Section A6.4.3 **Subchronic inhalation toxicity test in rats**

Annex Point IIA6.4.3

[redacted]

3.4.8 Urinalysis

3.5 Sacrifice and pathology

3.5.1 Organ Weights

3.5.2 Gross and histopathology

3.5.3 Other examinations

3.5.4 Statistics

3.6 Further remarks

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

Rales, gasping, dyspnea during the thirteen-weeks of dosing. No clinical signs of respiratory distress were noted during the recovery period.

4.1.2 Mortality

Mortalities were due to over-restraint in the nose-only tubes and not a result of exposure to o-xylene or the C9211M HQ test substance. No treatment-related mortalities were noted during the recovery period.

4.2 Body weight gain

Exposure to o-xylene resulted in statistically significantly decreased body weight gains when compared to the air control. The body weights of the C9211M HQ dosed rats were compared to the body weights of the o-xylene control rats. Group 5 (6.72 mg/m^3) male rats showed a statistically significant decrease in body weights in eight of thirteen weeks and the female rats showed a statistically significant decrease in body weights in eleven of the thirteen weeks of dosing. No body weight

		changes were seen during the recovery period.
4.3	**Food consumption**	Not measured
4.4	**Ophtalmoscopic examination**	Not conducted
4.5	**Blood analysis**	
4.5.1	Haematology	No effects
4.5.2	Clinical chemistry	No effects
4.5.3	Urinalysis	Not conducted
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Increase in absolute lung weights of Group 5 (6.72 mg/m^3) females only at 13 weeks.
4.6.2	Gross and histopathology	Histopathological evaluations at the 13-week necropsy revealed treatment-related observations in the nose, larynx and lungs. These observations were consistent with those of a respiratory tract irritant. There was no significant histopathological change in any other tissues.
4.7	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	[redacted]
5.2	**Results and discussion**	The increase in absolute lung weight was judged to be the result of edema of the lungs and consistent with a respiratory tract irritant. Histopathological evaluations at the 13-week necropsy revealed treatment-related observations in the nose, larynx and lungs. These observations were consistent with those of a respiratory tract irritant and are not a result of absorption or systemic toxicity. The occurrence and severity of these observations correlated with the amount of test material to which the animals were exposed. By the six month necropsy, recovery was seen in all tissues and the lungs no longer showed signs of histopathological lesions. There was no evidence of systemic toxicity up to and including the highest dose tested (6.72 mg/m^3).
5.3	**Conclusion**	
5.3.1	LO(A)EL	0.63 mg DCOIT/m^3, based on the histopathological changes seen in the nose and larynx.
5.3.2	NO(A)EL	0.02 mg DCOIT/m^3
5.3.3	Other	Not applicable
5.3.4	Reliability	[redacted]
5.3.5	Deficiencies	[redacted]

Document III-A / Sections A6.3 to A6.5

Section A6.4.3 Subchronic inhalation toxicity test in rats

Annex Point IIA6.4.3

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	8 November 2006
Materials and Methods	Agree with applicant's verison
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	Remarks to note: In the range-finding study *o*-xylene was found to increase lung weight. Being an irritant, *o*-xylene may contribute to the effect of DCOIT on respiratory irritation. The level of *o*-xylene in the vehicle control group was lower than the level in the highest exposure group so that the possible contribution of *o*-xylene to the respiratory irritation observed in high dose animals is not properly addressed.

Table A6.4.3-1. Results of thirteen-week subchronic inhalation toxicity study in rats

Document III-A / Sections A6.3 to A6.5

Parameter	Air Control		Vehicle Control		low dose (0.02 mg DCOIT/m³)		medium dose (0.63 mg DCOIT/m³)		high dose (6.72 mg DCOIT/m³)	
	m	f	m	f	m	f	m	f	m	f
number of animals examined	32	32	32	32	32	32	32	32	32	32
body weight changes	0/32	0/32	0/32	0/32	0/32	0/32	0/32	0/32	dec.	dec.
Organ: lung										
organ weight*	0/16	0/16	0/16	0/16	0/16	0/16	0/16	0/16	0/16	inc.
gross pathology*	brown foci 2/16	white foci 1/16	red foci 1/16	brown foci 1/16	red foci 3/16	none	red foci, brown foci 4/16	red foci 1/16	red foci, red-brown foci 4/16	red foci 1/16
microscopic pathology* goblet cell hyperplasia inflammation	0/16	0/16	0/16	0/16	0/16	0/16	0/16	0/16	9/16 6/16	2/16 5/16
Organ: nose microscopic pathology *										
inflammation	0/16	0/16	0/16	0/16	3/16	0/16	3/16	3/16	2/16	5/16
epithelial hyperplasia	0/16	0/16	0/16	0/16	3/16	0/16	4/16	9/16	4/16	11/16
goblet cell hyperplasia	0/16	0/16	1/16	1/16	3/16	0/16	1/16	7/16	6/16	14/16
Organ: larynx microscopic pathology *										
inflammation	0/16	0/16	0/16	0/16	0/16	0/16	5/16	9/16	15/16	16/16
hyperplasia	1/16	0/16	2/16	0/16	0/16	0/16	13/16	9/16	14/16	16/16
squamous metaplasia	0/16	0/16	0/16	0/16	0/16	0/16	14/16	16/16	16/16	16/16
hyperkeratosis	0/16	0/16	0/16	0/16	0/16	0/16	0/16	2/16	2/16	14/16

inc: increase / dec: decrease

Section 6.5 Annex IIA6.5	Chronic Toxicity	
	Justification for non-submission of data	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [X] **Other justification** [ ]	
Detailed justification:	**Please note that this summary is the same than the one presented in Section A6.7.** The waiving of the chronic/carcinogenicity study is argued in Report N° 08R-1002 which is included in Document IV-A. **Reference** Reference type: Study report Year: 2008 Report date: 8 January 2008 [redacted] **Data protection claimed.** Data owner : Rohm and Haas Company [redacted] Detailed justification is considered as confidential information.	

Section 6.5 **Chronic Toxicity**
Annex IIA6.5

Section 6.5 **Chronic Toxicity**
Annex IIA6.5

Document III-A / Sections A6.3 to A6.5

Section 6.5 **Annex IIA6.5**	**Chronic Toxicity**
	[redacted]
Undertaking of intended data submission [ ]	No further study planned.
	Evaluation by Competent Authorities
	Evaluation by Rapporteur Member State
Date	6 June 2008
Evaluation of applicant's justification	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 21 : Antifouling products

Document III-A (A6)
Study summaries – Active substance
Toxicological and metabolic studies

Part IV
Section A6.6: Genotoxicity studies
Section A6.7: Carcinogenicity study

TABLE OF CONTENT

Section A6.6.1/01 *In vitro* gene mutation study in bacteria

Annex Point IIA6.6.1 *Salmonella typhimurium*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Reference type: Study report Year: 1994 Report date: 11 April 1994 [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Rohm and Haas Company	
1.2.2			
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, US EPA 40 CFR Part 158.340 guideline 84-2, OECD guideline 471	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	RH-287 Technical (RH-287T)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	[redacted]	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	[redacted]	
3.2	**Study Type**	Bacterial reverse mutation test	

Section A6.6.1/01 — ***In vitro* gene mutation study in bacteria**
Annex Point IIA6.6.1 — *Salmonella typhimurium*

3.2.1	Organism/cell type	<u>*S. typhimurium*</u>: TA 1535, TA 1537, TA 98, TA 100	X
3.2.2	Deficiencies / Proficiencies	Not applicable	
3.2.3	Metabolic activation system	S9 mix was obtained from rats induced with Aroclor 1254, obtained from Molecular Toxicology, Inc. (Moltox). The S-9 mix consisted of: 4 mM NADP, 5 mM glucose-6-phosphate, 8 mM MgCl2, 33 mM KCl, 100 mM sodium phosphate buffer pH 7.4, and 10% liver homogenate (S-9) from Aroclor 1254 induced rats.	
3.2.4	Positive control	With metabolic activation: 2-anthramine (2ANTH) at 2 µg/plate for all 4 strains; Without metabolic activation: 2-nitrofluorene (2NF) at 3 µg/plate for TA98, sodium azide (SA) at 2 µg/plate for TA100 and TA1535 and 9-aminoacridine (9AA) at 100 µg/plate for TA1537.	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	0.3 to 300 µg/plate (concentrations were adjusted for % DCOIT)	X
3.3.2	Way of application	The test article solvent and solvent control was acetone. RH-287T was added to the test system by direct plate incorporation.	
3.3.3	Pre-incubation time	Not applicable	
3.3.4	Other modifications	Not applicable	
3.4	**Examinations**	See tables 6_6_1/01-1 and 6_6_1/01-2	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	without metabolic activation	Negative (not mutagenic)	
4.1.2	with metabolic activation	Negative (not mutagenic)	

Section A6.6.1/01 — ***In vitro* gene mutation study in bacteria**
Annex Point IIA6.6.1 — *Salmonella typhimurium*

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

[redacted]

5.2 Results and discussion

In the definitive assay, 0.3 to 300 µg/plate, the test article did not induce an increase in revertants when compared to solvent controls (Acetone). This was true for all tester strains both with and without metabolic activation. Toxicity was observed in all strains with metabolic activation at 100 µg/plate and greater, and without metabolic activation at 10 µg/plate and greater with the exception of TA1537 which was also toxic at 3 µg/plate.

An independent confirmatory assay was performed using doses ranging from 0.1 to 75 µg/plate. The test article did not induce an increase in revertants when compared to solvent controls. This was true for all tester strains both with and without metabolic activation. Toxicity was observed in all strains with metabolic activation at 75 µg/plate and greater, and without metabolic activation at 7.5 µg/plate and greater with the exception of TA1537 which was also toxic at 3 µg/plate.

5.3 Conclusion

Negative

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	14.November 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, with restrictions
Acceptability	Acceptable
Remarks	**Comments (2.3, 3.2.1, 3.2.4 and 3.3.1)** The study is well performed. However, OECD 471 suggest to use five test strains, TA 98, TA 100, TA 1535, TA 1537 and TA 102 or E.coli. The test criteria for a negative consideration of a substance is to test the substance up to 5000 µg/plate or the limit of solubility or the limit of toxicity. It is not as it is written in the study report A6_6_1_ref-01 at page 10 up to 300 µg/plate or the limit of solubility or the limit of toxicity.

Table A6.6.1/01-1. Table for *Salmonella typhimurium* Gene Mutation Assay – definitive assay

Concentration [µg/plate]	S-9	Mean revertants/plate TA98	Mean revertants/plate TA100	Mean revertants/plate TA1535	Mean revertants/plate TA1537
Acetone 0.0	+	38	117	20	14
RH-287 technical					
300	+	-- F	-- F	-- F	-- F
100	+	-- F	61 F_1	-- F	-- F
30	+	36	123	17	14
10	+	38	138	15	17
3	+	46	141	21	13
2-anthramine 2 µg/plate	+	1370 *	1658 *	334 *	196 *
Acetone 0.0	-	30	118	17	10
RH-287 technical					
30	-	-- F	-- F	-- F	-- F
10	-	-- F	-- F	10 F_1	-- F
3	-	36	77	15	-- F
1	-	36	133	16	15
0.3	-	40	129	16	11
2-nitrofluorene 3 µg/plate	-	611 *			
Sodium azide 2 µg/plate	-		733 *	733 *	
9-aminoacridine 100 µg/plate	-				931 *

Table A6.6.1/01-2. Table for *Salmonella typhimurium* Gene Mutation Assay – confirmatory assay

Concentration [µg/plate]	S-9	Mean revertants/plate TA98	Mean revertants/plate TA100	Mean revertants/plate TA1535	Mean revertants/plate TA1537
Acetone	+	46	125	20	14
RH-287 technical					
75	+	-- F	-- F	16	-- F
30	+	51	140	14	12
7.5	+	45	152	16	14
3	+	45	135	16	15
1	+	51	124	16	11
2-anthramine 2 µg/plate	+	1289 *	1558 *	282 *	165 *
Acetone	-	35	116	23	16
RH-287 technical					
7.5	-	-- F	-- F	-- F	-- F
3	-	30	88	16	-- F
1	-	39	141	18	15
0.3	-	39	132	23	17
0.1	-	32	115	24	15
2-nitrofluorene 3 µg/plate	-	1037 *			
Sodium azide 2 µg/plate	-		907 *	848 *	
9-aminoacridine 100 µg/plate	-				533 *

F = toxicity of strain F_1 = toxicity (1 out of 3) * = positive response, greater than or equal to 2 x solvent
F is excluded from calculations. -- = toxicity

Section A6.6.1/02 Genotoxicity in vitro – NNOMA (metabolite)

Annex Point IIA6.6.1/02 In-vitro gene mutation study in bacteria, *Salmonella typhimurium* and *Escherichia coli*

Official use only

		1 REFERENCE
1.1	**Reference**	Reference type: Study report Year: 2005 Report date: 7 September 2005 [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2		
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, US EPA 40 CFR Part 158, OECD guideline 471, US EPA OPPTS 870.5100
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	N-(n-Octyl) Malonamic Acid
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	The test substance is a metabolite of DCOIT.
3.1.2.1	Description	[redacted]
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	[redacted]
3.2	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	*S. typhimurium*: TA 1535, TA 1537, TA 98, TA 100 *Escherichia coli:* WP2 *uvr*A

Section A6.6.1/02		**Genotoxicity in vitro – NNOMA (metabolite)**
Annex Point IIA6.6.1/02		In-vitro gene mutation study in bacteria, *Salmonella typhimurium* and *Escherichia coli*
3.2.2	Deficiencies / Proficiencies	Not applicable
3.2.3	Metabolic activation system	S9 mix was obtained from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. The S9 mix consisted of: 1-% S9, 5 mM glucose-6-phosphate, 4mM ß-nicotinamide-adenine dinucleotide phosphate, 8 mM $MgCl_2$, 33 mM KCl in a 100 mM phosphate buffer at pH 7.4 and was prepared immediately before its use. The S9 was checked for sterility and its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.
3.2.4	Positive control	With metabolic activation: 2-anthramine at 1.0 µg/plate for all *Salmonella* 4 strains and at 10 µg/plate for E. coli; Without metabolic activation: 2-nitrofluorene at 1.0 µg/plate for TA98, sodium azide (SA) at 1.0 µg/plate for TA100 and TA1535 and 9-aminoacridine (9AA) at 75 µg/plate for TA1537 and methyl methanesulfonate at 1000 µg/plate for WP2 *uvr*A.
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate for initial assay 50, 150, 500, 1500 and 5000 µg per plate for confirmatory assay
3.3.2	Way of application	The test article solvent and solvent control was dimethylsulfoxide (DMSO). The test substance was added to the test system by direct plate incorporation.
3.3.3	Pre-incubation time	Not applicable
3.3.4	Other modifications	Not applicable
3.4	**Examinations**	see tables 6_6_1/05-1 and 6_6_1/05-2
3.4.1	Number of cells evaluated	Not applicable
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	Negative (not mutagenic)
4.1.2	with metabolic activation	Negative (not mutagenic)
4.2	**Cytotoxicity**	No

Document III-A / Sections A6.6 to A6.7

Section A6.6.1/02	**Genotoxicity in vitro – NNOMA (metabolite)**
Annex Point IIA6.6.1/02	In-vitro gene mutation study in bacteria, *Salmonella typhimurium* and *Escherichia coli*

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	US EPA 40 CFR Part 158, OECD guideline 471, US EPA OPPTS 870.5100, bacterial reverse mutation assay (Ames).
5.2	**Results and discussion**	Neither precipitate, appreciable toxicity nor positive mutagenic response were observed in either the initial or confirmatory assays.. Under the conditions of this study, N-(n-Octyl) Malonamic Acid did not induce a mutagenic effect in the Ames assay in either the presence or absence of Aroclor-induced rat liver S9.
5.3	**Conclusion**	Negative
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities

Evaluation by Rapporteur Member State

Date	14 November 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 without restrictions
Acceptability	Acceptable
Remarks	Positive control: 2-anthramine = 2- aminoanthracene (CAS. No: 613-13-8)

Table A6.6.1/02-1. Table for Gene Mutation Assay – Initial Assay

Concentration [µg/plate]	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9
Vehicle control	34	16	188	146	18	18	9	8	25	19
1.5	26	21	159	139	12	21	11	11	29	19
5.0	32	17	181	123	17	17	7	9	23	25
15	29	18	177	135	17	19	9	6	29	21
50	29	18	192	127	14	15	11	7	28	23
150	33	23	181	135	16	16	10	4	27	17
500	30	14	143	126	13	22	11	6	21	20
1500	27	19	194	129	18	23	9	11	26	19
5000	24	12	188	122	19	20	11	10	30	20
2-nitrofluorene (1.0 µg/plate)	--	203	--	--	--	--	--	--	--	--
2-aminoanthracene (1.0 µg/plate)	186	--	602	--	82	--	50	--	419	--
sodium azide (1.0 µg/plate)	--	--	--	705	--	260	--	--	--	--
9-aminoacridine (75 µg/plate)	--	--	--	--	--	--	--	99	--	--
Methyl methanesulfonate (1000 µg/plate)	--	--	--	--	--	--	--	--	--	389

-- no data--

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Table A6.6.1/02-2. Table for Gene Mutation Assay – Confirmatory Assay

Concentration [µg/plate]	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S 9	+ S9	- S9
Vehicle control	23	18	130	125	14	19	8	8	21	20
50	23	17	126	132	17	20	9	6	21	15
150	22	15	138	135	13	15	8	6	21	16
500	20	18	141	131	15	21	7	6	22	17
1500	23	15	125	108	14	17	8	7	23	21
5000	25	15	117	118	14	14	7	8	16	16
2-nitrofluorene (1.0 µg/plate)	--	232	--	--	--	--	--	--	--	--
2-aminoanthracene (1.0 µg/plate)	417	--	596	--	86	--	97	--	500	--
sodium azide (1.0 µg/plate)	--	--	--	623	--	419	--	--	--	--
9-aminoacridine (75 µg/plate)	--	--	--	--	--	--	--	607	--	--
Methyl methanesulfonate (1000 µg/plate)	--	--	--	--	--	--	--	--	--	182

-- no data--

Section A.6.6.2 In vitro cytogenicity study in mammalian cells

Section A6.6.2/01 *In vitro* cytogenicity study in mammalian cells

Annex Point IIA6.6.2

Official use only

1 REFERENCE

1.1 Reference

Reference type: Study report
Year: 1994
Report date: 4 November 1994
[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 473 and US EPA 40 CFR Part 158.135, guideline 84-2

2.2 GLP Yes

2.3 Deviations No

3 MATERIALS AND METHODS

3.1 Test material RH-287 technical (RH-287T)

3.1.1 Lot/Batch number [redacted]

3.1.2 Specification As given in section 2

Section A6.6.2/01 *In vitro* cytogenicity study in mammalian cells

Annex Point IIA6.6.2

3.1.2.1	Description	[redacted]
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	[redacted]
3.2	**Study Type**	*In vitro* mammalian chromosome aberration test
3.2.1	Organism/cell type	mammalian cell lines: Chinese Hamster Ovary (CHO)
3.2.2	Deficiencies / Proficiencies	Not applicable
3.2.3	Metabolic activation system	S-9 liver fraction from Aroclor 1254 induced rats and a cofactor pool.
3.2.4	Positive control	In the presence of metabolic activation, Cyclophosphamide (CP) was used as the positive control at 10 µg/ml. In the absence of metabolic activation, the positive control was Mitomycin-C (MMC) at 0.12 µg/ml.
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	0.1 - 0.7 µg/ml without activation and 3.0 -8.0 µg/ml with metabolic activation. Based on the toxicity expressed by the Mitotic Index, chromosome aberrations were scored from cells treated with 0.3, 0.6 and 0.7 µg/ml in the non-activated system and 6.0, 7.0 and 8.0 µg/ml in the activated system.
3.3.2	Way of application	Acetone diluent, test article was added to test medium
3.3.3	Pre-incubation time	Not applicable
3.3.4	Other modifications	Not applicable
3.4	**Examinations**	See tables A6.6.2/01-1 and A6.6.2/01-2
3.4.1	Number of cells evaluated	100 metaphases from each of 2 duplicate flasks resulting in 200 metaphases scored. In addition, the number of polyploid and endoreduplicated cells in a total of 100 dividing cells were scored.

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	Negative (Not mutagenic)
4.1.2	with metabolic activation	Negative (Not mutagenic)
4.2	**Cytotoxicity**	No

Document III-A / Sections A6.6 to A6.7

Section A6.6.2/01 ***In vitro* cytogenicity study in mammalian cells**

Annex Point IIA6.6.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

[redacted]

5.2 Results and discussion

Under the conditions of this study and according to the criteria set for evaluating the test results, RH-287 Technical was negative in the *in vitro* Chromosome Aberration Assay in CHO cells when tested with and without exogenous metabolic activation system.

5.3 Conclusion

5.3.1	Reliability	(1) valid without restrictions
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	14th November 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 without restrictions
Acceptability	Acceptable
Remarks	

Table A6.6.2/01-1. *In-Vitro* Chromosomal Analysis, Definitive

Without activation (21 hr treatment, 23 hr harvest)		**Untreated**	**Solvent**	**0.3 µg/ml**	**0.6 µg/ml**	**0.7 µg/ml**	**MMC, 0.12 µg/ml**
chromatid aberrations	deletions	0	0	0	0	0	2
	exchanges	0	0	0	0	0	22
	breaks	0	0	0	0	0	41
	intrachanges	0	0	0	1	0	50
chromosome aberrations	exchange	0	1	0	0	1	2
	breaks	0	0	1	1	0	12
	fragment	0	0	0	0	0	0
	intrachanges	0	0	0	0	0	1
Number of aberrations per cell		0.0	0.005	0.010	0.010	0.010	0.700
% cells with aberrations		0.0	0.5	1.0	0.5	1.0	46.0 *
Mean mitotic index		8.0	8.3	6.9	5.5	4.1	4.2
With activation (2 hr treatment, 20 hr harvest)		**Untreated**	**Solvent**	**6.0 µg/ml**	**7.0 µg/ml**	**8.0 µg/ml**	**CP, 10 µg/ml**
chromatid aberrations	exchange	0	0	0	0	0	51
	breaks	0	0	0	0	0	48
	intrachanges	0	0	0	0	1	133
	deletions						5
chromosome aberrations	intrachanges	0	0	1	1	0	9
	exchange	1	0	1	1	1	3
	breaks	2	0	0	1	1	39
	fragment	0	0	0	0	0	0
	damaged	0	0	0	0	0	3
Number of aberrations per cell		0.015	0.000	0.015	0.015	0.040	1.590
% cells with aberrations		1.0	0.0	1.5	1.5	2.5	74.5 *
Mean mitotic index		7.1	7.1	5.8	5.1	3.7	2.8

CP = Cyclophosphamide MMC = Mitomycin-C
*The positive controls showed statistically significant increases in the number and percentage of cells with aberrations.

Table A6.6.2/01-2. *In-Vitro* Chromosomal Analysis, Confirmatory

Without activation (21 hr treatment, 23 hr harvest)		**Untreated**	**Solvent**	**0.5 µg/ml**	**0.6 µg/ml**	**0.7 µg/ml**	**MMC, 0.12 µg/ml**
chromatid aberrations	deletions	0	0	0	0	0	0
	exchanges	0	0	0	0	0	28
	breaks	0	0	0	1	0	32
	intrachanges	0	0	1	0	0	55
chromosome aberrations	exchange	0	0	1	0	0	1
	breaks	0	1	0	0	0	10
	fragment	0	0	0	0	0	0
	intrachanges	0	0	1	2	0	5
	damaged	0	0	0	0	0	1
Number of aberrations per cell		0.000	0.005	0.015	0.015	0.005	0.705
% cells with aberrations		0.0	0.5	1.5	1.0	0.5	46.5 *
Mean mitotic index		6.4	6.4	5.7	4.1	3.4	3.1
Without activation (21 hr treatment, 47 hr harvest)		**Untreated**	**Solvent**	**0.5 µg/ml**	**0.6 µg/ml**	**0.7 µg/ml**	**MMC, 0.12 µg/ml**
chromatid aberrations	exchange	0	0	1	1	2	13
	breaks	1	0	0	1	1	19
	intrachanges	0	0	0	1	3	15
	deletions	0	0	0	0	0	0
chromosome aberrations	intrachanges	0	0	1	0	1	12
	exchange	0	1	0	1	1	22
	breaks	0	0	1	2	0	112
	fragment	0	0	0	0	0	0
	damaged	0	0	0	1	0	5
Number of aberrations per cell		0.005	0.005	0.015	0.080	0.040	1.215
% cells with aberrations		0.5	0.5	1.5	3.0	3.0	47.0 *
Mean mitotic index		7.8	8.2	6.4	5.8	5.7	5.3

With activation (2 hr treatment, 20 hr harvest)		Untreated	Solvent	6.0 µg/ml	7.0 µg/ml	8.0 µg/ml	CP, 10 µg/ml
chromatid aberrations	deletions	0	0	0	0	0	7
	exchanges	0	0	0	0	0	59
	breaks	0	0	0	1	0	52
	intrachanges	0	1	0	0	2	91
chromosome aberrations	exchange	0	0	0	1	1	2
	breaks	0	0	0	0	3	38
	fragment	0	0	0	0	0	0
	intrachanges	0	1	0	0	0	2
	damaged	0	0	0	0	0	3
Number of aberrations per cell		0.000	0.010	0.000	0.010	0.030	1.410
% cells with aberrations		0.0	1.0	0.0	1.0	2.5	63.0 *
Mean mitotic index		10.8	10.9	8.9	7.2	5.1	3.7
With activation (2 hr treatment, 44 hr harvest)		**Untreated**	**Solvent**	**6.0 ug/ml**	**7.0 ug/ml**	**8.0 ug/ml**	**CP, 10 ug/ml**
chromatid aberrations	exchange	0	0	1	0	1	3
	breaks	0	1	1	0	2	12
	intrachanges	0	0	0	0	3	4
	deletions	0	0	0	0	0	0
chromosome aberrations	intrachanges	0	0	0	1	0	19
	exchange	0	1	1	1	1	22
	breaks	0	1	0	2	2	67
	fragment	0	0	0	0	0	0
	damaged	0	0	0	0	0	2
Number of aberrations per cell		0.000	0.015	0.015	0.020	0.045	0.735
% cells with aberrations		0.0	1.5	1.5	1.5	3.0	32.5 *
Mean mitotic index		8.4	8.4	7.4	6.7	6.1	5.5

CP = Cyclophosphamide MMC = Mitomycin-C *
The positive controls showed statistically significant increases in the number and percentage of cells with aberrations.

Section A6.6.3/01 *In vitro* gene mutation assay in mammalian cells

Annex Point IIA6.6.3

Official use only

1 REFERENCE

1.1 Reference		Reference type: Study report Year: 1994 Report date: 4 November 1994 [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 476, US EPA 84-2
2.2 GLP	Yes
2.3 Deviations	No

3 MATERIALS AND METHODS

3.1 Test material		RH-287 Technical (RH-287T)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.2.1	Description	[redacted]
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	[redacted]
3.2 Study Type		*In vitro* mammalian cell gene mutation test

Section A6.6.3/01 *In vitro* gene mutation assay in mammalian cells

Annex Point IIA6.6.3

3.2.1	Organism/cell type	mammalian cell line: Chinese hamster Ovary (CHO), CHO-K1-BH4
3.2.2	Deficiencies / Proficiencies	mutants are deficient in HGPRT and resistant to 6TG.
3.2.3	Metabolic activation system	Aroclor induced rat liver homogenate, S9 mix , from male Sprague-Dawley rats
3.2.4	Positive control	Ethyl methanesulfonate, EMS (without activation) 0.5 µl/ml and 7,12-dimethylbenz(a)anthracene, DMBA (with activation) 5 µg/ml.
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Range-finding: 0.0025 to 5000 µg/ml. Definitive: 0.005, 0.025, 0.05, 0.1 and 0.5 µg/ml without activation and 0.5, 1.0, 2.5, 5.0, 10 and 25 µg/ml with activation. Confirmatory: 0.025, 0.05, 0.1, 0.2, 0.4, 0.5 and 0.75 µg/ml without activation and 2.5, 5.0, 6.0, 8.0, 9.0, 10 and 15 µg/ml with activation. All concentrations were prepared in acetone and corrected for DCOIT content.
3.3.2	Way of application	[redacted]
3.3.3	Pre-incubation time	none
3.3.4	Other modifications	none
3.4	**Examinations**	
3.4.1	Number of cells evaluated	The number of mutants were counted per 10^6 surviving cells

4 RESULTS AND DISCUSSION.

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	Negative (Not mutagenic)
4.1.2	with metabolic activation	Negative (Not mutagenic)
4.2	**Cytotoxicity**	Yes, the highest concentration of 0.75 µg/ml without activation and 15 µg/ml with activation.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

[redacted]

[redacted]

5.2 Results and discussion	All criteria for a valid assay were met. The dosing solutions were analysed and showed that the majority of the dosing solutions were within an acceptable range of the target levels (98-114%).
5.3 Conclusion	RH-287T did not cause a significant increase in the mutant frequency at the HGPRT locus in the presence or absence of metabolic activation.
5.3.1 Reliability	[redacted]
5.3.2 Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	15 November 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 without restrictions
Acceptability	Acceptable
Remarks	

Table A6.6.3/01-1. Table for CHO/HGPRT Gene Mutation Assay

Concentration [µg/ml]	Definitive Assay Number of mutant cells/10^6 surviving cells		Confirmatory Assay Number of mutant cells/10^6 surviving cells	
	-S9	+ S9	-S9	+ S9
acetone 0.0	5, 13		7, 7	
0.005	0, 5		----	
0.025	0, 22		7, 0	
0.05	7, 4		1, 12	
0.1	4, 5		7, 3	
0.2	--		7, 0	
0.4	--		--, 0	
0.5	9, 0		0, 0	
EMS (0.5 µl/ml)	351, 324 *		624, 488 *	
acetone 0.0		--, 3		7, 9
0.5		2, 12		----
1.0		0, 3		----
2.5		6, 10		0, 2
5.0		14, 4		2, 23
6.0		----		0, 0
8.0		----		3, 0
9.0		----		0, 15
10.0		6, 0		1, 22
DMBA (5.0 µg/ml)		452, 446 *		459, 390 *

-- = culture lost due to contamination
---- = dose level not part of assay
EMS = Ethyl methanesulfatonate
DMBA = 7,12-dimethylbenz(a)anthracene
* The positive controls, EMS and DMBA, caused significant increases in the number of mutants per 1 x 10^6 surviving cells.

Section A6.6.4/01 Genotoxicity *in vivo* micronucleus assay

Annex Point IIA6.6.4

Official use only

1 REFERENCE

1.1 Reference		Reference type: Study report Year: 2001 Report date: 29 May 2001 [redacted]
1.2 Data protection		Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2		
2.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study	Yes, OECD 474 and US EPA 870.5395
2.2 GLP	Yes
2.3 Deviations	No

3 MATERIALS AND METHODS

3.1 Test material		RH-287 Technical (RH-287T)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.2.1	Description	tan solid
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	[redacted]
3.1.2.4	Maximum tolerable dose	[redacted]
3.2 Test Animals		

3.2.1	Species	mice
3.2.2	Strain	adult CD-1
3.2.3	Source	Charles River Laboratories, Portage, Michigan, USA
3.2.4	Sex	male and female
3.2.5	Age/weight at study initiation	8 weeks, 21-31 g
3.2.6	Number of animals per group	5/sex/group per time point except high dose which had 9/sex/group per time point (24 and 48 hr time points)
3.2.7	Control animals	5/sex/group per time point
3.3	**Administration/ Exposure**	oral
3.3.1	Number of applications	1
3.3.2	Interval between applications	not applicable
3.3.3	Postexposure period	24 and 48 h after treatment
3.3.4	Vehicle	corn oil
3.3.5	Total volume applied	10 ml/kg
3.3.6	Dose applied	60, 300, 600 mg DCOIT/kg bw
3.3.7	Substance used as Positive Control	mitomycin-C at 2 mg/kg dosed by intraperitoneal injection
3.3.8	Controls	negative, solvent control was corn oil

3.4 Examinations

3.4.1 Clinical signs [redacted]

3.4.2 Tissue [redacted]

3.5 Further remarks none

4 RESULTS AND DISCUSSION

4.1 Clinical signs Diarrhea, anogenital staining, soft or scant faeces, passiveness and ataxia.

4.2 Haematology / Tissue examination Not applicable

4.3 Genotoxicity Negative (not mutagenic)

4.4 Other None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

[redacted]

5.2 Results and discussion

The test article did not induce an increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow cells of male or female mice when compared to the vehicle controls. A statistically significant decrease in the polychromatic/normochromatic ratio was observed in male and female mice treated with 600 mg DCOIT/kg bw at 48 hours which is indicative of cytotoxicity. An increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow cells of mice treated with mitomycin-C at 2.0 mg/kg indicated that the assay was sufficiently sensitive to detect induced cytogenetic damage.

5.3 Conclusion

Negative

5.3.1 Reliability

[redacted]

5.3.2 Deficiencies

[redacted]

Table A6.6.4/01-1. Table for Micronucleus Test *In Vivo* – males

State mean ± standard deviation state individual numbers for critical findings		control (corn oil)		control (2 mg/kg MMC)	low dose (60 mg DCOIT/kg)		mid dose (300 mg DCOIT/kg)		high dose (600 mg DCOIT/kg)	
Number of cells evaluated		2000+	2000+	2000+	2000+		2000+		2000+	
Sampling time (h)		24	48	24	24	48	24	48	24	48
Number of erythrocytes	**normochromatic (NCE)**	495 ± 86	520 ± 44	595 ± 28	483 ± 46	422 ± 58	438 ± 90	505 ± 96	497 ± 68	689 ± 86
	polychromatic 1 (PCE1)	571 ± 103	587 ± 88	519 ± 58	585 ± 49	647 ± 72	649 ± 97	538 ± 75	579 ± 53	390 ± 74
	polychromatic with micronuclei (MNP)	2 ± 2	1 ± 1	110 ± 25	2 ± 2	3 ± 2	3 ± 2	2 ± 1	2 ± 2	1 ± 1
	polychromatic 2 (PCE2)	1516 ± 119	1498 ± 61	1543 ± 50	1502 ± 46	1435 ± 86	1437 ± 86	1507 ± 73	1520 ± 60	1679 ± 93
	polychromatic, total (PCE total = PCE1 + PCE2)	2086 ± 33	2085 ± 36	2062 ± 31	2087 ± 37	2082 ± 15	2086 ± 23	2045 ± 27	2099 ± 31	2069 ± 55
Ratio of erythrocytes	**polychromatic / normochromatic (PNR ratio= PCE1/NCE))**	1.22 ± 0.45	1.14 ± 0.27	0.88 ± 0.12	1.23 ± 0.21	1.57 ± 0.36	1.58 ± 0.57	1.12 ± 0.37	1.20 ± 0.27	0.58 ± 0.18 *
	polychromatic with micro-nuclei / polychromatic, total (MNC %= MNP/(PCE1+PCE2) x 100)	0.11 ± 0.08	0.07 ± 0.06	5.32 ± 1.23 #	0.1 ± 0.11	0.14 0.09	0.16 ± 0.07	0.1 ± 0.04	0.12 ± 0.09	0.05 ± 0.05

* Indicates a statistically significant difference from control ($p<0.05$). Statistical Methods: Analysis of Variance followed by Dunnett's T-Test on Least Square Means.
\# Indicates a greater than 2 fold increase over corn oil control values.
MMC = mitomycin C
PCE1 : Polychromatic Erythrocytes used in combination with Normochromatic Erythrocytes to total at least 1000 cells and used to calculate the PCE/NCE ratio.
PCE2 : The remaining number of Polychromatic Erythrocytes recorded and added to PCE1 to total at least 2000 Polychromatic Erythrocytes.

Table A6.6.4/01-2. Table for Micronucleus Test *In Vivo* – females

State mean ± standard deviation state individual numbers for critical findings		control (corn oil)		control (2 mg/kg MMC)	low dose (60 mg DCOIT/kg)		mid dose (300 mg DCOIT/kg)		high dose (600 mg DCOIT/kg)	
Number of cells evaluated		2000+	2000+	2000+	2000+		2000+		2000+	
Sampling time (h)		24	48	24	24	48	24	48	24	48
Number of erythrocytes	**normochromatic (NCE)**	461 ± 109	499 ± 108	559 ± 108	448 ± 80	460 ± 39	448 ± 79	428 ± 27	527 ± 117	677 ± 154
	polychromatic 1 (PCE1)	609 ± 131	593 ± 101	609 ± 81	635 ± 73	614 ± 68	626 ± 72	625 ± 25	572 ± 98	443 ± 158
	polychromatic with micronuclei (MNP)	2 ± 1	3 ± 2	101 ± 16	2 ± 1	2 ± 1	2 ± 2	2 ± 1	2 ± 2	2 ± 1
	polychromatic 2 (PCE2)	1489 ± 144	1498 ± 73	1486 ± 75	1454 ± 85	1465 ± 50	1455 ± 80	1483 ± 70	1549 ± 94	1663 ± 176
	polychromatic, total (PCE total = PCE1 + PCE2)	2098 ± 83	2091 ± 46	2095 ± 35	2089 ± 16	2079 ± 52	2082 ± 20	2108 ± 66	2120 ± 99	2106 ± 43
Ratio of erythrocytes	**polychromatic / normochromatic (PNR ratio= PCE1/NCE))**	1.43 ± 0.57	1.27 ± 0.49	1.13 ± 0.32	1.48 ± 0.49	1.35 ± 0.26	1.46 ± 0.47	1.46 ± 0.11	1.18 ± 0.52	0.73 ± 0.43 *
	polychromatic with micro-nuclei / polychromatic, total (MNC %= MNP/(PCE1+PCE2) x 100)	0.09 ± 0.06	0.15 ± 0.08	4.81 ± 0.71 #	0.12 ± 0.04	0.11 ± 0.04	0.10 ± 0.07	0.11 ± 0.04	0.11 ± 0.09	0.11 ± 0.05

* Indicates a statistically significant difference from control ($p<0.05$). Statistical Methods: Analysis of Variance followed by Dunnett's T-Test on Least Square Means.
# Indicates a greater than 2 fold increase over corn oil control values.
MMC = mitomycin C

PCE1 : Polychromatic Erythrocytes used in combination with Normochromatic Erythrocytes to total at least 1000 cells and used to calculate the PCE/NCE ratio.

PCE2 : The remaining number of Polychromatic Erythrocytes recorded and added to PCE1 to total at least 2000 Polychromatic Erythrocytes.

Evaluation by Competent Authorities	
Evaluation by Rapporteur Member State	
Date	15 November 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 without restrictions
Acceptability	Acceptable
Remarks	

Section A6.6.5 **Annex Point IIA6.6.5**	**Genotoxicity *in vivo* second study**	
Section A6.6.6 **Annex Point IIA6.6.6**	**Germ cell effect**	
	Justification for non-submission of data	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	As outlined in the "Technical guidance document on data requirements in support of the directive 98/8/EC concerning the placing of biocidal products on the market": - For **section A6.6.5** : a second *in vivo* study has to be undertaken to examine whether mutagenicity or evidence of DNA damage can be demonstrated in tissue other than bone marrow only if negative results are obtained in Section A6.6.4 but positive results in some of the *in vitro* tests (Sections A6.6.1, A6.6.2, A6.6.3). - For **section A6.6.6** : a test to assess possible germ cell effects is required if positive result is obtained in section A6.6.4. Based on the negative results of the studies conducted in sections A6.6.1, A6.6.2, A6.6.3 (*in vitro* tests) and section A6.6.4 (*in vivo* bone-marrow cytogenetic test) it is not required to conduct studies for the sections A6.6.5 and A6.6.6.	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	15 November 2006	
Evaluation of applicant's justification	Agree with applicant's version.	

Conclusion	Agree with applicant's version.
Remarks	

Section A6.6.7 **Annex Point IIA6.6.7**	**Genotoxicity studies, further studies**	
	Justification for non-submission of data	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	[redacted] [redacted] [redacted]	X
	[redacted] [redacted] [redacted]DETAILED JUSTIFICATION IS CONSIDERED AS CONFIDENTIAL INFORMATION	X
Undertaking of intended data submission [ ]	No further studies planned.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	15 November 2006	

Section A6.6.7 Annex Point IIA6.6.7	Genotoxicity studies, further studies
Evaluation of applicant's justification	Agree with applicant's version. **Comment:** In addition to the above mentioned in vitro and in vivo genotoxicity tests on DCOIT a Bacteria Gene Mutation assay (Ames test) was performed on the major metabolite N-(n-octyl) malonamic acid (NNOMA). The study was negative.
Conclusion	Agree with applicant's version.
Remarks	

A6.7 Carcinogenicity study

Section 6.7 Annex Point IIA6.7	Carcinogenicity study	
	Justification for non-submission of data	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	**Please note that this summary is the same than the one presented in Section A6.5.** The waiving of the chronic/carcinogenicity study is argued in Report N° 08R-1002 which is included in Document IV-A. **Reference** Reference type: Justification Year: 2008 Report date: 8 January 2008 [redacted]	

Section 6.7 Annex Point IIA6.7	Carcinogenicity study

[redacted]

Data protection claimed.

Data owner : Rohm and Haas Company

[redacted]

DETAILED JUSTIFICATION IS CONSIDERED AS CONFIDENTIAL INFORMATION

[redacted]

Section 6.7 **Carcinogenicity study**
Annex Point IIA6.7

Section 6.7 **Annex Point IIA6.7**	**Carcinogenicity study**
	[redacted]
Undertaking of intended data submission [ ]	No study planned.
	Evaluation by Competent Authorities
	Evaluation by Rapporteur Member State
Date	6 June 2008

Section 6.7 **Annex Point IIA6.7**	**Carcinogenicity study**
Evaluation of applicant's justification	Agree with applicants justification
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 21 : Antifouling products

Document III-A (A6)
Study summaries – Active substance
Toxicological and metabolic studies

Part V
Section A6.8: Reproductive toxicity
Section A6.9: Neurotoxicity study
Section A6.10: Mechanistic study
Section A6.11: Studies on other routes of administration
Section A6.12: Medical data in anonymous form
Section A6.13 to A6.17: additional information

TABLE OF CONTENT

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

Section A6.8 Reproductive toxicity

Section A6.8.1a/01 Teratogenicity Study (Rabbit)

Annex Point IIA6.8.1

Official use only

		1 REFERENCE
1.1	**Reference**	Reference type: Study report Year: 1986 Report date: 14 January 1986 [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, US EPA OPP 83-3
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Marine Antifoulant C-9211
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Test substance was a dilution of DCOIT technical in xylene at 40%.
3.1.2.1	Description	[redacted]
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	[redacted]
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White

3.2.3	Source	[redacted]
3.2.4	Sex	Females
3.2.5	Age/weight at study initiation	[redacted]
3.2.6	Number of animals per group	[redacted]
3.2.7	Control animals	Yes, vehicle and solvent
3.2.8	Mating period	Artificially inseminated with semen from males of the same stain and same supplier as the females
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	
		rabbit: day 7-19 post mating
3.3.2	Postexposure period	10 days
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	Gavage: 5, 25, 70 mg DCOIT/kg bw/day
3.3.5	Vehicle	methylcellulose
3.3.6	Concentration in vehicle	[redacted]
3.3.7	Total volume applied	[redacted]
3.3.8	Controls	Vehicle and solvent (amount of xylene as in the highest dose group)
3.4	**Examinations**	
3.4.1	Body weight	[redacted]
3.4.2	Food consumption	[redacted]
3.4.3	Clinical signs	[redacted]
3.4.4	Examination of uterine content	[redacted]
		[redacted]
		[redacted]
3.4.5	Examination of foetuses	
3.4.5.1	General	[redacted]
3.4.5.2	Skeletal	[redacted]
3.4.5.3	Soft tissue	[redacted]
3.5	**Further remarks**	[redacted]

4 RESULTS AND DISCUSSION.

4.1 Maternal toxic Effects

[redacted]

X

4.2 Teratogenic / embryotoxic effects

No biologically meaningful differences in the mean numbers of viable fetuses, implantation sites, corpora lutea, postimplantation loss, or mean fetal weights and sex ratios were observed in the xylene control, 5.0 and 25.0 mg/kg/day groups. Only 23 fetuses were available for evaluation at a dose level of 70.0 mg/kg/day which is insufficient for an evaluation of teratogenicity; however, there were no signs of a teratogenic response in this dose group. Fetal malformation and variation data did not indicate an adverse response to treatment in any dose group.

4.3 Other effects

None

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	This study is compliant with US EPA OPP 83-3 Guideline. There were no guideline deviations.	
5.2	**Results and discussion**	See conclusion	
5.3	**Conclusion**	DCOIT applied as a 40% dilution in xylene (C-9211 formulation) produced maternal toxicity at all the dose levels tested expressed primarily by body weight loss. The effect was dose-related. Maternal toxicity at the 5.0 mg DCOIT/kg/day dose level was minimal; the trend in the xylene control group was similar to the 5.0 mg a.i./kg/day group. A dose level of 70.0 mg a.i.DCOIT/kg/day was decidedly excessive for a teratology study. Although a dose level of 25.0 mg DCOIT/kg/day approached an excessive level for maternal toxicity, DCOIT was not teratogenic at a dose level of 25.0 mg DCOIT/kg/day or less when administered orally to pregnant rabbits throughout the major period of organogenesis.	X
5.3.1	LO(A)EL maternal toxic effects	Maternal toxicity at the 5.0 mg DCOIT/kg bw/day level was minimal; the trend in the xylene control group was similar to the 5.0 mg DCOIT/kg bw/day group.	X
5.3.2	NO(A)EL embryotoxic / teratogenic effects	25.0 mg DCOIT/kg bw/day	X
5.3.3	Reliability	[redacted]	
5.3.4	Deficiencies	[redacted]	

Evaluation by Competent Authorities

Evaluation by Rapporteur Member State

Date	6 November 2006, revised 12 January 2010
Materials and Methods	Agree with applicant's version.
Results and discussion	See conclusion

Comment (4.1): The LOAEL for maternal toxicity was set at 5 mg/kg bw/day and was based on a minimal (not statistically significant) change in body weight. However, a NOAEL at 5 mg/kg bw/day should have been considered based on the following: It is described in the study description that in the xylene control group, the mean body weight and the mean body weight gains were affected by the xylene exposure, primarily in the latter days of treatment. The trend in the xylene control group was similar to that in the 5 mg/kg bw/day dose group, and the changes in body weight at 5 mg/kg bw/day is therefore considered attributed to xylene exposure, and not to exposure to DOICT.