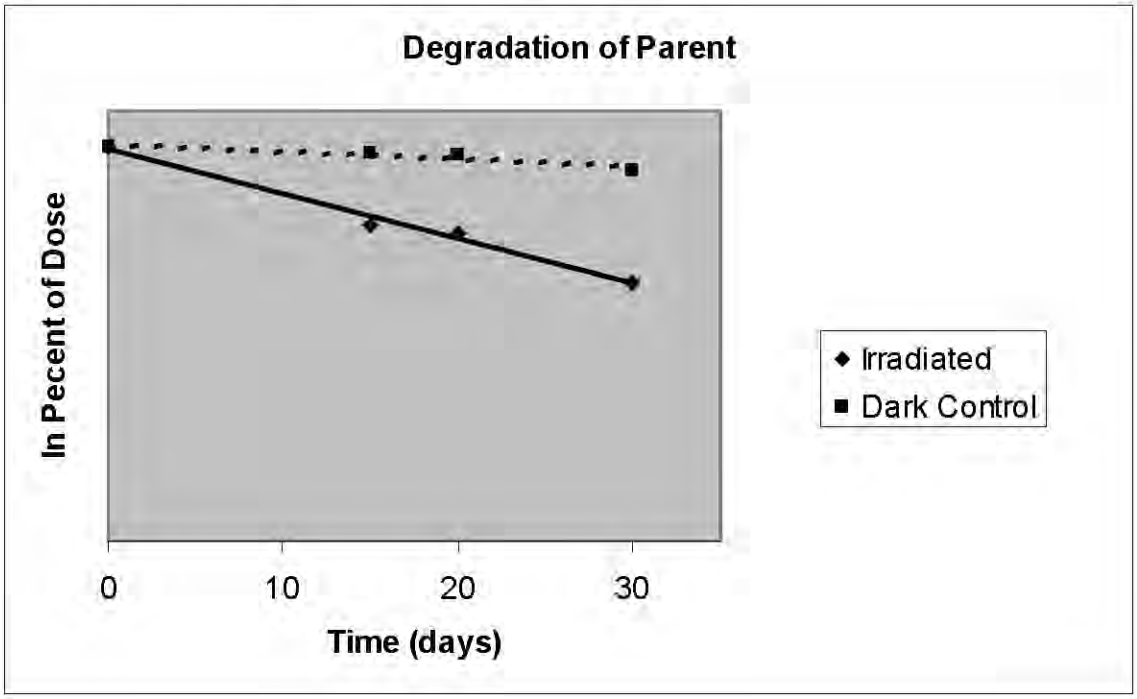
Section A7.1.1.1.2	Phototransformation in water including identity of transformation products – **TABLES AND FIGURES**

Table A7.1.1.1.2-1: Structure and Quantitation of Photodegradates

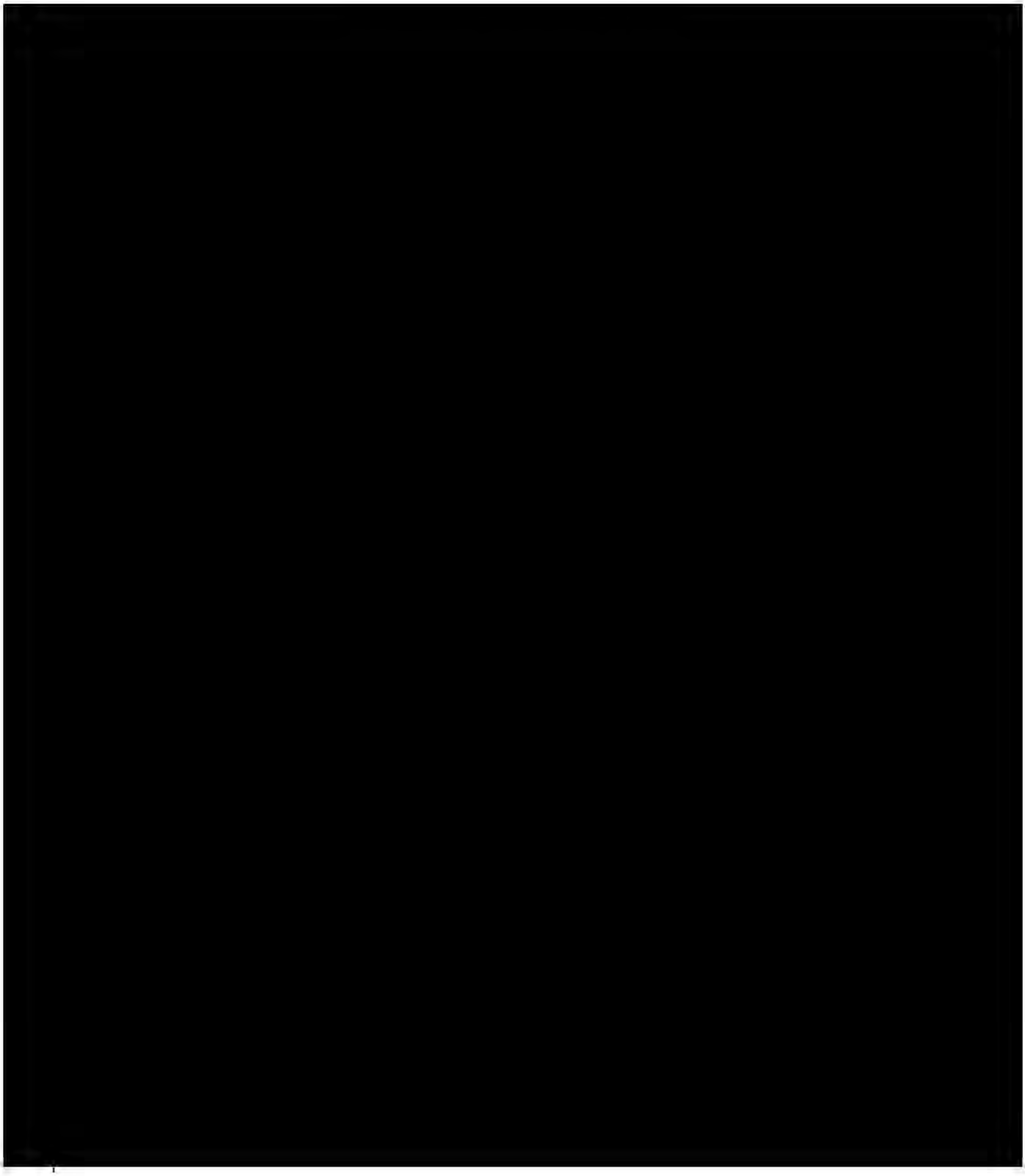
Compound	Structure	Percent of Total Activity
Carbon dioxide	CO_2	18.5
Parent		5.3
4,5-dichloro-3-(n-octyl) thiazole		1.9
N-(n-octyl) oxamic acid		31.4
Unidentified[1]		3.0
Unidentified[1]		4.3
Unidentified[1]		1.8
Unidentified[1]		3.1
N-(octyl) carbamic acid		5.0
4 unidentified degradates		5.1
Sum of all degradates in the aquous fraction but mainly N-(n-octyl) carbamic acid		10.9

[1] Insufficient quantity isolated to obtain a definitive mass spectral identification

Figure A7.1.1.1.2-1: Dissipation of Parent Compound



Document III-A / Section A7.1.1



Document III-A / Section A7.1.1

Section A7.1.1.2.1 Ready Biodegradability
Annex Point IIA7.6.1.1

Official use only

1 REFERENCE

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

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes. OECD 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**	DCOIT (RH-25,287)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2.
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	[redacted]
3.1.5	Composition of Product	[redacted]
3.1.6	TS inhibitory to microorganisms	[redacted]

Document III-A / Section A7.1.1

Section A7.1.1.2.1 **Ready Biodegradability**
Annex Point IIA7.6.1.1

		[redacted]	
3.1.7	Specific chemical analysis	[redacted]	
3.2	**Reference substance**	[redacted]	
3.2.1	Initial concentration of reference substance	[redacted]	
3.3	**Test ing procedure**		
3.3.1	Inoculum / test species	[redacted]	
3.3.2	Test system	[redacted]	
3.3.3	Test conditions	[redacted]	
3.3.4	Method of preparation of test solution	[redacted]	
3.3.5	Initial TS concentration	[redacted]	x
3.3.6	Duration of test	[redacted]	
3.3.7	Analytical parameter	[redacted]	
3.3.8	Sampling	[redacted]	

[redacted]

3.3.9 Intermediates/ degradation products

3.3.10 Nitrate/nitrite measurement

3.3.11 Controls

3.3.12 Calculations/Statistics

[redacted]

4 RESULTS

4.1 Degradation of test substance

4.1.1 Graph

The percent biodegradation for flasks containing DCOIT (2 replicate flask), sodium benzoate (2 replicate flask), DCOIT + sodium benzoate, and $HgCl_2$ only is presented in Figure A7.1.1.2-1.

The percent biodegradation of the test item was calculated based on a total carbon content (TOC) of 0.47 mg C/mg DCOIT. The CO_2 produced in flask containing only DCOIT was slightly less than that of the inoculum controls (no additions). The abiotic control (DCOIT + $HgCl_2$) was about 10% of the TOC on Day 28.

The percent biodegradation of the reference item was based on total carbon content of 0.58 mg C/mg sodium benzoate. The reference item

was degraded by an average of extent of 76% by day 14 thus confirming the suitability of the activated sludge. By Day 28 the sodium benzoate was completely degraded.

The extent of biodegradation of sodium benzoate in the presence of DCOIT was slightly delayed over the course of the experiment compared to sodium benzoate alone.

4.1.2 Degradation

$$\% \text{ degradation} = \frac{\text{mg } IC_{prod} \text{ in the test flask - mg } IC_{prod} \text{ in blank}}{\text{mg TOC}} \times 100$$

	% degradation at the end of incubation (mean)
Test item[1]	-15.7
Procedure control (Sodium Benzoate)[1]	94.8
Toxicity control[1]	32.4
Abiotic control[2]	9.9

[1] Corrected for the inoculum controls
[2] Corrected for the abiotic blank

4.1.3 Other observations

The CO_2 production of DCOIT in the test media was slightly lower than of the inoculum controls.

The extent of biodegradation of sodium benzoate in the presence of DCOIT was slightly delayed over the course of the experiment compared to sodium benzoate alone. Rapid degradation of 25 % occured with the first five days of incubation. Afterwards, the extent of biodegradation only increased slightly. Within 14 days of exposure, biodegradation amounted to 29 % and corresponded to 32 % by the end of the test. x

4.1.4 Degradation of TS in abiotic control

Degradation of DCOIT in abiotic control corresponds to approximatively 10 %.

4.1.5 Degradation of reference substance

See Figure A7.1.1.2-1.

4.1.6 Intermediates/ degradation products

Not applicable.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

DCOIT was investigated for its ready biodegradability in a 28-day CO_2 Evolution (Modified Sturm) test according to EU Commission Directive 92/69/EEC C.4-C (1992) and OECD Guideline for testing of Chemicals N° 301 B (1992).

To each of nine 5 L flasks, 2400 to 3000 ml of test water containing mineral salts (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 added. The flasks were aerated overnight with CO_2-free air to purge the system of CO_2. The morning after purging, 32 mg/L of the test item, DCOIT, was added

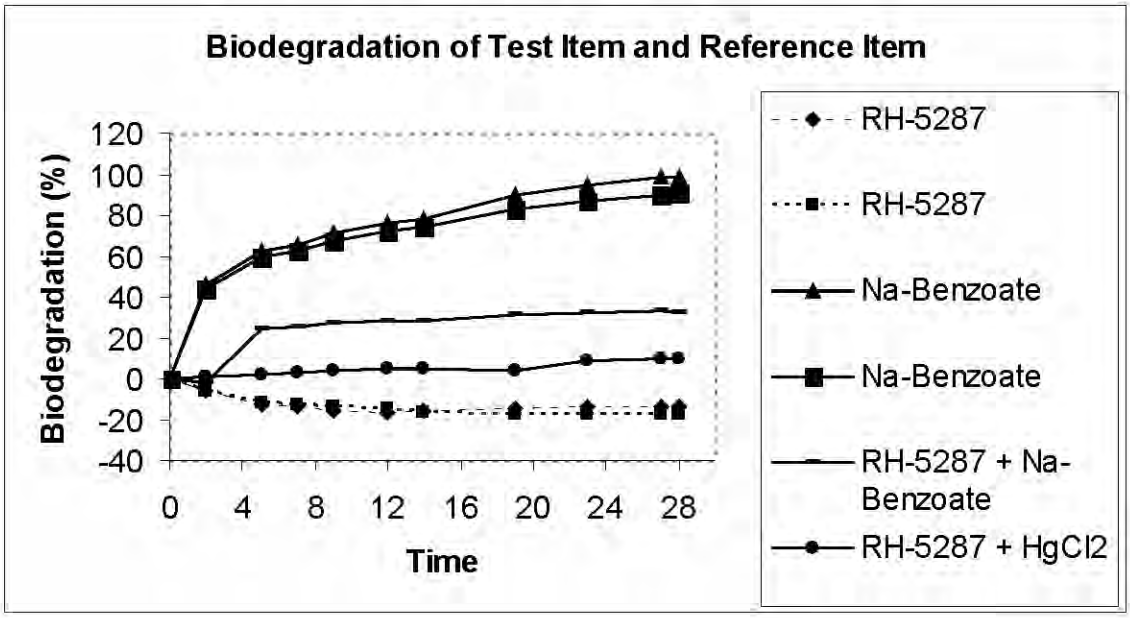
		to four flasks. To one of these flask, 10 mg/L of $HgCl_2$ was added (abiotic control) while to another flask 25.7 mg/L of the reference item, sodium benzoate, was added. To 2 procedure control flasks, only sodium benzoate (25.7 mg/L) was added while to 2 additional flasks neither the test substance nor the reference substance was added. The final flask contained only $HgCl_2$ (10 mg/L). The flasks were made up to a volume of three liters. Two 0.05 M NaOH traps were 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 a five ml sample was withdrawn from each of the first NaOH absorber in series. Additionally on Days 14 and 28 samples were drawn from the second NaOH absorber to correct for any carryover CO_2. Total inorganic carbon was quantitated by a TOC analyzer. After sampling on Day 28, 1ml of concentrated HCl was added to each flask and the flask aerated overnight to drive residual CO_2 into absorber allowing for quantitation of dissolved CO_2.	
5.2	**Results and discussion**	The test item, DCOIT, was found to be not ready biodegradable under the test conditions within 28 days. In the abiotic control containg DCOIT and poisoned test medium, no significant degradation was noted at the end of the 28-day exposure period (<10 %). In the toxicity control containing both DCOIT and the reference item sodium benzoate, biodegradation was slightly delayed over the course of the experiment compared to sodium benzoate alone. In the procedure controls, sodium benzoate was degraded to an average extent of 76 % by exposure day 14, confirming suitability of the activated sludge. By the end of the test, the reference item was degraded completely.	x
5.3	**Conclusion**	DCOIT was found to be not biodegradable under the tests conditions within 28 days. However, testing biocides for ready biodegredability may not be relevant since biocides which are toxic to the inoculum may give false negative test results which may lead to requirements for further tests. In addition, the biocidal concentration of this compound is less than 3 ppm (whereas the concentration used in this ready biodegradation study was 32 ppm). Therefore higher tier studies have been performed and are summarized in Document IIIA section A7.1.2. They demonstrate that DCOIT is rapidly biodegradable with a half-life of less than 48 hours.	x
5.3.1	Reliability	1-valid without restrictions.	x
5.3.2	Deficiencies	No	x

Document III-A / Section A7.1.1

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	5 September 2007
Materials and Methods	**Comment (3.3.5)**: The test concentration exceeds the water solubility of DCOIT.
Results and discussion	**Comment (4.1.3 and 5.2):** Agree with applicant's version regarding the result that DCOIT was found to be not ready biodegradable under the test conditions within 28 days. However, in the toxicity control, containing both DCOIT and the reference item sodium benzoate, the biodegradation of sodium benzoate was totally inhibited after 5 h compared to sodium benzoate alone. These data suggest that DCOIT inhibits the bacteria at the test concentration of 32 ppm, something which is not unexpected since the minimum inhibitory concentration for several bacteria is less than 0.25 ppm. According to OECD 301 the test substance can be assumed to be inhibitory if in a toxicity test, containing both the test substance and a reference compound, less than 35% degradation (based on total DOC) or less than 25% (based on total ThOD or ThCO2) occurred within 14 days. In order to get information of the biodegradability of DCOIT in a STP, the test series should in principle be repeated, using a lower concentration of test substance (if this can be done without seriously impairing the accuracy of the DOC determination) and/or a higher concentration of inoculum, but not greater than 30 mg solids/l. However, higher tier studies on biodegradation have been performed for the aquatic compartment and therefore no further testing is considered necessary at the moment.
Conclusion	**Comment (5.3):** Agree with applicant's version regarding the result that DCOIT was found to be not biodegradable under the tests conditions within 28 days. However, DCOIT inhibited the microorganisms at the test concentration. Higher tier studies on biodegradation have been performed for the aquatic compartment. These are summarized in Document IIIA section A7.1.2.
Reliability	**Comment (5.3.1 and 5.3.2):** Due to the inhibition of the inoculum and the test concentration exceeding the water solubility, the reliability is changed from 1 to 2 – valid with restrictions.
Acceptability	Acceptable with the restrictions noted above
Remarks	-

Section A7.1.1.2.1	Ready Biodegradability – **TABLES AND FIGURES**

Figure A7.1.1.2.1-1 Biodegradation of the test item and the reference item during incubation period



Document III-A / Section A7.1.1

Section A7.1.1.2.2 **Annex Point IIA7.6.1.2**	**Inherent biodegradability**	
	Justification for non-submission of data	Official use only
Other existing data [x] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The test protocol is unsuitable for compounds with biocidal activity. Carbon dioxide, the end point measured in this study, has a limit of detection of about 2 ppm. However, DCOIT demonstrates biocidal activity as measured by its minimal inhibitory concentration to microbes at less than 0.1 ppm. The OECD guidelines recognize this biocidal/dosage paradox and states that this test may not be applicable for biocidally active compounds. It is also mentioned in the The Technical Guidance document on data requirements in support of the Directive 98/8/EC concerning the placing of biocidal products on the market (chapter 3 section 7.0.2.2.2) that simulation tests are generally preferred to inherent biodegradability test. Higher tier simulation test in water, water/sediment microcosms, and soil have been performed in lieu of the Inherent Biodegradation study. They are summarized in Document IIIA section A7.1.2 and A7.2.	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	5 October 2006	
Evaluation of applicant's justification	Agree with the applicant that the test protocol is unsuitable for compounds with biocidal activity.	
Conclusion	Agree with Applicant's justification.	
Remarks	-	

Document III-A / Section A7.1.1

Section A7.1.1.2.3 **Annex Point IIA7.6.1.2**	**Biodegradation in seawater**	
	Justification for non-submission of data	Official use only
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The Technical Guidance document on data requirements in support of the Directive 98/8/EC concerning the placing of biocidal products on the market specifies (chapter 3 section 7.1.1.2.3) that if a substance is to be used in marine environment, a seawater biodegradation test according to OECD306 may be performed. It is also mentioned that alternatively, a water/sediment degradation study in seawater may be done. A water/sediment study in seawater has been performed and is summarized in section A7.1.2. The biodegradation test in seawater is therefore not required.	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	5 October 2006	
Evaluation of applicant's justification	Agree with Applicant's justification. Besides the performed seawater/sediment studies (IIIA7.1.2.2.2 c and d) an aerobic aquatic degradation study with estuarine surface water (IIIA7.1.2.2.1) has been conducted.	
Conclusion	Applicant's justification is acceptable according to the Technical Notes for Guidance on Data Requirement.	
Remarks	-	

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

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

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

Product type 8: Wood preservatives

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

Part II

Fate and behaviour in the environment

Section A7.1.2: Fate and behaviour in water

Biodegradation in aquatic systems (simulation tests)

TABLE OF CONTENTS

Document III-A / Section A7.1.2

Section A7.1.2.1.1 Annex Point	**Biological sewage treatment-Aerobic biodegradation**	
	Justification for non-submission of data	Official use only
Other existing data [x] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	No.	

Document III-A / Section A7.1.2

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	28 November 2007
Evaluation of applicant's justification	STP are exposed to DCOIT in PT 8 at the application stages and due to leaching from treated wood in the Noise Barrier Scenario. It can not be concluded from the activated sludge respiration inhibition study that DCOIT is rapidly biodegraded in STP (see evaluation of study summary IIIA7.4.1.4). From the test on ready biodegradability (IIIA7.1.1.2.1) it can only be concluded that DCOIT is not readily biodegradable at the test concentration tested, which inhibited the inoculum. Therefore, no information is available on biodegradation of DCOIT in STPs. However, as shown in IIIA7.1.3.a DCOIT adsorbs to the activated sludge of a STP at a typical sludge concentrations of 3-9 g sludge/L of solution expected in a waste treatment plant and is unlikely to remain in the aqueous phase. Results from higher tier degradation studies in water/sediment systems show that DCOIT is rapidly biodegraded by microorganisms.
Conclusion	A rate constant for DCOIT in STP can not be established. Therefore a rate constant of 0 h^{-1} is anticipated in a STP and adsorption to sludge is taken into account. No further testing is considered necessary at the moment.
Remarks	-

Section A7.1.2.1.2 Annex Point	Biological sewage treatment-Anaerobic biodegradation	
	Justification for non-submission of data	Official use only
Other existing data [x]	Technically not feasible [ ] Scientifically unjustified [ ]	
Limited exposure [x]	Other justification [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	No.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	28 November 2007	
Evaluation of applicant's justification	Agree with applicant's justification	
Conclusion	No further testing considered necessary	
Remarks	-	

Document III-A / Section A7.1.2

Section A7.1.2.2.1 Aerobic aquatic degradation study – Biodegradation in Estuarine Surface Water

Annex Point IIIA XII 2.1

Official use only

1 REFERENCE

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

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes. OECD Draft Document for New Guideline 309: Aerobic Mineralization in Surface Water Simulation Biodegradation Test.
2.2	**GLP**	Yes.
2.3	**Deviations**	GLP deviation was minor: plate counts determining microbial activity were not conducted under GLP.

3 METHOD

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 substance**	[redacted]
3.2.1	Nature of reference substances	[redacted]

Section A7.1.2.2.1 **Aerobic aquatic degradation study –**
Annex Point IIIA XII 2.1 **Biodegradation in Estuarine Surface Water**

(chromatography) [redacted]

3.3 Test ing procedure

3.3.1 Water characterization [redacted] X

3.3.2 Test system [redacted] X

[redacted]

3.3.3	Test conditions	[redacted]
3.3.4	Method of preparation of test solution	[redacted]
3.3.5	Initial TS concentration	5, 10, 25, 100, 1000 ppb
3.3.6	Duration of test	144 hours
3.3.7	Sampling	[redacted]
3.3.8	Intermediates/ degradation products	[redacted]

[redacted]

3.3.10 Statistics

[redacted]

4 RESULTS

4.1 Preliminary study

Initially, surface waters from several areas along the U.S. Atlantic Coast were examined for their physical and chemical characteristics and compared to the OECD guideline criteria. The water from Port Penn, Delaware was chosen because it met all the guideline criteria.

A preliminary experiment at 10 ppb and 18°C was performed to estimate the half-life and determine the appropriate sampling intervals. The estimated half-life was 26.3 hours. After 120 hours, about 4% of the applied radioactivity still remained as parent.

4.2 Definitive

Document III-A / Section A7.1.2

Experiment

4.2.1 System feasability

Sterile controls had about 18% degradation of parent after 144 hour incubation. Thus biological degradation is the primary route for dissipation of parent. Biodegradation of aniline, the reference standard, was about 81% after 144 hrs. Thus the system is operating satisfactorily.

Parent method validation :

The HPLC limit of quantitation (LOQ) for ^{14}C DCOIT was determined as 1 ppb.

The recovery of fortified parent is tabulated below.

Conc/Temp	% Recovery	% RSD	No. Samples
5 ppb/18°C	101	0.69	9
25 ppb/18°C	95.3	2.77	9
10 ppb/10°C	91.9	3.78	8
100 ppb/10°C	94.6	13.9	8

Overall recovery was 95.7 ± 5.3% (n=34). This validates the extraction procedure and analytical method used for parent quantitation.

4.2.2 Distribution and recovery of ^{14}C-activity

Tables A7.1.2.2.1-2, A7.1.2.2.1-3, and A7.1.2.2.1-4 summarize the distribution between the methylene chloride, aqueous, and biomass phases at the various dosing concentrations and temperatures. The ^{14}C-activity in the methylene chloride phase decreases with time while the activity in the aqueous phase correspondingly increases with time. Parent partitions into the methylene chloride phase so that the activity present in this phase gives an initial estimate of its half-life. The recovery of applied ^{14}C-activity ranges from about 65% to 117% and the overall recovery from the six test runs is 94.36 ± 11.36%.

4.2.3 Half-life

The half-life of DCOIT ranged from 4 hrs to 32 hrs and was dependent on the temperature and initial concentration of DCOIT (Table A7.1.2.2.1-5). A graphical presentation of the kinetics for samples dosed at 5 ppb (18°C) and 10 ppb (10°C) is presented in Figures A7.1.2.2.1-1 and A7.1.2.2.1-2, respectively. The longer half-life observed with increasing concentration of DCOIT is due to the biocidal activity (and thus microbial inhibition) of the compound. Quantitation of parent in the experiments incubated at 18°C is presented in Tables A7.1.2.2.1-6, A7.1.1.2.1-7, A7.1.2.2.1-8, and A7.1.2.2.1-9. At 1000 ppb, around 60% of the ^{14}C-activity after 48 hours is parent and conditions have been established such that the parent biocide effectively controlled microbial growth in this system and degradation of DCOIT has ceased. Thus sub-biocidal concentrations of DCOIT rapidly biodegrade in biologically active estuarine water.

4.2.4 Metabolite profile

There were seven metabolites (M1 to M7) of DCOIT detected. Tables A7.1.2.2.1-6 – A7.1.2.2.1-9 summarize the results. Metabolite profiling was only performed on the samples incubated at 18°C. Generally, M1 and M2 increase steadily with time. At all dosing concentrations except 1000 ppb, M1 and M2 exceed 10% of the applied dose by the 72 hr sampling interval. M3-M7 gradually increase with time. Only M4 exceeds 10% of the applied dose, and only in samples dosed at 100 ppb. In the 1000 ppb dosed samples, parent biodegradation essentially ceases at 48 hours. Metabolite production is also severely reduced. These results indicate that at 1000 ppb, DCOIT was operating as an effective biocide in this system. $^{14}CO_2$ was monitored in the 1000 ppb dosing level and it reached at maximum after 144 hours at 0.21%

4.2.5 Identification of metabolites

Table A7.1.2.2.1-10 summarizes the metabolite identification. The metabolites were identified by mass spectroscopy as the following:

- M7, 1,2-dichloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid;
- M5, 1-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid;
- M4, 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid
- M2, N-(n-octyl) oxamic acid.

M3 and M6 were present in small amounts and no peak could be observed by HPLC using a radioactivity detector. The metabolites were isolated by TLC and analyzed by LC-MS. Negative ionization was effective with a molecular ion at 296 (molecular weight, 297). The exact mass determination was essentially identical to M4 and M5. An A+2 peak at 298 indicates the presence of one chlorine atom. The small quantity of material present prevented additional analysis.

M1 is a very polar peak and showed multiple ions, and thus multiple compounds by LC-MS. Quantitation of the multiple ions showed that none were greater than 10%.

4.2.6 Metabolic pathway

A metabolic pathway is presented in Figure A7.1.2.2.1-3.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The test guideline followed was the OECD Draft Document for New Guideline 309: Aerobic Mineralization in Surface Water Simulation Biodegradation Test. There are no deficiencies.

Bottles containing either 100 or 200 ml of estuarine surface water collected at Port Penn, Delaware, USA were dosed at 5 ppb, 25 ppb, or 100 ppb. The samples were placed into a dark incubator at 18°C. Another set of bottles were dosed at 10 ppb and 100 ppb and incubated in the dark at 10°C. A final bottle containing 1000 ml of surface water was dosed at 1000 ppb and incubated at 18°C. At periodic intervals between 0 and 144 hours, duplicate samples were removed for analysis. The water was partitioned with methylene chloride and the resulting aqueous, organic, and biomass (emulsion) phases quantitated by radioassay. The aqueous and organic phases were concentrated, chromatographed, parent and metabolites quantitated, and metabolites identified by LC-MS.

5.2 Results and discussion

DCOIT biodegrades very rapidly in the estuarine water studied. The half-life varied from 4-32 hours and was dependent on the dosing concentration of DCOIT and the incubation temperature.

The amount of ^{14}C activity in the methylene chloride fraction (fraction containing parent) decreased with time while the activity in the aqueous fraction increased. This indicates that DCOIT is being degraded, probably by ring cleavage and the formation of more polar alkyl metabolites. Chromatography revealed the presence of seven isothiazolone ring cleaved metabolites. The major metabolite was identified by mass spectroscopy as N-(n-octyl) oxamic acid. Also identified by mass spectroscopy were three sulfonic acid analogs: 1,2-dichloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid, 1-chloro-2-(n-octylcarbamoy)l-1-ethene sulfonic acid and 2-chloro-2-(n-octylcarbamoyl)-1-ethylene sulfonic acid. Insufficient quantities of two additional metabolites were isolated for definitive structure determination, however, they also appear to be chlorinated sulfonic acids similar to the above.

5.3 Conclusion

Similar to the results in other media (*e.g.* water:sediment microcosms, soil), DCOIT rapidly biodegrades in estuarine water. Half-life ranged

		from 4-32 hours and was dependent on dosing concentration of DCOIT and incubation temperature. Metabolism involved cleavage of the isothiazolone ring, leading to the formation of N-(n-octyl) oxamic acid and various sulfonic acids.
5.3.1	Reliability	1-valid without restrictions
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	13 September 2007
Materials and Methods	**Comment (3.3.1):** According to additional information provided by the applicant. salinity was 12.6 g/l.
	Comment (3.3.2): The test samples at concentrations of 5, 25 and 100 ppb at 18°C were used for determining kinetics and metabolite profiling, not metabolite identification.
Results and discussion	**Comment (4.2.1):** The LOQ was 1 ppb in this test. According to OECD Guideline 309, the lowest dosing level in the test should 10 times the LOQ, that means 10 ppb. Thus, a half life of 4 hrs for the 5 ppb dosing level should not be used, because the test concentration it too low. However, the LOQ for surface/drinking/seawater is actually as low as 0.02 µg/l as demonstrated in analytical methods for the determination of residues in different media (see Doc. III A4.2.c/01 and 02). Therefore the 5 bbp result is considered valid.
Conclusion	**Comment (5.3):** DCOIT rapidly biodegrades in estuarine water. This can be regarded as primary degradation as no CO_2 was developed (see mass balances presented in tables -2, -3 and -4). The half-life ranged from 4-32 hours and was dependent on dosing concentration of DCOIT and incubation temperature. Metabolism involved cleavage of the isothiazolone ring, leading to the formation of N-(n-octyl) oxamic acid and various sulfonic acids.
Reliability	1, valid without restrictions
Acceptability	Acceptable
Remarks	-

Section A7.1.2.2.1	Aerobic aquatic degradation study – Biodegradation in Estuarine Surface Water – **TABLES AND FIGURES**

Table A7.1.2.2.1-1: Parameters of Test Water

During the course of the study, three water samples were collected (two for 18 °C treatment and one for 10°C). The key parameters of the test waters were measured at the begining, middle and end of the experiment.

Test Stage	pH	Temp. (°C)	Oxygen (mg/L)	Bacteria (cell/ml)	TOC (ppm)	Conductivity (mohms/cm)	Total Nutrients	
							N (ppm)	P (ppm)
Sampling	6.88	18.2	8.92	3.2×10^4	8.8	10.07	25.4	2.9
T = 48 hr	7.02	17.8	8.84	N/A[a]	N/A	N/A	N/A	N/A
End of Test	7.10	17.8	9.12	N/A	6.5	9.5	8.75	3.1
Sampling	7.02	19.8	8.80	2.7×10^4	6.0	10.67	4.4	3.0
T= 24 hr	7.10	17.9	9.07	N/A	N/A	N/A	N/A	N/A
End of Test	7.08	18.1	9.23	N/A	3.0	11.47	1.4	3.8
Sampling	7.26	19.3	9.12	9.0×10^4	xxx[b]	xxx	xxx	xxx
T = 72 hr	7.02	10.5	9.07	N/A	N/A	N/A	N/A	N/A
End of Test	7.13	10.6	9.01	N/A	4.0	5.68	4.6	0.8

[a] N/A = Not Applicable or Not Analyzed, [b] xxx = sample lost.

Table A7.1.2.2.1-2: Distribution Between Methylene Chloride, Aqueous, and Biomass Phases

Sample Time (hrs)	Percent of Applied ^{14}C-Activity Applied (mean of replicate samples)							
	5 ppb/18°C				25 ppB/18°C			
	Methylene Chloride	Aqueous	Biomass	Recovery	Methylene Chloride	Aqueous	Biomass	Recovery
0	87.00			87.00	95.36			95.36
1	78.46	5.99	23.54	107.99	88.80	2.77	18.16	109.63
3	60.98	4.83	37.31	103.12	62.56	8.51	1959	90.66
6	38.23	13.93	33.12	85.28	63.04	10.02	34.34	107.40
10					70.08	13.66	36.37	120.11
12	35.28	19.63	32.82	87.75				
24	22.26	24.86	43.30	90.42	35.68	16.80	38.93	91.41
48	15.01	30.85	41.74	87.60	28.40	17.92	46.26	92.58
72	11.29	37.27	35.99	84.55	16.05	28.48	52.80	97.33
144	8.21	51.48	49.69	109.38	15.38	49.60	51.80	116.78
Average Recovery				94.51± 10.50				103.24± 11.79

Table A7.1.2.2.1-3: Distribution Between Methylene Chloride, Aqueous, and Biomass Phases

Sample Time (hrs)	Percent of Applied ^{14}C-Activity (mean of replicate samples)							
	100 ppb/18°C				1000 ppb/18°C			
	Methylene Chloride	Aqueous	Biomass	Recovery	Methylene Chloride	Aqueous	Biomass	Recovery
0								
1	89.59	1.44	16.65	107.68	98.17	1.79	0.49	100.45
3	68.36	4.31	20.84	93.51	96.97	2.07	0.58	99.62
6	59.70	5.74	29.90	95.34	92.58	2.25	1.55	96.46
10					79.81	4.74	1.73	86.42
12	64.37	7.85	27.82	100.04				
24	27.82	15.49	53.11	96.42	82.37	5.09	2.37	89.99
48	20.11	16.32	56.09	92.52	56.50	6.62	2.53	65.73
72	18.00	20.99	63.64	101.63	53.96	9.98	4.37	68.47
144	15.64	27.14	69.68	112.46	59.62	15.64	3.22	78.69
Average Recovery				99.95± 7.07				85.73± 13.58

Table A7.1.2.2.1-4: Distribution Between Methylene Chloride, Aqueous, and Biomass Phases

Sample Time (hrs)	Percent of Applied ^{14}C-Activity (mean of replicate samples)							
	10 ppb/10°C				100 ppb/10°C			
	Methylene Chloride	Aqueous	Biomass	Recovery	Methylene Chloride	Aqueous	Biomass	Recovery
0	102.16	2.52	0.19	104.87	103.75	2.38	0.15	106.28
1	95.78	3.82	1.44	101.03	97.77	3.00	0.80	101.57
3	86.20	5.92	2.47	64.59	97.37	3.81	1.09	102.27
6	84.60	4.64	2.58	91.82	92.18	3.26	1.01	96.45
10	83.80	8.92	6.46	99.18	98.57	2.73	0.31	101.60
24	66.09	23.27	9.46	98.82	81.41	10.06	2.08	93.55
48	31.45	37.11	11.29	79.85	45.97	42.18	2.18	90.33
72	20.79	44.77	19.04	84.60	30.93	42.10	7.88	80.90
144	8.09	69.00	16.72	93.81	15.28	67.76	13.61	90.65
Average Recovery				94.28± 7.99				95.96± 7.92

Table A7.1.2.2.1-5: Half-Life of DCOIT in Estuarine Water

Concentration (µg/L)	Temperature (°C)	Half-Life (hr)[a]	R^2 [b]
5	18.1 ± .16	4.2	0.96
25	18.0 ± 0.30	11.9	0.92
100	18.1 ± 0.16	11.7	0.91
10	10.3 ± 0.25	20.2	0.94
100	10.3 ± 0.25	31.9	0.98

[a] Half-life determined from a plot of the ln % DCOIT versus time. From the slope of the line, $t_{½}$ = ln2/slope.
[b] Correlation coefficient of the line, ln % DCOIT versus time.

Table A7.1.2.2.1-6: Metabolite Profiling, 5 ppb at 18°C

Time (hr)	Component—Percent of Applied ^{14}C-Activity (mean of replicate samples)							
	Parent[1]	M1	M2	M3	M4	M5	M6	M7
0	100[2]							
1	69.7	1.94	2.47	0.80	1.00	0.58	0.76	0.79
3	51.1	3.30	5.64	2.13	1.54	1.45	2.26	5.67
6	28.4	3.72	6.37	2.92	1.65	1.69	2.44	3.67
12	15.3	4.48	11.05	4.21	2.66	2.03	3.00	5.82
24	1.4	6.21	15.37	5.83	2.87	2.78	4.13	6.91
48	ND[3]	6.00	15.71	6.78	3.93	3.08	3.44	6.69
72	ND	14.41	23.72	6.66	3.94	3.66	4.74	8.27
144	ND	45.68	10.15	5.03	2.42	2.20	3.18	6.70

[1] Parent quantitated by HPLC
[2] Time 0, 5.21 ppb
[3] ND = no parent detected

Table A 7.1.2.2.1-7: Metabolite Profiling, 25 ppb at 18°C

Time (hr)	Component—Percent of Applied ^{14}C-Activity (mean of replicate samples)							
	Parent[1]	M1	M2	M3	M4	M5	M6	M7
0	100[2]							
1	107.4	1.45	1.65	0.44	0.37	0.52	0.82	1.46
3	73.0	1.84	5.45	1.94	1.39	1.82	1.73	1.02
6	52.5	3.49	7.64	3.14	2.73	2.51	2.33	2.05
10	28.4	5.84	10.87	3.44	3.43	3.12	2.78	2.04
24	16.5	7.13	16.91	4.10	4.47	4.15	2.96	2.12
48	10.3	10.28	17.20	4.54	5.92	3.58	1.81	2.34
72	1.1	14.84	18.20	4.79	4.38	4.90	3.16	2.13
144	ND[3]	27.52	23.04	6.27	6.21	6.37	4.74	3.49

[1] Parent quantitated by HPLC
[2] Time 0, 21.62 ppb [3] ND = no parent detected

Table A7.1.2.2.1-8: Metabolite Profiling, 100 ppb at 18°C

Time (hr)	Component—Percent of Applied ^{14}C-Activity (mean of replicate samples)							
	MDC Phase[1]	M1	M2	M3	M4	M5	M6	M7
0	100[2]							
1	87.51	1.32	2.26	0.75	2.58	1.51	2.81	2.14
3	88.98	1.77	3.53	1.29	3.73	2.32	4.75	2.01
6	78.1	2.36	4.94	2.19	4.63	2.30	4.97	2.47
12	57.8	2.83	5.49	2.06	4.62	2.60	4.34	3.33
24	15.2	6.30	13.26	4.11	7.50	4.47	4.13	4.14
48	5.0	6.95	15.17	4.67	7.85	4.02	5.12	4.99
72	2.35	10.05	22.91	6.76	11.86	5.87	7.02	6.35
144	ND	12.17	24.44	7.31	11.81	8.91	8.87	7.67

[1] Parent quantitated by HPLC
[2] Time 0, not analyzed. Used the nominal concentration, 100 ppb.
[3] ND = no parent detected

Table A7.1.2.2.1-9: Metabolite Profiling, 1000 ppb at 18°C

Time (hr)	Component—Percent of Applied ^{14}C-Activity (mean of replicate samples)							
	Parent[1]	M1	M2	M3	M4	M5	M6	M7
0								
1		0.33	0.48	0.32	0.61	0.45	0.27	0.43
3		0.29	0.29	0.27	0.44	0.34	0.20	0.02
6		0.24	0.27	0.31	0.25	0.36	0.08	0.49
10		0.31	0.63	0.52	0.48	0.51	0.19	0.79
24		0.68	1.03	1.06	0.74	1.78	0.82	2.21
48		0.68	1.26	1.41	1.22	1.92	0.85	0.37
72		0.89	2.20	1.53	1.76	3.40	0.64	0.90
144		1.23	3.43	1.55	4.68	9.35	0.79	1.60

[1] Not determined since the 1000 ppb samples were to assist with metabolite identification only

Table A7.1.2.2.1-10: Structure of Metabolites

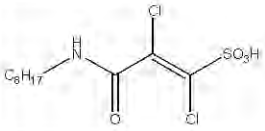
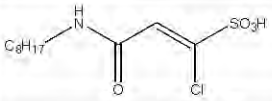
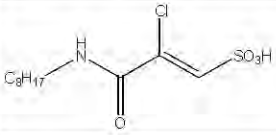
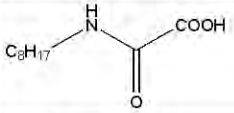
Component	Structure	Name	Maximum Percent of Applied ^{14}C-Activity
M7		1,2-dichloro-2-(n-octyl carbamoyl-1-ethene sulfonic acid	8.27
M6	$C_{11}H_{20}NO_4SCl$		8.87
M5		1-chloro-2-(n-octyl carbamoyl-1-ethene sulfonic acid	9.35
M4		2-chloro-2-(n-octyl carbamoyl-1-ethene sulfonic acid	11.81
M3	$C_{11}H_{20}NO_4SCl$		7.31
M2		N-(n-octyl) oxamic acid	24.44
M1	Multiple compounds		

Figure A7.1.2.2.1-1: Kinetic Analysis of DCOIT Dosed at 5 ppb and Incubated at 18°C

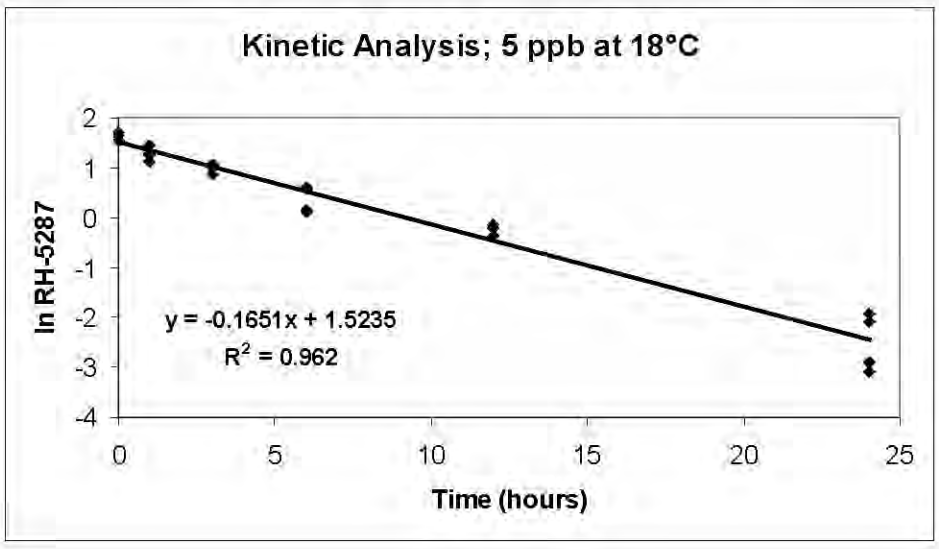
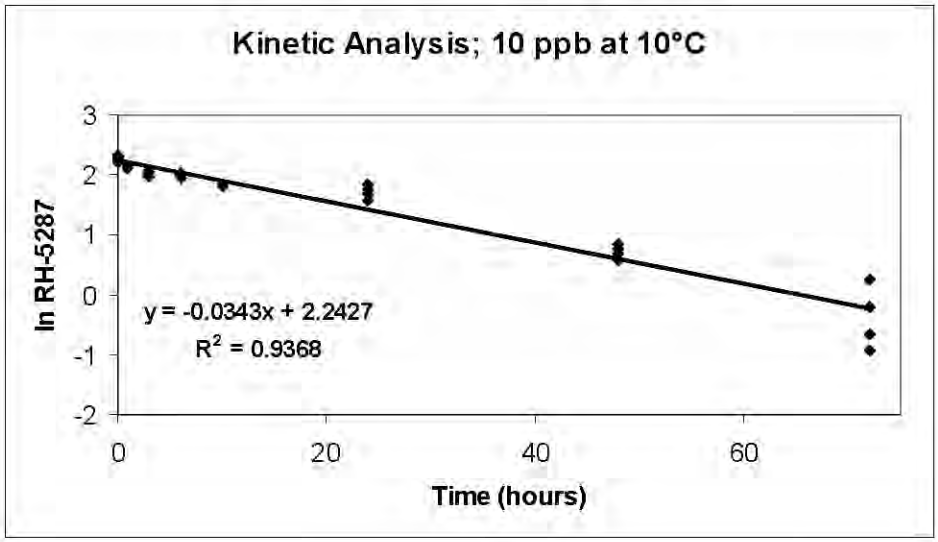


Figure A7.1.2.2.1-2: Kinetic Analysis of DCOIT Dosed at 10 ppb and Incubated at 10°C



[redacted]

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

Annex Point IIIA XII 2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Rohm and Haas Company	
1.2.2			
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline Study**	Yes. OECD Guideline 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.a **Fresh Water/Sediment Degradation study - Aerobic**
Annex Point IIIA XII 2.1

substances

[redacted]

3.3 Sediment and Water Characterization

[redacted]

3.4 Test procedures

3.4.1 Test system

[redacted]

3.4.2 Preparation of test solution

[redacted]

3.4.3 Initial Test substance concentration

[redacted]

Document III-A / Section A7.1.2

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

[redacted]

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.a **Fresh Water/Sediment Degradation study - Aerobic**
Annex Point IIIA XII 2.1

[redacted]

3.4.9 Analytical methods

[redacted]

3.4.10 Degradation products

[redacted]

4 **RESULTS**

4.1 Preliminary studies

Preliminary studies using Bury Pond water and sediment were conducted to establish the dosing concentration, the extraction procedures and the sampling interval for the aerobic 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.a-2, -3 and -4) it was determined to dose both the aerobic systems at 1 mg/l (1ppm).

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 aerobic non-sterile system dosed at 1 ppm is presented in Table A7.1.2.2.2.a-5. The total radioactivity in the water phase decreased rapidly from 90.5% (Day 0.042) to 1% of applied radioactivity at termination of the study (Day 101). Solvent extractable residues increased slowly with time from 1.6% of the applied radioactivity on Day 0.042 to 39.5% on Day 13. By Day 101 solvent extractable residues had declined to 25% of the applied radioactivity. ^{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 101 when it comprised 10.7% of the applied activity. Nonextractable (bound) residues comprised 11.2% of the applied on Day 0.042 and increased to 61.7% on Day 101. Recovery of

applied ^{14}C-activity ranged from 92.8% (Day 7) to 103.2% (Day 0.042) and averaged 97.01 ± 4.93%.

Table A7.1.2.2.2.a-6 provides the quantitation of parent as a percent of applied radioactivity in the water phase in both 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 13 it comprised about 1% of the applied radioactivity. From the results in Table A7.1.2.2.2.a-5 and A7.1.2.2.2.a-6 the percent parent in the water phase can be calculated. On Day 1, DCOIT comprised 99.2% of the water phase radioactivity and this decreased to 89.5% on Day 3 and 19.6% on Day 13.

Sterile system :

Table A7.1.2.2.2.a-7 provides the distribution of ^{14}C-activity between the different phases for the sterile system dosed with 1 ppm ^{14}C DCOIT. Microbial examination of the system showed that the system was sterile at Day 0 but by Day 30 bacterial contamination had occurred. Thus the distribution results are similar to the non-sterile system. Sterility was demonstrated at Day 0. Quantitation of parent compound (as percent of applied) is presented in Table A7.1.2.2.2.a-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. x

The concentration of parent (as percent applied) in the non-sterile system is presented in Table A7.1.2.2.2.a-6. The half-life was determined by best-fit linear regression analysis using ModelMaker software. The degradation constant;

k (days^{-1}): 0.341

and correlation coefficient

r^2: 0.97

The dissipation kinetics are expressed below

DT_{50} (days): 2.0

DT_{75} (days): 4.1

DT_{90} (days) 6.7

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.a-8 and A7.1.2.2.2.a-9, respectively.

In water, the primary metabolite identified through Day 7 was parent. Parent decreased with time accounting for 89.1% of the applied activity on Day 0.042 and 1% on Day 13. No other major metabolite was detected in the water phase as the total non-parent metabolites were 0.6% of the applied activity on Day 0 and increased to 4.1% on Day 13.

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.a-9). Essentially all were less than 10% of the applied dose. N-(n-octyl) malonamic acid and N-(n-octyl) acetamide were identified by cochromatography with standards. The major metabolites were peaks U,V,and W which were poorly resolved. A pool of these peaks x

		were combined, purified by additional TLC, and identified by mass spectroscopy. The results appear in Table A7.1.2.2.2.a-10. With the additional TLC, two bands of metabolites were initially observed; major band (accounting for 60-75% of the activity) and a minor band (25-40%). 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. CO_2 was identified as a major metabolite comprising 10.2% of the applied activity by Day 101.	
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.a-11 and A7.1.2.2.2.a-12. The insoluble fraction of humin contains about 7-40% of the applied radioactivity which correlates to over half of the bound residue. 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% 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.a-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. Air (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, 13, and 101 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. 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.	x
5.2 Results and Discussion		In the non-sterile aerobic fresh water:sediment microcosm studied, the half-life of DCOIT was 2 days. There was a steady decrease of ^{14}C activity in the water phase which correlated with a steady increase in the	x

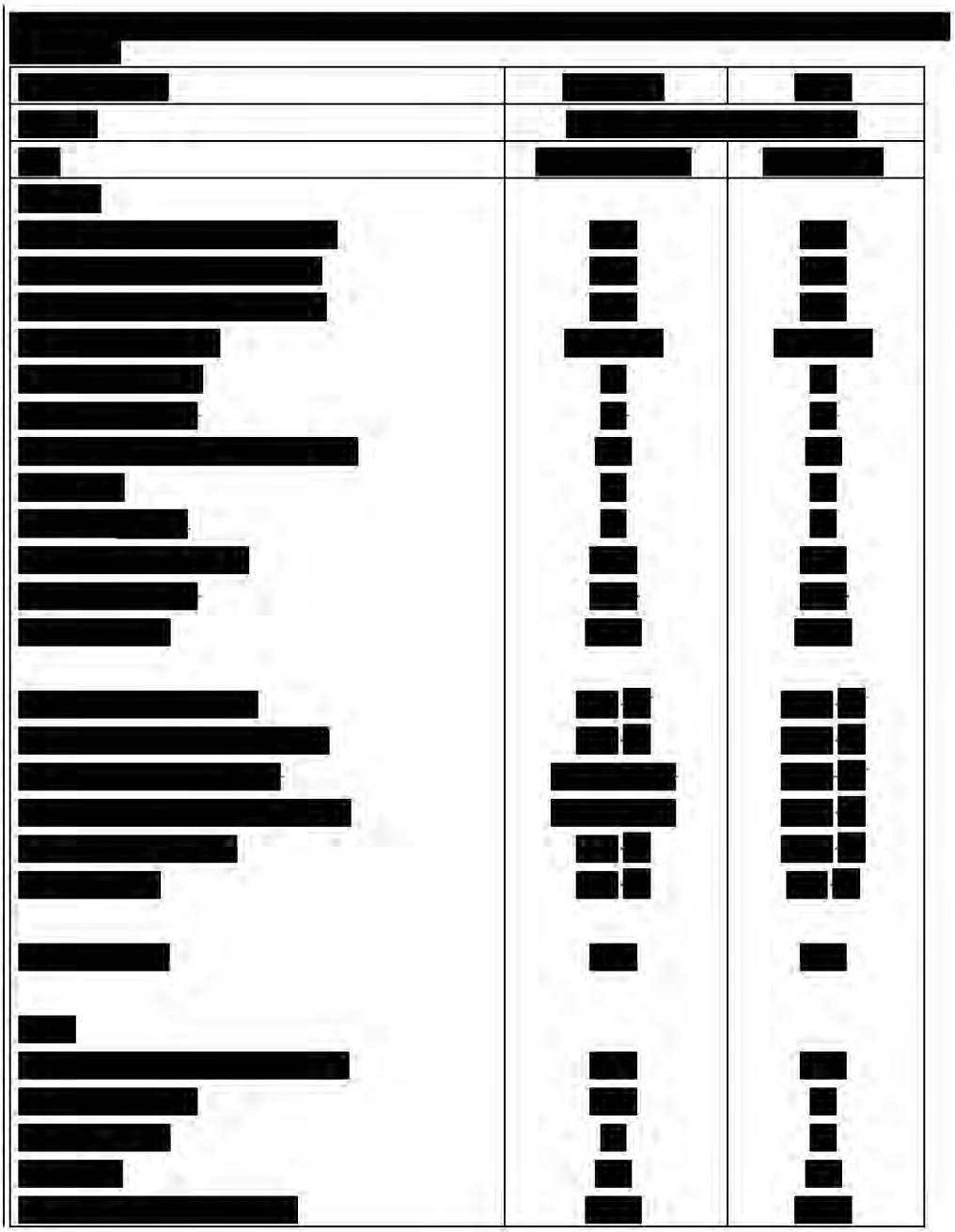
		sediment. On Day 0.042, 90.5% of the applied activity was in the water phase but by Day 101 that had decreased to 1%. ^{14}C-activity in the solvent extractable sediment residue increased from 1.6% of the applied radioactivity on Day 0.042 to 39.5% on Day 13. Activity in the bound residue increased to 61.7% of applied radioactivity on Day 101. By day 101, 10.7% of the applied activity was $^{14}CO_2$. The distribution of metabolites is presented in Table A7.1.2.2.2.a-7, 8, and 9 and a metabolic pathway is presented in Figure A7.1.2.2.2.a-1. Except for $^{14}CO_2$, no metabolites was detected at greater than 10% of applied radioactivity. N-(n-octyl) malonamic acid, N-(n-octyl) acetamide, 3,3'dithiobis-(n-octyl)-3-chloropropenamide and 2-chloro-3-(formyldithio)-N-octylpropenamide were identified by either cochromatography with standards or LC-MS. Similar results were obtained for the sterile system. The microbial growth in the 30 day sterile samples indicated that these samples were either not successfully sterilised or were contaminated after treatment.	
5.3	**Conclusion**	DCOIT rapidly biodegrades in fresh water:sediment microcosm with a half-life of 2 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

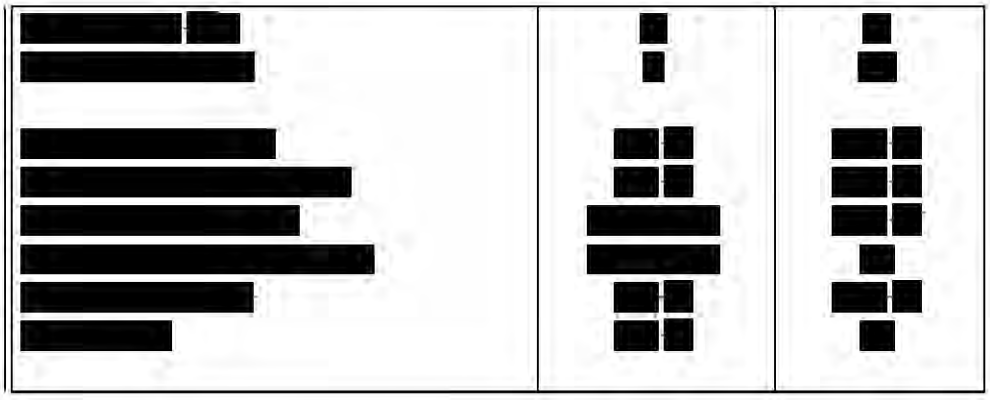
Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	29 June 2007, revised 22 January 2009, revised 3 June 2010
Materials and Methods	**Comment (2.3)**: There is one Guideline deficiency: At least two different sediment types and their associated waters are required for this study. A coarse texured sediment, low in organic matter is lacking. **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-chloropropenamide and 2-chloro-3-(formyldithio) –N-octylpropenamide, were identified by mass spectroscopy and two others, N-(n-octyl) malonamic acid and N-(n-octyl) acetamide, by cochromatography with a standard. **Comment (4.2.5):** After 101 days, 61.7 % 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 (5.1):** There is one Guideline deficiency: At least two different sediment types and their associated waters are required for this study. Coarse textured sediment, low in organic matter is lacking. **Comment (5.2)**. Parent DCOIT initially disappeared faster in the sterile control than in the biotic samples, which could be due to abiotic degradation or contamination. Considerable abiotic degradation of parent was also demonstrated in the anaerobic study (A7.1.2.2.2b), where the sterile control was not contaminated. Nucleophiles produced by autoclaving may explain the rapid initial disappearance of DCOIT in these cases. 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. 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





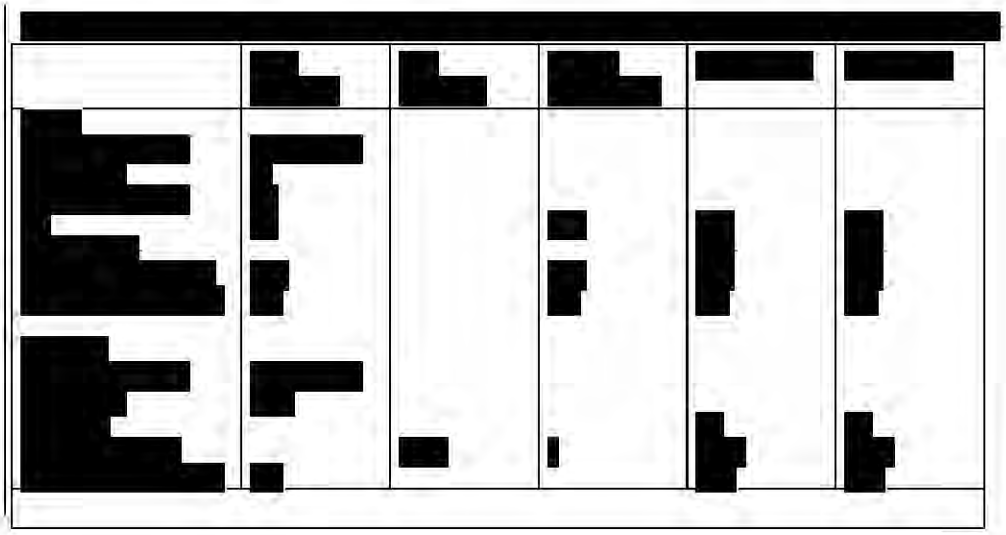


Table A7.1.2.2.2.a-3: Distribution of Radioactivity in Preliminary Studies Following Dosing With ^{14}C DCOIT in in % applied radioactivity

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

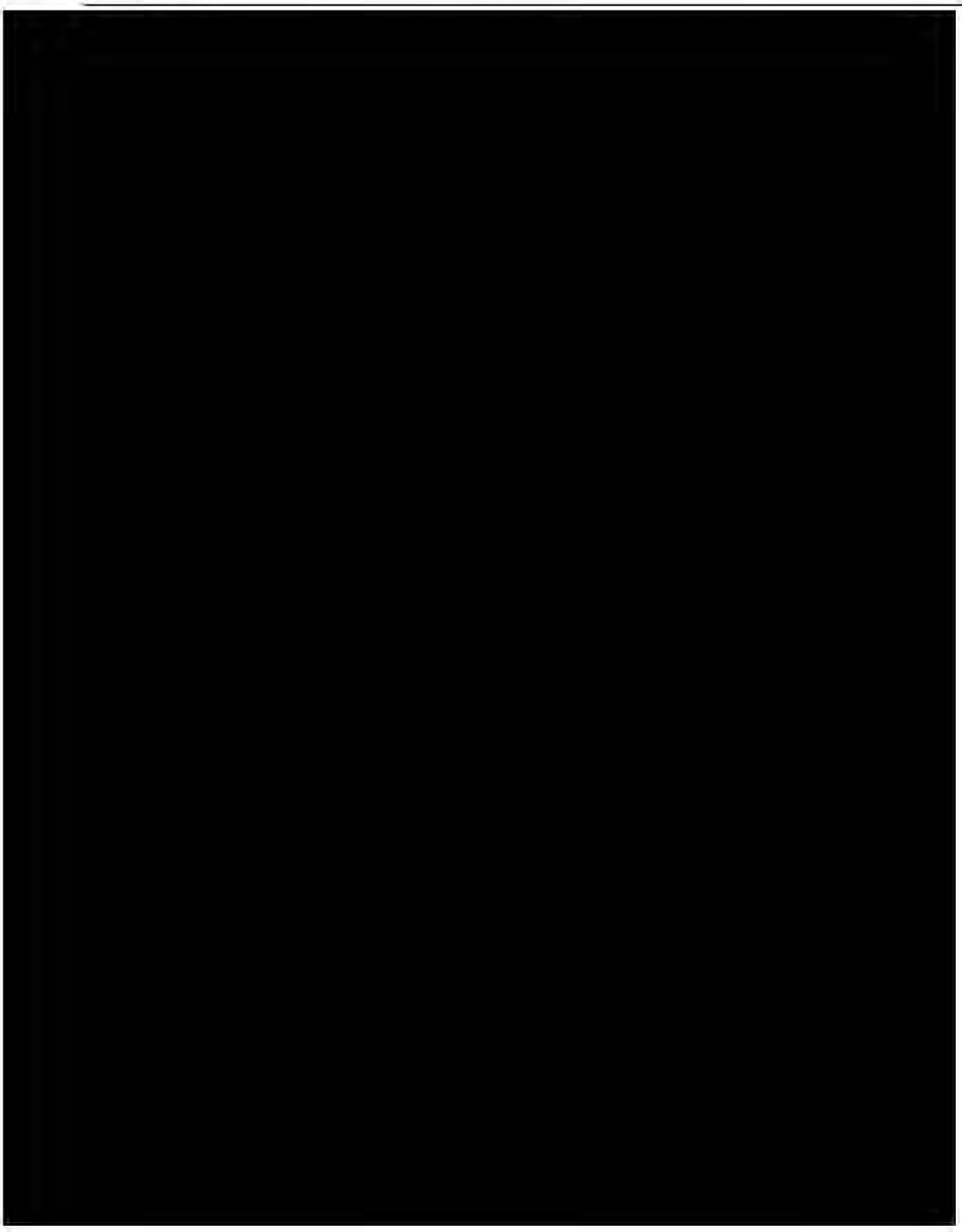
[1]MZ 496 = 3,3'dithiobis-(n-octyl)-3-chloropropenamide and MZ 308 = 2-chloro-3-(formyldithio)-N-octylpropenamide

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			



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**	[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]	

substances

3.3 Sediment and Water Characterization

x

3.4 Test procedures

3.4.1 Test system

x

3.4.2 Preparation of test solution

3.4.3 Initial Test substance

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

	concentration	[redacted]
3.4.4	Duration of test	[redacted]
3.4.5	Sampling details	[redacted]
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.b **Fresh Water/Sediment Degradation study - Anaerobic**
Annex Point IIIA XII 2.1

[redacted]

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.

Sterile system :

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. x

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

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.	

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

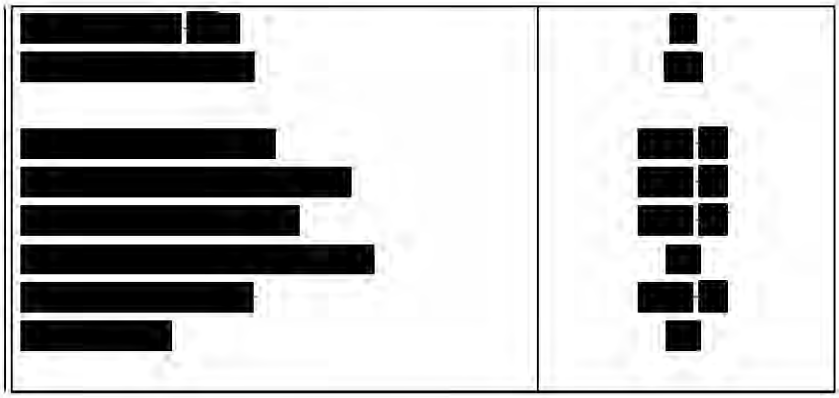
	Physical and chemical characterization of the system such as pH, temperature, Eh and TOC were determined periodically throughout the in-life study. Volatiles traps were periodically replaced and the removed trap quantitted by LSC.	
5.2 Results and Discussion	In the anaerobic fresh water:sediment microcosm 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]



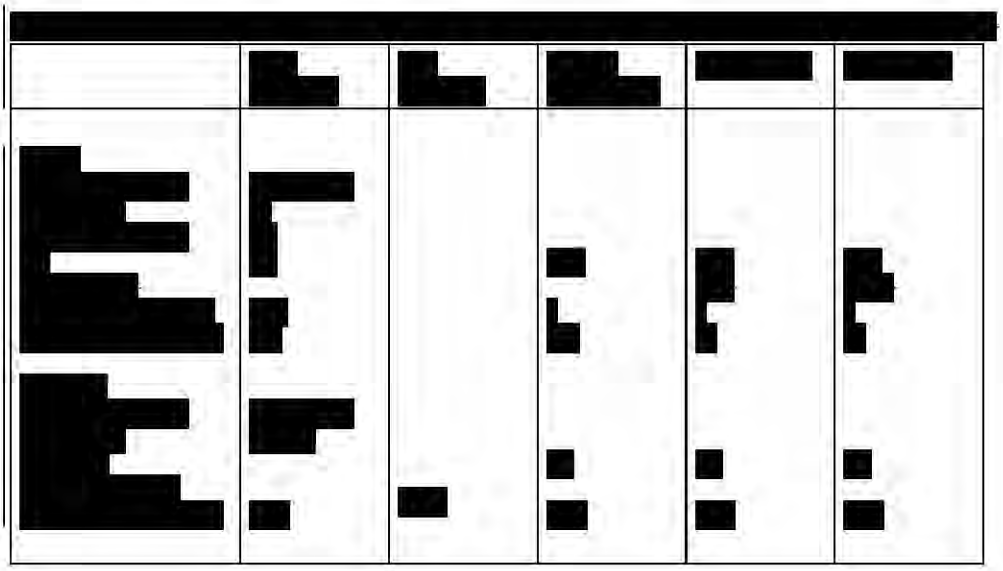


Table A7.1.2.2.2.b-3: Distribution of Radioactivity in Preliminary Study Following Dosing With ^{14}C RH-5287 in Preliminary Studies in % applied radioactivity

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

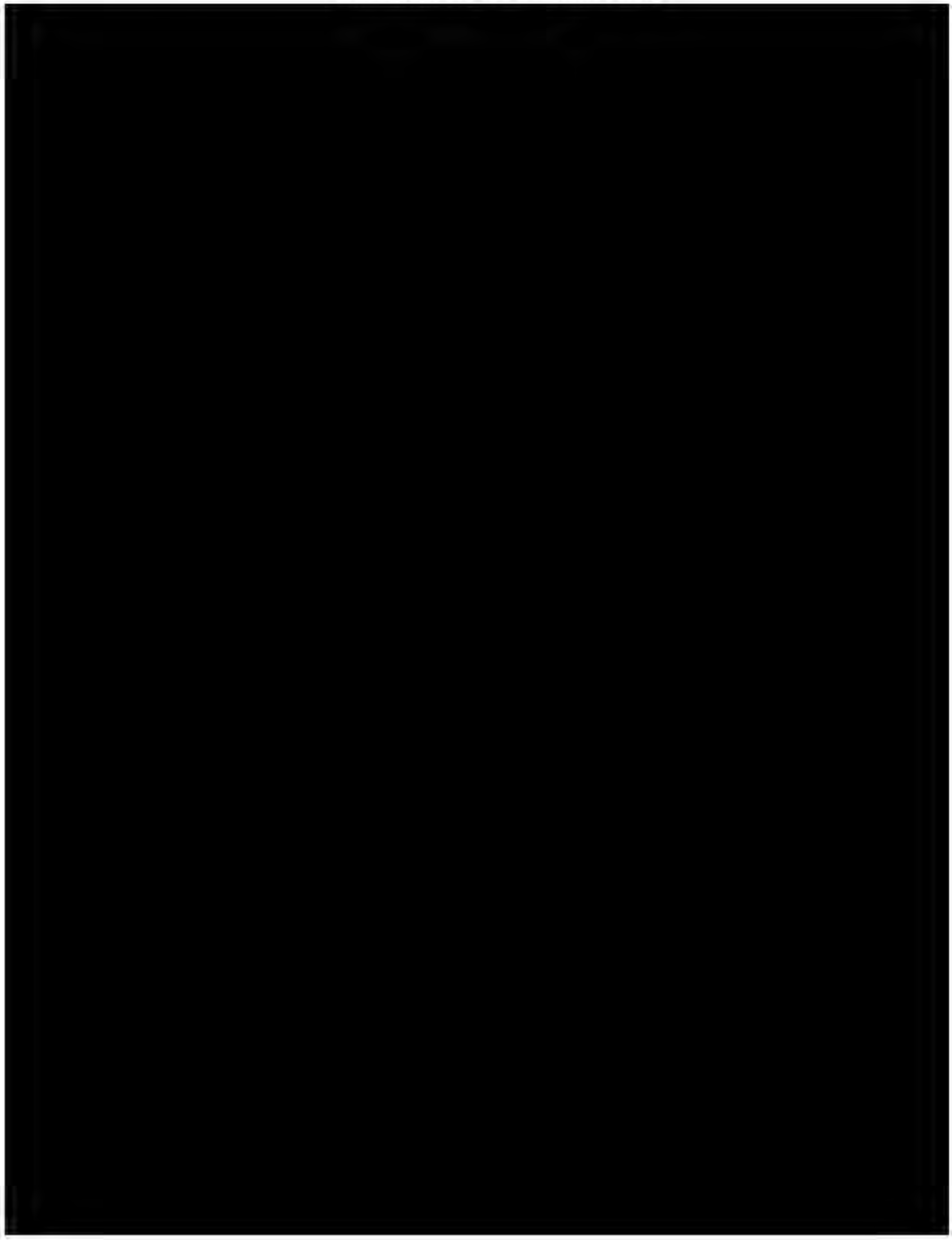
[1] MZ 496 = 3,3'dithiobis-(n-octyl)-3-chloropropenamide and MZ 308 = 2-chloro-3-(formyldithio)-N-octylpropenamide

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			



Document III-A / Section A7.1.2

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	<u>Kinetics and some metabolite identification:</u> [redacted] <u>Supplemental metabolites identification:</u> [redacted] <u>Extractability and Stability of DCOIT in Sediment:</u> [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2		
1.2.3	Criteria for data	[redacted]

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

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.

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]

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

substances

[redacted]

3.3 Sediment and Water Characterization

[redacted]

3.4 Test procedures

3.4.1 Test system

[redacted]

Document III-A / Section A7.1.2

Section A7.1.2.2.2.c **Sea Water/Sediment Degradation study - Aerobic**
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

Document III-A / Section A7.1.2

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]

Document III-A / Section A7.1.2

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

[redacted]

3.4.8 Bound residues- extent and nature

[redacted]

3.4.9 Analytical methods

[redacted]

3.4.10 Degradation products

[redacted]

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

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

Document III-A / Section A7.1.2

	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.

Document III-A / Section A7.1.2

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	102.36	0.23	1.17	103.75
Room Temp.	112.36	0.21	1.86	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	90.87	1.13	0.94	91.94
Room Temp.	126.96	0.24	0.82	128.02
Day 189				
Frozen	110.73	0	0.47	111.20
Room Temp.	106.24	0.46	0.39	107.08
Day 224				
Frozen	106.32	0.26	1.18	107.76
Room Temp.	103.76	0.27	1.15	105.17
Average	105.07 ± 9.80	0.48 ± 0.51	1.64 ± 2.16	107.10 ± 9.25

[1] Average of duplicate samples

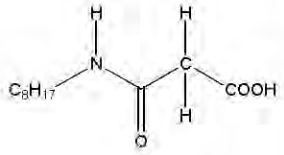
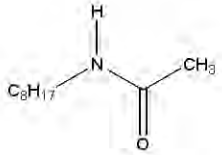
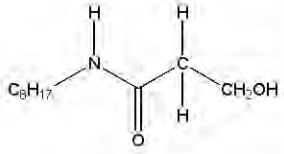
Table A7.1.2.2.2.c-10: Quantitation of DCOIT in Sediment Stored at Room Temperature and Frozen a nd Subsequently Soxhlet Extracted

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]

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: [redacted] Nature of bound residues: [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. X

- 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.

- 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) ([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

[redacted]

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

[redacted]

3.4.9 Analytical methods

[redacted]

3.4.10 Degradation products

[redacted]

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

	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
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

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

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.

Document III-A / Section A7.1.2

	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

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**	[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]

Section A7.1.2.3/01
Annex Point IIA7.6.1.1

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

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

Section A7.1.2.3/01 **Ready Biodegradability of metabolites –**
Annex Point IIA7.6.1.1 **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	-

Document III-A / Section A7.1.2

Section A7.1.2.3/01	Ready Biodegradability of metabolites – N-(n-octyl) Malonamic Acid
	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]

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

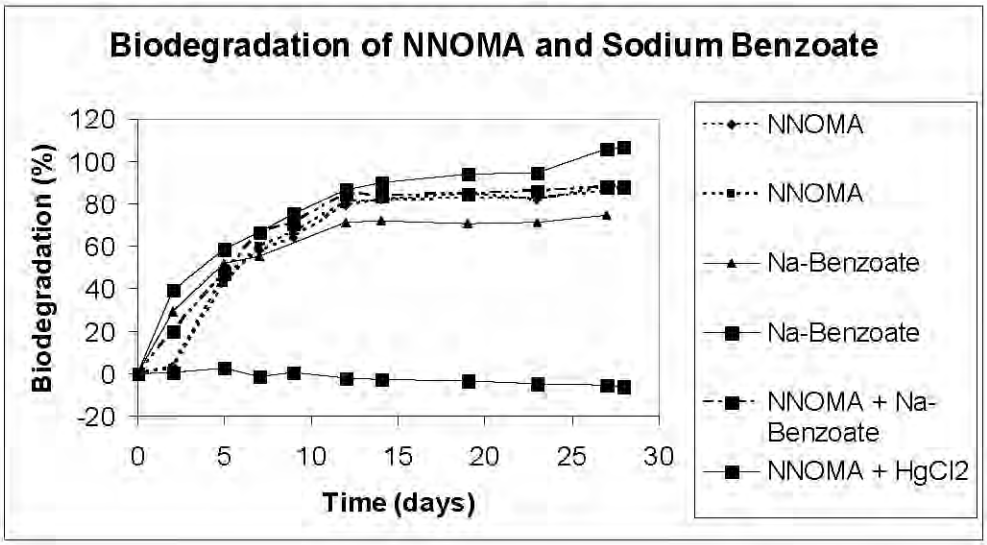
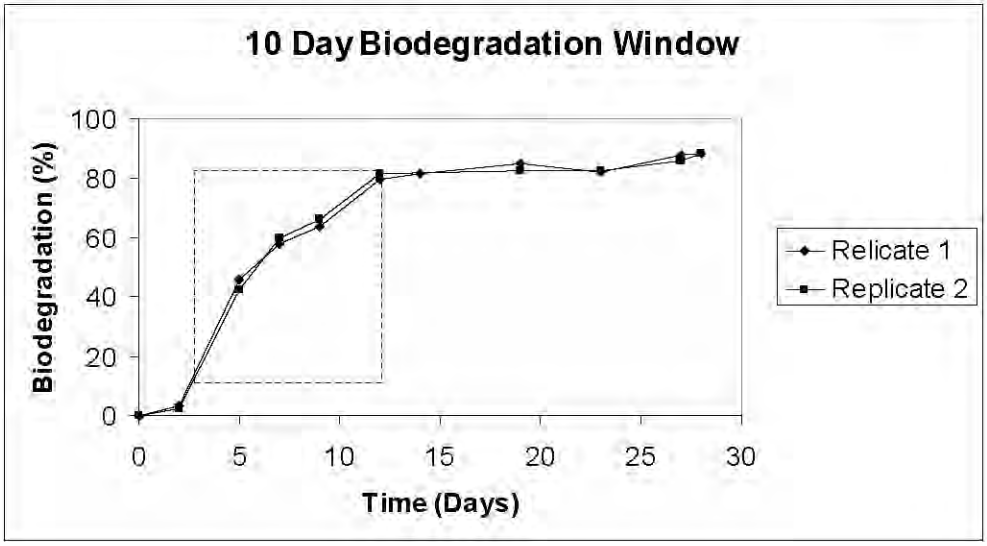


Figure A7.1.2.3/01-2: 10 Day Window for Biodegradation of N-(N-Octyl) Malonamic Acid (NNOMA)



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

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2		
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes. OECD 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	**Testing 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	[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]

[redacted]

4 RESULTS

Document III-A / Section A7.1.2

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]

[redacted]

[redacted]	[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

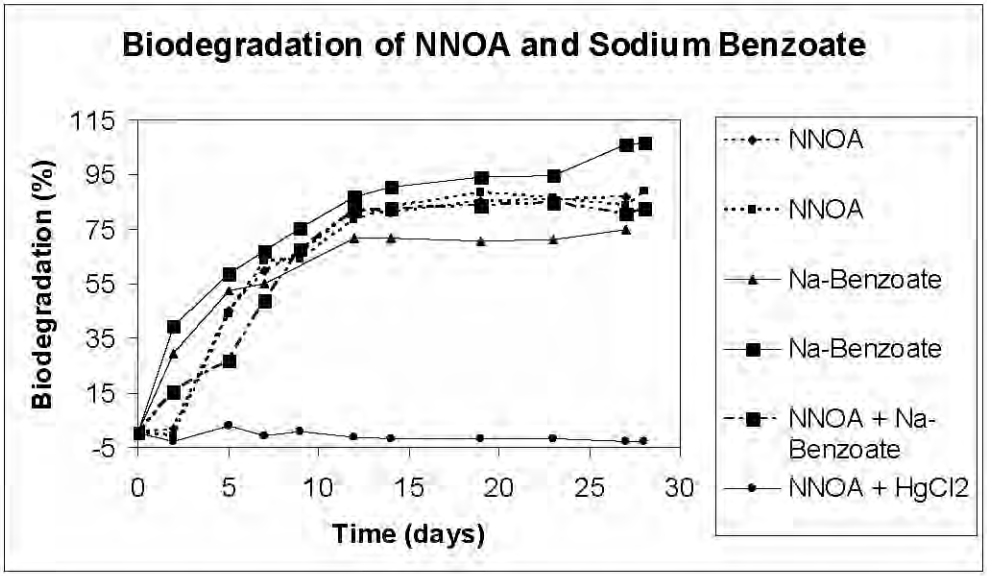
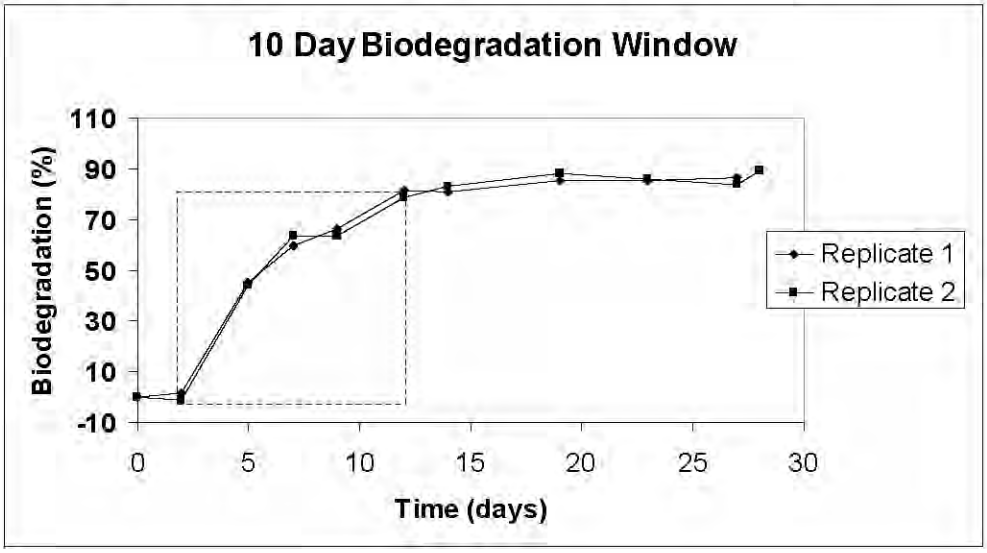


Figure A7.1.2.3/02-2: 10-Day Window for Biodegradation of N-(N-Octyl) Acetamide (NNOA)



Document III-A / Section A7.1.2

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**	[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]
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]

Document III-A / Section A7.1.2

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]

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

Document III-A / Section A7.1.2

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

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.

5.3.1 Reliability

1-valid without restrictions.

Document III-A / Section A7.1.2

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	-

Document III-A / Section A7.1.2

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

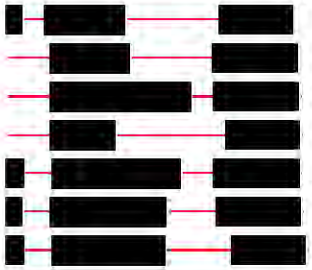
TABLES AND FIGURES

[redacted]

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

[redacted]

[redacted]

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