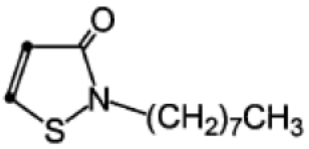
Section A7.1.2.1.1-01 Annex Point IIIA XII.2.1		Biological sewage treatment, aerobic biodegradation	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] (2002): [redacted] OIT [redacted]: Simulation test - aerobic sewage treatement. [redacted] Unpublished report, [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Thor GmbH	
1.2.2	Company 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 303A: Activated Sludge Units	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] OIT [redacted], 2-n-octyl-4-[4,5-^{14}C]-isothiazolin-3-one	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	 • denotes position of [^{14}C]-radiolabel	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	Water solubility 0.4 g/L (20°C); MW: 213.3 g/mol; K_{OC} in sewage sludge: 6740 mL/g	X
3.1.5	Composition of Product	Not applicable.	
3.1.6	TS inhibitory to microorganisms	Toxic effects possible depending on the concentration	X
3.1.7	Specific chemical analysis	Effluent containing residual OIT was diluted with acetic acid 1.5% v/v. Sludge samples were extracted with acetonitrile for 16 h by shaking and then filtered. Extracts were diluted with 1.5% v/v acetic acid. OIT was analysed with HPLC-UV using external standardisation. HPLC details:	X

Section A7.1.2.1.1-01 **Annex Point IIIA XII.2.1**		**Biological sewage treatment, aerobic biodegradation**	
		Column: [redacted] Mobile phase: [redacted] Flow rate: [redacted] UV-detection: [redacted]	
3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance	Not applicable	
3.3	**Test ing procedure**		
3.3.1	Inoculum / test species	see table A7_1_2_1_1-1	
3.3.2	Test system	see table A7_1_2_1_1-2	
3.3.3	Test conditions	see table A7_1_2_1_1-3	
3.3.4	Method of preparation of test solution	250 mg test item dissolved in demineralised water, ultrasonicated and made up to 25 L.	
3.3.5	TS concentration in influent	[redacted], expressed as active substance	
3.3.6	Duration of test	Acclimatisation phase (no test item addition): 28 days Adaptation phase: 34 days Plateau phase: 25 days	X
3.3.7	Analytical parameter	Specific analysis of OIT; DOC removal	
3.3.8	Sampling	DOC in effluent: at least 3x a week OIT concentration in effluent: at least 3x a week, altogether 23 valid values obtained in 25 days OIT adsorbed to surplus sludge: at least weekly Influent DOC and OIT concentration: with each new batch Volume of influent and of removed sludge: at least weekly Suspended solids: at least weekly Oxygen, temperature, pH: at least weekly	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Nitrate/nitrite measurement	No (not applicable)	
3.3.11	Controls	The determination of DOC in the activated sludge unit served as control for viability of the sludge.	X
3.3.12	Statistics	Only mean and standard deviation calculations	

<table>
<tr><td colspan="2">Section A7.1.2.1.1-01
Annex Point IIIA XII.2.1</td><td>Biological sewage treatment, aerobic biodegradation</td><td></td></tr>
<tr><td colspan="2"></td><td></td><td></td></tr>
<tr><td colspan="2"></td><td>4 RESULTS</td><td></td></tr>
<tr><td>4.1</td><td>Degradation of test substance</td><td></td><td></td></tr>
<tr><td>4.1.1</td><td>Graph</td><td>See Figure 7_1_2_1_1-1</td><td></td></tr>
<tr><td>4.1.2</td><td>Degradation</td><td>Results of the specific analysis of [redacted] OIT [redacted]:
<table>
<tr><th>Parameter</th><th>Range</th><th>Phase end</th><th>No. of values</th><th>Mean CV</th></tr>
<tr><td colspan="5">Adaptation phase</td></tr>
<tr><td>Elimination</td><td>72 to > 99%</td><td></td><td>26</td><td>-</td></tr>
<tr><td>Adsorption</td><td>0.0 to 3.5%</td><td>1.2</td><td>10</td><td>-</td></tr>
<tr><td>Biodegradation</td><td>77.3 to 99.0%</td><td>84.8</td><td>10</td><td>-</td></tr>
<tr><td colspan="5">Plateau phase</td></tr>
<tr><td>Elimination</td><td>84 to 84%</td><td>92%</td><td>23</td><td>89%
3.6%</td></tr>
<tr><td>Adsorption</td><td>0.6 to 1.1%</td><td>n.d.</td><td>4</td><td>0.95%
25%</td></tr>
<tr><td>Biodegradation</td><td>82.9 to 89.0%</td><td>n.d.</td><td>4</td><td>87%
3.0%</td></tr>
</table>
n.d. - not determined

Biodegradation was calculated as difference between elimination of [redacted] OIT [redacted] and adsorption, following OECD 303A.</td><td>X</td></tr>
<tr><td>4.1.3</td><td>Other observations</td><td>No pre-test for volatilisation was carried out, since [redacted] OIT [redacted] is well soluble in water and not volatile.

No pre-test for adsorption was carried out, since the sorption of [redacted] OIT [redacted] to sludge was to be determined in the main test.</td><td></td></tr>
<tr><td>4.1.4</td><td>Degradation of TS in abiotic control</td><td>Not applicable. An abiotic control is not part of OECD 303A</td><td></td></tr>
<tr><td>4.1.5</td><td>Degradation of reference substance</td><td>Not applicable. A reference substance is not part of OECD 303A</td><td></td></tr>
<tr><td>4.1.6</td><td>Intermediates/ degradation products</td><td>Not applicable, degradation products were not analysed.</td><td></td></tr>
<tr><td colspan="2"></td><td>5 APPLICANT'S SUMMARY AND CONCLUSION</td><td></td></tr>
<tr><td>5.1</td><td>Materials and methods</td><td>The biodegradation of [redacted] OIT [redacted] was assessed in a simulation test according to OECD 303A (activated sludge units). A single sludge unit was fed with municipal sewage sludge, synthetic sewage and tap water.

During the acclimation phase, the sludge was allowed to establish itself in the sludge unit. During the adaptation phase, [redacted] OIT [redacted] was dosed to the influent at a concentration of 7.27 mg/L, and the sludge was allowed to adapt to the test item. During the plateau phase, the test</td><td></td></tr>
</table>

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	item dosing continued to determine the elimination, sorption and biodegradation of [redacted] OIT [redacted] Specific analysis by HPLC-UV was used to determine concentrations of [redacted] OIT [redacted] The DOC in influent and effluent was also determined, to obtain data on DOC elimination and thus on viability of the sludge.	
5.2 Results and discussion	**Elimination of [redacted] OIT [redacted]:** Test item dosing started after the activated sludge unit had stabilised. The adaptation phase was completed after 34 days, when the elimination of Acticide OIT 100% was > 80%. Thereafter, the plateau phase was run for 25 days. Elimination of Acticide OIT 100% was 86% at the start of the plateau phase and ranged from 84 - 94% throughout the plateau phase. Adsorption of [redacted] OIT [redacted] to sludge was low, ranging from < 0.1 - 3.5% during the adaptation phase and 0.6 - 1.1% during the plateau phase. Higher sorption could be expected judging from the high K_{OC} for [redacted] OIT [redacted] of 6740 mL/g determined in sewage sludge in another study. Thus, [redacted] OIT [redacted] must be degraded before significant sorption to sludge constituents can take place. The fraction of [redacted] OIT [redacted] eliminated but not adsorbed can be assigned to degradation. Therefore, degradation amounted to 69.6 - 99.0% already during the adaptation phase, and to 82.9 - 89.0% during the plateau phase. The arithmetic mean elimination of [redacted] OIT [redacted] during the plateau phase was 89%. The mean fraction sorbed to sludge was 0.95%. Deducing the sorbed fraction from the eliminated fraction, a mean fraction biodegraded of 88% was calculated for [redacted] OIT [redacted] No lag phase was apparent for elimination of [redacted] OIT [redacted] In fact, elimination reached very high levels directly at the start of the adaptation phase. Since adsorption was shown to be very minor, the elimination must be due to degradation. The absence of a lag phase could indicate either abiotic degradation processes or the presence of microorganisms in municipal sewage sludge that are able to co-metabolise OIT without adaptation. The latter appears likely, since the use of OIT is widespread with diffuse emissions to municipal STPs. **Validity criteria:** [redacted] OIT [redacted] was recovered from the dosing solution at 73 - 85%, and therefore actual concentrations were used in all evaluations. Test item concentrations were stable during up to 3 days of storage of dosing solutions. The DOC elimination was > 80% throughout the acclimatisation, adaptation and plateau phase, except for one day during acclimatisation and the first day of the adaptation phase (77%). Therefore, the activated sludge was viable and the test was suitable to obtain information on the biodegradation of the test substance. Slight deviations from the guideline values for pH and temperature occurred, as well as suspended solids concentrations exceeding the guideline value of 3 g/L up to 3.90 g/L. Those points were not considered to influence the test result.	X

Section A7.1.2.1.1-01 **Annex Point IIIA XII.2.1**	**Biological sewage treatment, aerobic biodegradation**	
5.3 Conclusion	The activated sludge unit test following OECD 303A showed that [redacted] OIT [redacted] is degraded in aerators of municipal sewage treatment plants to a large extent and without a lag phase. A representative mean fraction degraded of 88% was calculated. Adsorption to sludge was shown to be very low, with a representative mean value of 1%. It is deemed likely that the degradation of Acticide OIT 100% observed in the present study is biotically mediated. The test was shown to be valid by DOC elimination > 80% throughout all phases, and no relevant deviations from the guideline or the study protocol.	
5.3.1 Reliability	1 (valid without restrictions)	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	03 Nov 2009	

Section A7.1.2.1.1-01 Annex Point IIIA XII.2.1	Biological sewage treatment, aerobic biodegradation	
Materials and Methods	Applicant's version is considered acceptable noting the following: **General:** The report is badly written and confusing. For example, the use of a control is not well documented and it is not clear which results relate to the control. **Section 2.3:** There is deviation from the OECD guideline in terms of temperature, pH and amount of suspended solids. Evaluations procedures were changed because no losses by volatisation of the test item were considered likely. No preliminary test was performed to check the adsorption due to the chemical characteristics of the test item. To maintain the concentration of suspended solids in the range of 1 to 3 g/l the mean sludge age was higher than 6 to 10 days. A different acclimatisation and inoculation procedure was used due to technical reasons. These deviations were not considered by the CA to have impacted on the quality and integrity of the study. It is also noted however, that no attempt has been made to analyse for the presence of metabolites, therefore the study should only be relied upon to refine the assessment of fate of the parent compound. **Section 3.1.4:** Report states water solubility as 0.5 g/L at 20 ˚C; this also seems to differ from the water solubility in the Physchem section (IIA3.5) which is reported as 406 mg/l at 20 ˚C for pH 4 and 5. Please see A7.1.3-03 for K_{oc} value. **Section 3.1.6:** This comment is very vague. No toxic effects were observed in the study, as it is stated in the OECD guideline that any inhibition will be noticed as the presence of the test substance decreasing the % removal of DOC of the organic medium. This does not occur. **Section 3.1.7:** The UK CA note that **t**he sludge samples were transferred to 50 ml centrifuge tubes with 10 ml of acetonitrile, shaken for 16 hrs, and then filtered (0.45 µm). The filtered extract was diluted with 1.5% acetic acid and quantitated by HPLC. Recovery of fortified control sludge was 95% **Section 3.3.6:** One day from previous category is counted in both the adaptation and plateau phases; these should therefore read 33 and 24 days respectively. ***Section 3.3.****8: It is unclear whether OIT is tested within the effluent only or the effluent and DOC, as such the UK CA are of the opinion that the results should only be utilised to report on the fate of OIT, and cannot be utilised to disregard the formation of metabolites that were persistent to degradation in the STP.* **Section 3.3.11**: Organic medium according to OECD 303A. Nominal DOC concentration in influent ≥100 mg/l.	
Results and discussion	Applicant's version is considered acceptable noting the following:**General:** It is not clear which data relates to the control; this is probably the data reported as 'organic medium', but this has not been specifically stated. **Section 4.1.2 (Table):** Plateau phase elimination range in summary should read 84- 94 % (according to report). **Section 4.1.3:** Adsorption of OIT to sludge was expected and so was determined by HPLC analysis in surplus sludge removed during the study. **Table A7_1_2_1_1-3:** Test conditions – an approximate ratio of 5:1 for synthetic sludge:domestic sludge was used. The raw data below is added to the robust study summary for completeness.	

Section A7.1.2.1.1-01 **Annex Point IIIA XII.2.1**	**Biological sewage treatment, aerobic biodegradation**	
Conclusion	Applicant's version is considered acceptable, noting the following: **Section 5.1:** Test conditions – an approximate ration of 5:1 for synthetic sludge: domestic sludge was used. Surplus sludge (102 ml/d) was removed continuously with a pump (with a few exceptions 7 to 12 Dec and 2 to 11 Jan). An aeration vessel (3 l) and a separator (1.5 l) were used. The LOQ was 0.033 to 0.036 mg/l (see table 18, p42 of report). **Section 5.2:** The applicant has not justified the no-effect of deviations from the pH and temperature ranges stated in the guidelines. In the absence of any information in other parts of the dossier which indicate a possible pH effect, the variation in this study has been considered acceptable within the range likely for such a study **Section 5.2:** Volatility has not been measured.	
Reliability	2 The study contained minor methodological deviations which do not affect the quality of results.	
Acceptability	Acceptable	
Remarks	All endpoints and data presented in the summary and tables have been checked against the original study. Metabolites are not identified or quantified, while this is not a requirement, as the OIT was directly measured it would appear that the metabolites could have been identified in the same manner. The potential for persistent metabolites to have been formed and released in the effluent is further discussed in the AII summary document, taking into account other information on route of degradation available from other simulation studies in natural water and soil.DOC elimination was >80 % throughout the study and therefore the study is acceptable. All endpoints and data presented have been checked against the original study and are correct.	
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A7_1_2_1_1-1: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Mixed
Strain	Mixed
Source	Sewage treatment plant treating mainly municipal sewage
Sampling site	[redacted]
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Aeration at room temperature until use. Dilution with tap water.
Pretreatment	Acclimatisation phase, adaptation phase
Initial cell concentration	[redacted] g/L dry matter

Table A7_1_2_1_1-2: Test system

Criteria	Details
Culturing apparatus	Activated sludge units: aeration vessel (3 L), separator (1.5 L), dimensions according to OECD 303A
Number of culture flasks/concentration	No replicates
Aeration device	Membrane pump
Measuring equipment	Oxygen, pH, temperature: [redacted]
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_2_1_1-3: Test conditions

Criteria	Details
Composition of medium	Organic medium: synthetic sewage according to OECD 303A and domestic sewage ([redacted]) to obtain mean DOC concentration in influent of about 100 mg C/L
Additional substrate	Yes (synthetic and domestic sewage, see above)
Test temperature	Temperature in aeration vessel: 19.3 - 24.7°C throughout the whole test
pH	pH in the aeration vessel: 5.05 - 7.83 throughout the whole test
Aeration of dilution water	Not applicable
Suspended solids concentration	2.67 - 3.90 g/L during the plateau phase
Sludge wastage and mean sludge age	Continuous removal of sludge with a pump at approximately 102 mL/day, resulting in a mean sludge age of 30 days. The sludge age was necessary to maintain the suspended solids concentration in the desired range of 1 - 3 mg/L
Other relevant citeria	Mean hydraulic retention time 5.5 h. Influent flow of 0.55 L/h maintained by dosing synthetic sewage at 0.05 L/h, domestic sewage at 0.10 L/h, and tap water (containing test item stock solution during adaptation and plateau phase) at 0.40 L/h All solutions pumped directly into the activated sludge units.

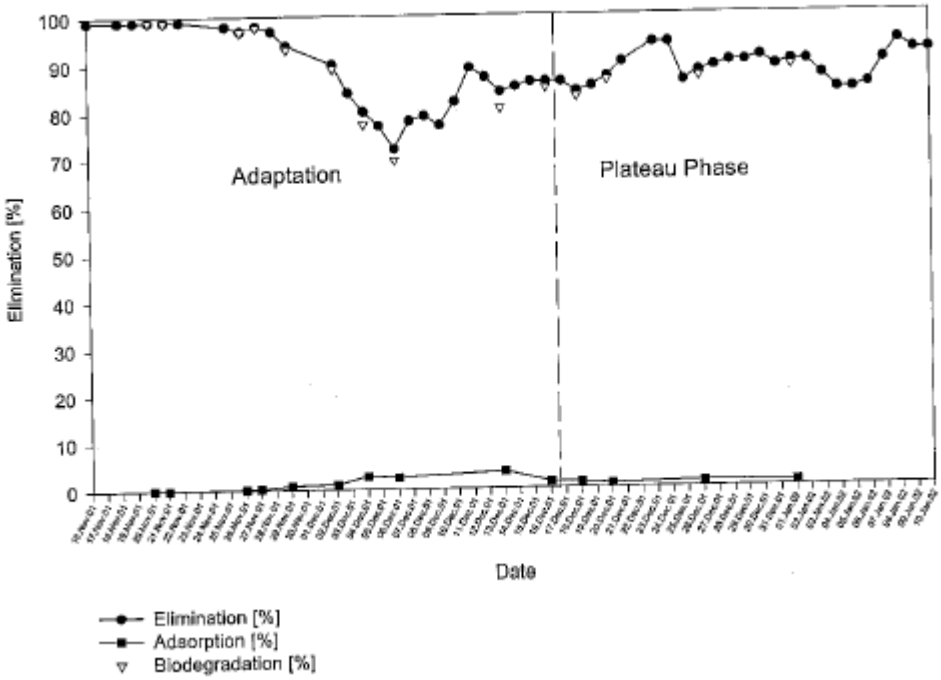

Figure A7_1_2_1_1-1: Elimination, biodegradation and adsorption of [redacted] OIT [redacted] during adaptation and plateau phase

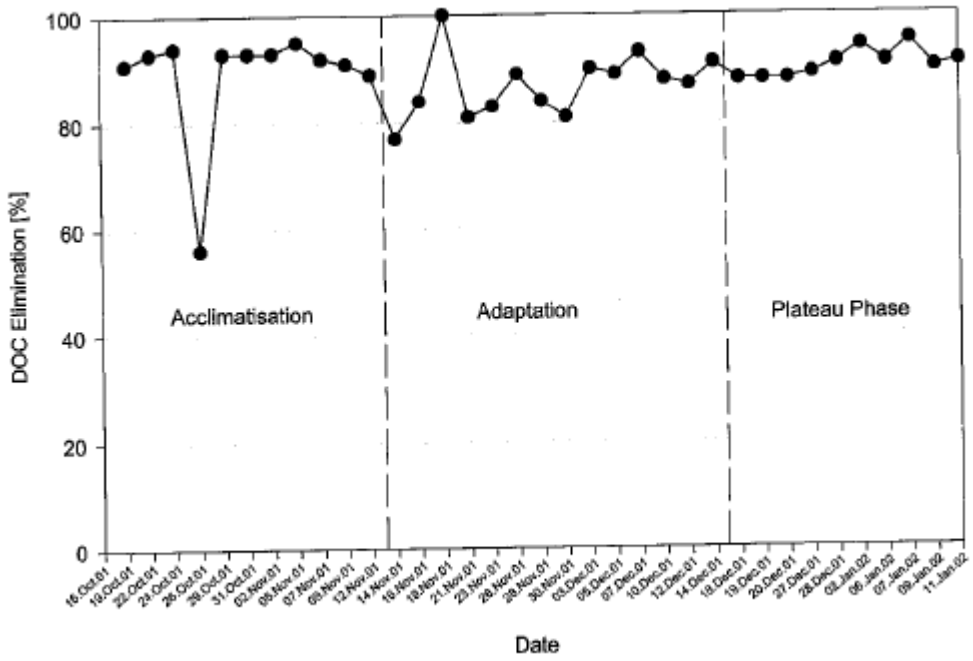

Figure A7_1_2_1_1-2: Elimination of DOC in th e organic medium during the test