Section A7.1.1.2.1-01 Annex Point IIA VII.7.6.1.1		Biodegradability (ready)	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Noack M. (2002): [redacted] OIT [redacted]: Ready Biodegradability Closed Bottle Test.- [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Thor GmbH	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; OECD guideline No. 301 D for testing of chemicals (adopted 17 July 1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] OIT [redacted] (CAS 26530-20-1), a clear yellow-brown liquid. Active ingredient: 2-Octyl-2H-isothiazol-3-one Empirical formula: $C_{11}H_{19}NOS$ ThOD (theoretical oxygen demand) = 2.28 mg O_2/mg	
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	Water solubility: 0.5 g/L (20 °C); MW: 213.34 g/mol	
3.1.5	Test substance inhibitory to microorganisms	Toxic effects possible depending on the concentration	
3.2	**Reference substance**	Yes: Sodium acetate puriss. [redacted] CAS 127-09-3, [redacted]	
3.2.1	Initial concentration of reference substance	10 mg/L; ThOD= 0.78 mg O_2/mg	
3.3	**Testing procedure**	The ready biodegradability of test substance [redacted] OIT [redacted] was investigated over a period of 28 days under aerobic conditions at a mean temperature of 20°C in the dark in order to determine the rate of biodegradation. BOD (biochemical oxygen demand) bottles of 300 mL volume were filled with aerated oxygen saturated mineral nutrient medium and the test compound (where needed) was added from a stock solution with a	X

Section A7.1.1.2.1-01 **Annex Point IIA VII.7.6.1.1**		**Biodegradability (ready)**	
		syphon to the desired final concentration of 1.5 (toxicity control) or 3 mg/L (test suspension). Each bottle was inoculated with 0.2 mL of sewage sludge filtrate and closed without air bubbles. The following set of incubation flasks (two replicates per sampling interval) was prepared: • 5x2 bottles containing test item (3.0 mg/L) and inoculum (test suspension) • 5x2 bottles containing only inoculum (inoculum control) • 5x2 bottles containing reference substance (10 mg/L) and inoculum (functional control) • 5x2 bottles containing test item (1.5 mg/L) and reference substance (5 mg/L) and inoculum (toxicity control) At all sampling intervals (days 0, 7, 14, 21, 28) the oxygen concentration of the duplicates of the above 4 test groups was determined. The pH of the test solutions was measured at the beginning and at the end of the test.	X X
3.3.1	Inoculum / test species	For information see Table A7.1.1.2.1-1	
3.3.2	Test system and test conditions	For information see Table A7.1.1.2.1-2	
		4 RESULTS	
4.1	**Degradation of test and reference substance**	The test item [redacted] OIT [redacted] did not reach the pass level of a biodegradation > 60% neither in the 10 day-window nor after 28 days. [redacted] OIT did not even reach the level of 10% (begin of biodegradation) throughout the study. Therefore, the test item has to be classified as not readily biodegradable. The reference substance sodium acetate reached the pass level > 60% degradation after 5 days, thereby fulfilling the validity criteria of the test. The toxicity control showed degradation of 26% after 14 days and 35% after 21 days. These values are only slightly higher than the critical value of 25% degradation (OECD guideline 301), and indicate that the test item may be inhibitory to microorganisms. This is corroborated by the fact that the test item concentration in the toxicity control (1.5 mg/L) was only half of the concentration in the test suspension (3.0 mg/L) and no respiration occurred in the test item vessels. The oxygen uptake and depletion data of all four test bottle groups together with the BOD and biodegradation calculations are presented in Tables A7.1.1.2.1-3 to A7.1.1.2.1-5.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The ready biodegradability of test substance [redacted] OIT [redacted] was investigated over a period of 28 days under aerobic conditions at a mean temperature of 20°C in the dark in order to determine the rate of biodegradation. BOD (biochemical oxygen demand) bottles of 300 mL volume were filled with oxygen saturated mineral nutrient medium, and the test	

Section A7.1.1.2.1-01 **Annex Point IIA VII.7.6.1.1**	**Biodegradability (ready)**	
	compound was added to the desired final test concentration. Each bottle was inoculated with 0.2 mL of sewage sludge filtrate. The test bottle battery consisted of four different sets of incubation flasks (two replicates per sampling interval): 10 bottles each of 'test suspension', 'inoculum control', 'functional control' and 'toxicity control'. Flasks were sampled at days 0, 7, 14, 21 and 28 days after inoculation, oxygen consumption was recorded at every sampling interval and the pH was measured at the beginning and at the end of the test.	
5.2 Results and discussion	The test item [redacted] OIT [redacted] did not reach the pass level of a biodegradation > 60% neither in the 10 day-window nor after 28 days. [redacted] OIT did not even reach the level of 10% (begin of biodegradation) throughout the study. In the 4 incubation sets pH varied between 7.12 and 7.16 at the start of the study and between 6.95 and 7.36 at the end of the study	
5.3 Conclusion	The test item [redacted] OIT [redacted] has to be assessed as not readily biodegradable.	X
5.3.1 Reliability	2	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	29 Oct 2009	
Materials and Methods	Applicant's version is considered acceptable noting the following: **3.3:** The procedure should have been repeated using a lower concentration of test substance or a higher concentration of inoculums in the test suspension, in order to rule out the inhibitory effects of the test substance on the microbial populations. **3.3:** It would also have been more appropriate to have conducted the toxicity control with 3.0 mg/L test item, as in the test suspension. **3.3:** There are some minor deviations from the temperature and pH stated in OECD Guideline 301 (which specifies temperature 21 ˚C and pH 7.4). However, these are not expected to have had an adverse effect on the quality of the study.	
Results and discussion	Applicant's version is considered acceptable noting the following: **Table A 7.1.1.2.1-3:** Guideline OECD 301D (P36) states that the oxygen demand on day 28 of the study from the inoculum control bottle should not exceed 1.5 mg/L. Table A 7.1.1.2.1-3 shows that this value is 3.52 mg/L. There are no significant deviations in measured temperature or pH at this stage in the study and the concentration of sewage sludge added, the source of the sewage sludge and the number of colony forming units are all within the recommended parameters as stated in guideline OECD 301D. Therefore, the applicant's assessment that this will not affect the quality or integrity of the study is acceptable. It is noted that the applicant states that they believe that the test substance may be inhibitory to microorganisms.	

Section A7.1.1.2.1-01 Annex Point IIA VII.7.6.1.1	Biodegradability (ready)	
Conclusion	Applicant's version is considered acceptable noting the following: **5.3:** The applicant states that the toxicity control shows that there may be some inhibitory effects on the micro-organism populations. Guideline OECD 301 states that under these circumstances, the procedure should be repeated with either a lower concentration of test substance, or a higher concentration of the inoculum in the test suspension. As this has not been carried out, it is not possible to draw the conclusion that the test substance is not readily biodegradable.	
Reliability	2 Study conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of relevant results.	
Acceptability	Acceptable.	
Remarks	All endpoints and data presented have been checked against the original study and are correct. The UK CA note that following Guideline OECD 301 the procedure should be repeated with either a lower concentration of test substance, or a higher concentration of the inoculum in the test suspension. While this has not been carried out, due to the higher tier sewage simulation study submitted (A7.1.2.1.1-01.) and the fact that the test resulted in a negative result (not ready biodegradable), the UK CA are of the opinion that the study represents an adequate screening test.	
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A 7.1.1.2.1-1: Information on the Inoculum

Parameter	Details
Kind of inoculum	Activated sludge
Source/origin	Municipal sewage treatment plant comprising mostly domestic sewage and hardly chemical waste
Sampling site	[redacted]
- Preparation of inoculum for study conduct - pre-treatment - inoculum quantity	The activated sludge was filtered through a folded paper filter. The first 200 mL of the filtrate were not used. Only the second filtrate served as inoculum for the study. The sludge was neither pre-conditioned (i.e. aerated) nor pre-adapted (to the test substance) before study start. Per 300 mL test bottle, 0.2 mL inoculum were added.

Initial cell concentration	10^4 – 10^6 CFU/L

Table A 7.1.1.2.1-2: Information on Test System and Test Conditions

Parameter	Details
Test type	Closed bottle test (OECD 301 D)
Test medium	Mineral nutrient solution acc. to OECD 301 D
Test flasks	BOD bottles with glass stoppers (volume 300 mL)
Test duration	28 days
Test temperature	Nominally 20 - 21 ºC, maintained in an incubator and measured daily (min and max deviations were 19.3 and 21.9 ºC)
Initial test compound concentration	3 mg/L (selection based on the calculated ThOD of the test item of 2.28 mg O_2/mg), hence the ThOD in the test suspension bottle group corresponds to 3x2.28 = 6.84 mg O_2/L.
Analytical parameter	Oxygen consumption, BOD
Sampling	On days 0, 7, 14, 21 and 28 in duplicates
Equipment	pH-meter [redacted], Oximeter [redacted]
Controls	Three types of control flasks were incubated, sampled and analysed in the same way as the test item bottle set: • inoculum controls (mineral medium plus inoculum, no test item) • functional controls (mineral medium plus reference substance sodium acetate plus inoculum, no test item) • toxicity controls (mineral medium plus test item plus reference compound plus inoculum)
Nitrate/nitrite measurement	No (oxidation of the test item nitrogen molecule was not expected, therefore nitrification was not considered)

Table continued on next page

Calculations	The ThOD of a compound (without nitrification) is calculated according to: **ThOD** = $\frac{16*(2c+0.5*(h-cl-3n)+3s+2.5p+0.5na-o)}{MW}$ Where MW = molecular weight and the small letters in the formula represent the index of atoms of the substance example $C_cH_hCl_{cl}N_nNa_{na}O_oP_pS_s$ acc. to OECD 301 D. For [redacted] OIT [redacted] ($C_{11}H_{19}NOS$), one obtains a ThOD of 2.28 mg O_2/mg [redacted] The BOD_L of a solution is obtained according to: **BOD_L** (mg O_2/L) = (m_{t0} - m_{tx}) - (m_{b0} – m_{bx}) providing the BOD_S of a test substance by: **BOD_S** (mg O_2/mg) = BOD_L/C_t Where C_t (mg/L) = test concentration of test or reference item and m_{t0}, m_{tx},, m_{b0} and m_{bx} signify the O_2 concentration of a test substance bottle on day 0, on day x, the O_2 concentration of the inoculum control on day 0 and on day x, respectively The biodegradation is given by: **Biodegradation** (%) = $\frac{BOD_S\ (mg\,O_2/mg)}{ThOD\ (mg\,O_2/mg)}$ x 100

Table A 7.1.1.2.1-3: Oxygen uptake/depletion of inoculum control bottles

Sampling interval (days)	O_2 (mg/L)			
	Replicate 1	Replicate 2	Mean value	O_2 -depletion
0	11.32	11.30	11.31	-
7	10.18	10.34	10.26	1.05
14	9.81	10.13	9.97	1.34
21	10.42	10.52	10.47	0.84
28	7.84	7.73	7.79	3.52

Table A 7.1.1.2.1-4: Oxygen depletion, BOD and biodegradation in [redacted] OIT [redacted] and functional control (sodium acetate) bottles

Sampling interval (days)	[redacted] OIT [redacted] 3.0 mg/L ThOD = 2.28 mg O_2/mg						Functional control (sodium acetate), 10 mg/L ThOD = 0.78 mg O_2/mg					
	O_2 (mg/L)			BOD (mg O_2/L resp. mg O_2/mg)		Degr. (%)	O_2 (mg/L)			BOD (mg O_2/L resp. mg O_2/mg)		Degr. (%)
	Repl	Rep2	mean	BOD_L	BOD_S		Rep1	Rep2	mean	BOD_L	BOD_S	
0	11.40	11.55	11.48	-	-	-	11.50	11.48	11.49	-	-	-
7	10.82	10.80	10.81	-0.38	-0.13	0	3.87	3.66	3.77	6.67	0.67	86
14	10.39	10.79	10.59	-0.45	-0.15	0	3.38	3.36	3.37	6.78	0.68	87
21	11.04	11.24	11.14	-0.50	-0.17	0	4.49	7.26[1]	4.49	6.16	0.62	79
28	9.05	9.06	9.06	-1.10	-0.37	0	3.04	2.72	2.88	5.09	0.51	65

[1] sample considered as outlier, not considered in calculations

Table A 7.1.1.2.1-5: Oxygen depletion, BOD and biodegradation in toxicity control [redacted] OIT [redacted] plus sodium acetate) bottles

Sampling interval (days)	[redacted] OIT [redacted] 1.5 mg/L (ThOD = 2.28 mg O_2/mg) plus sodium acetate, 5mg/L (ThOD = 0.78 mg O_2/mg); $ThOD_{gesamtsubstanz}$ = 1.13 mg O_2/mg_{gesamt}					
	O_2 (mg/L)			BOD (mg O_2/L resp. mg O_2/mg)		Degr. (%)
	Repl	Rep2	mean	BOD_L	BOD_S	
0	11.61	11.56	11.59	-	-	-
7	8.40	8.25	8.33	2.21	0.34	30
14	8.26	8.48	8.37	1.88	0.29	26
21	8.13	8.15	8.14	2.61	0.40	35
28	6.16	6.44	6.30	1.77	0.27	24