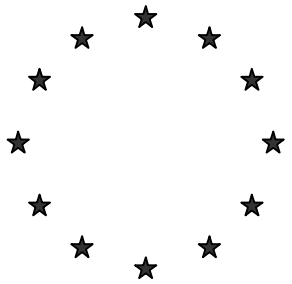
COMPETENT AUTHORITY REPORT



1,2-Benzisothiazol-3-(2*H*)-one (BIT) (PT 6)

Document III-A

Active Substance

RapporteurMemberState: Spain

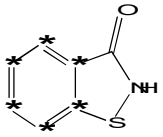
Section A7

Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.1

HYDROLYSIS AS A FUNCTION OF PH AND IDENTIFICATION OF BREAKDOWN PRODUCTS (01)

Annex Point IIA7.6.2.1

		1 REFERENCE	**Officialuse only**
3.1	**Reference**	**A7.1.1.1.1/01** [redacted] (2007) ^{14}C-BIT Hydrolytic Stability: [redacted] 17 May 2007	
3.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		4 GUIDELINES AND QUALITY ASSURANCE	
4.1	**Guideline study**	Yes. OECD Guideline 111, Hydrolysis as a Function of pH (April 2004) and US EPA OPPTS 835.2110, Hydrolysis as a Function of pH (January 1998)	
4.2	**GLP**	Yes	
4.3	**Deviations**	None	
		5 MATERIALS AND METHODS	
5.1	**Test material**	^{14}C-BIT  * site of ^{14}C label	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As specified in the study guidelines, ^{14}C-BIT was employed. Specifications for the ^{14}C-materials are listed elsewhere.	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.1.1.1.1	**HYDROLYSIS AS A FUNCTION OF PH AND IDENTIFICATION OF BREAKDOWN PRODUCTS (01)**
Annex Point IIA7.6.2.1	

3.1.3 Purity	[redacted]
3.1.4 Further relevant properties	Specific activity of the ^{14}C-test material was 53.57 mCi/g
5.2 Reference substance	• Water solubility is greater than 0.7 g/L.
3.2.1 Initial concentration of reference substance	No reference substances were employed to validate the hydrolysis study. The following compound was used as chromatography reference standards. • ^{12}C-BIT, [redacted]
5.3 Test solution	A treatment solution of ^{14}C-BIT was prepared by dissolving 20.018 mg in 16.85 mL of acetonitrile. Actual concentrations of the test solutions, determined from the Time 0 samples, are tabulated below.

Dosing Concentration (µg/g)

pH 4	pH 7	pH 9
9.73	9.56	9.75

A non-radiolabeled treatment solution was prepared by dissolving 5.794 mg of ^{12}C-BIT in 4.8 mL of acetonitrile. This solution was used for dosing samples that were used for sterility and pH examinations.

5.4 Testing procedure	
3.4.1 Test system	The guidelines employed for this study, OECD 111 and OPPTS 835.2110, are designed as a tiered approach. The first tier is to measure the stability of the test material at pH 4, 7, and 9 for 5 days at 50°C. If the compound is stable at elevated temperatures, no additional testing is required. BIT was stable so the only testing performed was Tier 1. pH 4, 7, and 9 buffers were prepared as outlined in Table A7.1.1.1.1-1. The buffers were degassed by sonication and then purged with nitrogen to exclude dissolved oxygen. Thirty-six vials were prepared, twelve for each pH. To each vial, 3 mL of the appropriate buffer solution was added, the headspace purged with nitrogen and the vial sealed with crimped PTFE-lined septa. The vials were then sterilized by autoclaving. Prior to dosing the vials were placed in a water bath maintained in the dark at 50 ± 0.2°C. For each pH, the 12 vials were dosed and employed as described in the table below.

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.1.1 **HYDROLYSIS AS A FUNCTION OF PH AND IDENTIFICATION OF BREAKDOWN PRODUCTS (01)**

Annex Point IIA7.6.2.1

Number of Vials	^{14}C-BIT (µl)	^{12}C-BIT (µl)	Use
2	25		0 hour samples
2	25		5 day samples
2	25		Spare samples
2			Pre-application pH determination
2		25	Post-application sterility determination
2		25	Post-application pH determination

The study was initiated by injecting ^{14}C-BIT into the buffered solution through the septa.

Samples dosed with ^{14}C-BIT were removed for analysis immediately (Time 0) and at Day 5. The samples were placed in ice water and aliquots removed for radioassay. Additional aliquots were transferred to vials for HPLC analysis.

The pH was determined in duplicate samples from each pH after sterilization and prior to dosing with BIT. The pH was again measured in two additional vials dosed with ^{12}C-BIT on Day 5.

Two samples from each pH dosed with ^{12}C-BIT were remove on Day 5 and the their sterility examined by counting colony forming units on agar plates incubated at 35°C for 2 days.

Prior to study initiation, it was found that BIT did not adsorb to the glass walls of the vials used.

3.4.2 Temperature	The temperature of the water bath used was 50 ± 0.2°C.
3.4.3 pH	pH 4.0 ± 0.2 pH 7.0 ± 0.2 pH 9.0 ± 0.2
3.4.4 Duration of the test	The duration of the test at pH 4, 7, and 9 was 5 days.
3.4.5 Number of replicates	Duplicate vials were removed at Time 0 and Day 5.

Section A7 Subsection A7.1.1.1.1 Annex Point IIA7.6.2.1	Ecotoxicological Profile Including Environmental Fate and Behaviour HYDROLYSIS AS A FUNCTION OF PH AND IDENTIFICATION OF BREAKDOWN PRODUCTS (01)	
3.4.6 Sampling	Sampling intervals were: pH 4: 0 and 5 days pH 7: 0 and 5 days pH 9: 0 and 5 days Aliquots were removed immediately after sampling for radioassay. Additional aliquots were taken for chromatographic analysis.	
3.4.7 Analytical methods	Radioassay was performed using Packard liquid scintillation counters. Thin layer chromatography (TLC) was performed on 250 µm thick silica gel plates (Whaman). The development solvent was ethyl acetate:methanol:acetonitrile:acetic acid (90:5:5:1). Solutions were cochromatographed with non-radiolabeled BIT. Radiolabeled compounds were detected using a phophorimager while non-labeled compounds visualized with a UV lamp (254 nm). Aliquots were analyzed by HPLC using a modified C-18 column and a binary gradient composed of 0.5% aqueous formic acid and 0.5% methanolic formic acid. Detection employed a ^{14}C-flow through monitor and/or UV detector (254 nm). Representative samples at each pH were analyzed by LC-MS to confirm the presence of parent. Analysis employed a modified C-18 column and a binary gradient composed of 0.5% aqueous formic acid and 0.5% methanolic formic acid. The LC effluent was introduced in to the MS via an API interface and positive ionization was employed.	
	6 RESULTS	
6.1 pH, storage, and sterility stability	After 5 days of incubation the pH of the buffer solutions were stable; 4.0, 7.0, and 9.0. Overnight storage at room temperature of the acetonitrile dosing solution resulted in no degradation of BIT. Examination of the buffer solutions after 5 days incubation showed they were still sterile (no detectable colony forming units).	
6.2 Material Balance	The material balance was determined by radioassaying the hydrolysis solutions at Day 0 and 5 and the results expressed as a percent of applied radioactivity in Table A7.1.1.1.1-2. Recovery was greater than 97% with the average being 98.6 ± 1.7%.	
6.3 Quantitation of parent and hydrolytic products	Table A7.1.1.1.1-3 contains the replicate average data for the quantitation, as a percent of applied, of parent compound and total hydrolytic degradates at the three pH's. Quantitation in µg/g is presented in Table A7.1.1.1.1-4. These results show that parent compound is stable at pH 4, 7 and 9 since BIT comprises over 97% of the applied radioactivity. Thus there is essentially no degradation OIT	

Section A7 Subsection A7.1.1.1.1 Annex Point IIA7.6.2.1	Ecotoxicological Profile Including Environmental Fate and Behaviour HYDROLYSIS AS A FUNCTION OF PH AND IDENTIFICATION OF BREAKDOWN PRODUCTS (01)	

		observed at pH 4, 7 and 9.	
6.4	**Hydrolysis rate constant (k_h)**	There is no rate constant since BIT did not hydrolyze under the test conditions. Thus no higher tier testing is required.	
6.5	**Dissipation time**	Since BIT did not hydrolyze, the dissipation time (DT_{50}) cannot be determined.	
6.6	**Specification of the transformation products**	The transformation products were insignificant since BIT did not hydrolyze under the test conditions.	
		7 APPLICANT'S SUMMARY AND CONCLUSION	
7.1	**Materials and methods**	The Guidelines followed were OECD 111, Hydrolysis as a Function of pH and US EPA OPPTS 835.2110, Hydrolysis as a Function of pH. The tier one test examined the stability of the test compound at pH 4, 7, and 9 for 5 days at 50°C. If the compound is stable, no further testing is required. Sterile and degassed pH 4, 7, and 9 buffers were prepared and dosed at nominal 10 ppm with ^{14}C-BIT. The buffered aliquots were incubated in the dark at 50 ± 0.2°C and duplicate samples removed on Day 0 and Day 5. Solutions were radioassayed and chromatographed to quantitate parent.	
7.2	**Results and discussion**	In pH 4, 7, and 9 buffers no significant hydrolysis of BIT was observed after 5 days of incubation at 50°C. As a result, the compound is considered hydrolytically stable and no additional tiered testing is required. Over 97% of the applied radioactivity was recovered as BIT after the 5 day incubation.	
5.2.1	kh	Not determined since BIT was stable at pH 4, 7, and 9.	
5.2.2	DT_{50}	Not determined since BIT was stable at pH 4, 7, and 9.	
5.2.3	r^2	Not determined since BIT was stable at pH 4, 7, and 9.	
7.3	**Conclusion**	Following the tier 1 guidelines, BIT was found to be hydrolytically stable at an elevated temperature and thus no additional testing is	

Section A7	Ecotoxicological Profile Including Environmental Fate and Behaviour
Subsection A7.1.1.1.1 Annex Point IIA7.6.2.1	HYDROLYSIS AS A FUNCTION OF PH AND IDENTIFICATION OF BREAKDOWN PRODUCTS (01)

		required. This study fulfils the requirement for determining the effect of aqueous hydrolysis on the fate of BIT in the environment. As discussed further in Doc. III-A sections A7.1.1.1.2, BIT rapidly photodegrades. Additionally, BIT rapidly biodegrades (7.1.1.2.1). Therefore, hydrolysis will have minimal, if any influence on the fate of MI and on its risk assessment.	
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 RAPPORTEURMEMBERSTATE
Date	*November 2010*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted.*
Conclusion	*BIT was found to be hydrolytically stable.* *This study fulfils the requirement for determining the effect of aqueous hydrolysis on the fate of BIT in the environment.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.1.1.1.1-1: Type and composition of buffer solutions

pH	Type of buffer (final molarity)	Composition
4	0.05 M Phthalate	5.108 g potassium hydrogen phthalate made up to 500 mL with water. The pH was 4.03

7	0.05 M Phosphate	3.0407 g KH_2PO_4 made up to 500 mL with water. The pH was adjusted with 0.05 NaOH to 6.95.
9	0.01 M Sodium Tetraborate-HCl	4.768 g of $Na_2B_40_7•10H_2O$ made up to 500 mL with water. The pH was 9.01.
The pH of the bulk buffer solutions were adjusted to 4.0 ± 0.2, 7.0 ± 0.2, and 9.0 ± 0.2.		

Table A7.1.1.1.1-2: Recovery of Applied ^{14}C-Activity

pH	Material Balance as a Percent of Applied Radioactivity (%)[1]	
	Day 0	Day 5
4	99.4	98.6
7	97.2	99.0
9	98.9	98.5

[1]average of duplicate samples

Table A7.1.1.1.1-3: Percent of Parent and Hydrolytic Products

pH	Sampling Day	Percent of Applied Activity (%)[1]		
		BIT	Other	Total
4	0	98.3	1.1	99.4
	5	97.7	0.8	98.6
7	0	96.6	0.6	97.2
	5	98.5	0.5	99.0
9	0	98.4	0.4	98.9
	5	97.2	1.2	98.5

[1]Average of duplicate samples.

Table A7.1.1.1.1-4: Concentration of Parent and Hydrolytic Products

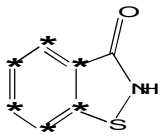
pH	Sampling Day	Percent of Applied Activity (%)[1]		
		BIT	Other	Total
4	0	9.73	0.11	9.84
	5	9.67	0.08	9.76
7	0	9.56	0.05	9.62
	5	9.75	0.05	9.80
9	0	9.75	0.04	9.79
	5	9.63	0.12	9.75

[1]Average of duplicate samples.

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER**

Annex Point IIA7.6.2.2

			Officialuse only
		1 REFERENCE	
	1.1 Reference	**A7.1.1.1.2/01** [redacted] June 2007 Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	OECD Draft Guideline: Phototransformation of Chemicals in Water – Direct and Indirect Photolysis (August 2000)	
	2.2 GLP	Yes	
	2.3 Deviations	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	^{14}C-BIT  * site of ^{14}C label	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As specified in the study guidelines, ^{14}C-material was employed.	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER**

Annex Point IIA7.6.2.2

	Specifications for the ^{14}C-material are listed below.	
3.1.3 Purity	[redacted]	
3.1.4 Radiolabelling	53.57 mCi/g	
3.1.5 UV/VIS absorption spectra and absorbance value	The absorption spectra for BIT at pH 5, 7, and 9 are presented in Figures A7.1.1.1.2-1, A7.1.1.1.2-2, and A7.1.1.1.2-3, respectively.	
3.1.6 Further relevant properties	• Water solubility is greater 0.7 g/L. • The Henry's Law constant is 1.45 x 10^{-5} Pa m^3 mol^{-1} • The compound is hydrolytically stable at pH 5, 7, 9	
3.2 Reference substances	No reference substances were employed to validate the photolysis study. The compounds listed in Table A7.1.1.1.2-1 were used as chromatography and mass spectral reference standards.	
3.3 Test solution	A ^{14}C-BIT stock solution was prepared by dissolving 20.018 mg with 16.85 mL of acetonitrile. A ^{12}C-BIT stock solution was prepared by dissolving 5.794 mg with 4.8 mL acetonitrile The pH 5, 7, and 9 buffers were prepared as follows. • pH4: 1.544g of ammonium acetate was dissolved in 1L of water and the pH adjusted with 0.05M NaOH • pH 7: 2.727g of KH_2PO_4 was dissolved in 1L of water and the pH adjusted using either 0.05M NaOH or 0.05M HCl • pH 9: 3.819g of $Na_2B_40_7$•$10H_2O$ was dissolved in 1L of water and the pH adusted with either 0.05M NaOH or 0.05M HCl Aliquots (25mL) of the buffer solutions were transferred to glass vessels and sterilized in an autoclave.	
3.4 Testing procedure		
3.4.1 Tier 1 Screen	The UV-VIS spectrum of BIT was recorded over the wavelength range of 295-800 at pH 5, 7, and 9 (Figures A7.1.1.1.2-1, A7.1.1.1.2-2, and A7.1.1.1.2-3, respectively). The loss of compound is calculated as follows: $Percent\ \ loss\ =\ 100[e^{KaT}]$ Eq 1 Where T = 30 days $Ka\ =\ \sum_{\lambda 297.5}^{\lambda 800} \varepsilon_\lambda L_\lambda$ Eq 2 ε_λ is the molar adsorption coefficient	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER**

Annex Point IIA7.6.2.2

		L_λ is the solar irradiance
3.4.2	Test System for Tier 2 and 4	Test Vessels The glass test vessels had an inlet and an outlet as well as an injection port. Their height was approximately 41 mm and the diameter approximately 24 mm (yielding an exposure surface of 452.6 mm^2). 25 mL buffer portions were added to each vessel. Vessels to be irradiated were fitted with quartz lids while the dark control vessels were sealed with a crimped PTFE-lined cap. Bacterial air filters were attached to the inlet and outlet prior to autoclaving the unit. Properties of the light source A Hanau Suntest Xenon lamp was used as the light source. Radiation below 290 nm was removed with a filter. The spectral properties and intensity was measured using an LI-1800 spectroradiometer. Traps To each irradiation unit four traps were attached to capture evolved volatiles. The traps contained ethanediol (25 g) to collect polar organic volatiles, 2% paraffin in xylene (25 g) to collect non-polar organic volatiles, and two 2M NaOH (25 g) to collect CO_2. Temperature The vessels were placed into a cooling block and the temperature maintained at 20 ± 3°C by circulating temperature controlled water through the block and thus around the vessels. The temperature on the dark control samples were maintained at 20 ± 3°C in a similar manner to the irradiated.
3.4.3	Tier 2 (preliminary kinetics)	For each pH, 6 vessels containing 25 mL of the buffer solution were prepared and dosed with either 0.1 µg/mL ^{14}C-BIT or 10 µg/mL ^{14}C-BIT. The system is described below.

Sample Type	^{14}C BIT µg/mL	Irradiated	Number of Samples
Time 0	0.1	NA	1
Day 1, 2, 7	0.1	Yes	1
Time 0	10	NA	1
Day 1, 2, 7	10	Yes	1
Dark Control	0.1	No	1
Dark Control	10	No	1

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER**

Annex Point IIA7.6.2.2

		NA = Not Applicable The experiment was initiated by injecting ^{14}C-BIT into the glass test vessel through the septum on the injection port. After application of BIT the Time 0 samples were removed, radioassayed and chromatographed. The samples to be irradiated were placed under the xenon lamp while the controls were placed in a dark chamber. On Days 1, 2, and 7 aliquots were removed from the irradiated samples, radioassayed, and chromatographed. The dark controls were only analyzed on Day 7. Selected samples were analyzed by LC-MS to confirm the presence of parent. The rate constant was determined by non-linear regression and the loss determined by equation 1 (section 3.4.1 above). The irradiation intensity was 42 Watts/m^2 (between 300 and 400 nm) resulting in the samples receiving the equivalent of 12 days of natural sunlight (30°N-50°N latitude) in the 7 days of xenon lamp exposure.	
3.4.4	Tier 4 (Definitive test)	For each pH, 22 glass vessels containing 25 mL of buffer solution were sterilized. To 16 sterile vessels a nominal 10 µg/mL ^{14}C-BIT was added through the injection port septum and the vessels gently swirled. Fourteen vessels were placed under the xenon lamp and the volatile traps connected. Humidified air was pulled through the system to remove volatiles from the test vessel. The remaining two dosed vessels were analyzed immediately as Time 0 samples. In addition the following samples were prepared; duplicate dark controls containing 10 µg/mL ^{14}C-BIT, duplicate samples without BIT to check the pH at Time 0, duplicate irradiated samples containing ^{12}C-BIT to check the pH and solution sterility at the end of the exposure period, and duplicate dark control samples containing ^{12}C-BIT to check the pH and solution sterility at the end of the exposure period. At various intervals, duplicate vessels were removed for analysis. Aliquots of solution were radioassayed and chromatographed (HPLC). In addition representative samples were analyzed by LC-MS to confirm the presence of parent and for identification of photodegradtes. The volatiles traps and a polyurethane bung placed between the glass vessel and the traps were radioassayed when their respective glass vessel was removed for analysis. The bung was soaked in acetonitrile and the extract radioassayed. The presence of CO_2 was confirmed in selected samples of the NaOH traps by precipitation with $BaCl_2$. The irradiation intensity was 25 Watts/m^2 (between 300 and 400 nm) resulting in the samples receiving the equivalent to 1 day of natural sunlight (30°N-50°N latitude) for every day of exposure under the xenon lamp.	
3.4.5	Duration of the test	The duration of the Tier 2 test was 7 days (equivalent to 12 days of natural sunlight) The duration of the Tier 4 test was 30 days (equivalent to 30 days of	

		natural sunlight)
3.4.6	Number of replicates	In the Tier 2 test single samples were employed In the Tier 4 test duplicate samples were employed
3.4.7	Sampling	In the Tier 1 test, irradiated samples were taken on Days 0, 1, 2 and 7. The dark control was analyzed on Day 7. In the Tier 4 test, the following schedule was employed for irradiated samples. • pH 5: 0, 2, 4, 8 hours and 1, 15 and 30 days • pH 7: 0, 0.5, 1, 2 hours and 1, 15, 30 days • pH 9: 0, 0.5, 1, 2 hours and 1, 15, 30 days The dark controls dosed with ^{14}C-BIT were analyzed on Day 30. Sterility and pH samples were analyzed at the start of the exposure period and on Day 30.
3.4.8	Analytical methods	Radioassay was performed using Packard liquid scintillation counter. Radiopurity and aliquots from the buffer solutions were analyzed by HPLC using a modified C-18 column and a binary gradient composed of 0.5% aqueous formic acid and 0.5% methanolic formic acid. Detection employed a ^{14}C-flow through monitor and/or UV detector (254 nm). Thin layer chromatography (TLC) was used for radiopurity determination. Silica gel plates (250 µm thick) were developed with ethyl acetate:methanol:acetonitrile:acetic acid (90:5:5:1). Solutions were cochromatographed with non-radiolabeled BIT. Radiolabeled compounds were detected using a phophorimager while non-labeled compounds visualized with a UV lamp (254 nm). Representative samples were analyzed by LC-MS (ion trap) to confirm the presence of parent. Analysis employed a modified C-18 column and a binary gradient composed of 0.5% aqueous formic acid and 0.5% methanolic formic acid. Detection was by a radioactivity flow monitor and the mass spectrometer. The LC effluent was split between the two detectors and introduction in to the MS via an API interface and positive and negative ionization were employed. For metabolite identification, accurate masses were obtained using an LC-Fourier Transform MS. A modified C-18 column was employed with a gradient consisting of 0.5% aqueous formic acid and 0.5% formic acid in acetonitrile. The LC effluent was introduced into the MS via an API interface and both positive and negative ionization was employed.
3.5	**Transformation products**	-

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER**

Annex Point IIA7.6.2.2

3.5.1	Method of analysis for transformation products	Transformation products were quantitated by HPLC and identified by LC-MS.	
		4 RESULTS	
4.1	**Tier 1**	The UV/VIS spectra for BIT at pH 5, 7, and 9 are presented in Figures A7.1.1.1.2-1, A7.1.1.1.2-2, and A7.1.1.1.2-3, respectively. The maximum possible rate constants determined for BIT at pH 5, 7, and 9 are 994 day^{-1}, 953 day^{-1}, and 965 day^{-1}, respectively. These rate constants predicte that photolysis could account for 100% loss of BIT over a 30 day period at all three pH's. Therefore additional testing is necessary.	
4.2	**Tier 2 (preliminary kinetics test)**	The distribution and recovery of ^{14}C-activity from Tier 2 testing is presented in Table A7.1.1.1.2-2. Over 94% of the applied activity remained in the buffer solution with less than 1% being found in volatile organic traps and less than 10% as evolved $^{14}CO_2$. Quantitation of BIT at Day 0, 1, 2, and 7 is presented in Table A7.1.1.1.2-3. The results demonstrate that photolysis could account for 100% loss of BIT within 30 days. Therefore additional testing, Tier 4, is required.	
4.3	**Tier 4 (definitive test)**		

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER**

Annex Point IIA7.6.2.2

4.4.1	Distribution and recovery	The results from the distribution and recovery of applied activity are presented in Table A7.1.1.1.2-4. The results are similar to that observed in the Tier 2 test. For pH 5 irradiated samples, over 93% of the applied activity was detected in the buffer solution. Less than 0.3% was found in the volatile organic traps and less than 4% in the CO_2 trap. For the dark control sample, 99.6% was detected in the buffer solution and no volatiles were detected. The mean recovery of ^{14}C-activity was 98.8 ± 2.2%. The results for the pH 7 irradiated samples were similar to pH 5. Over 86% of the applied activity was detected in the buffer solution. Less than 0.5% was in the volatile organic traps. By Day 30, 9.1% of the applied activity was present as CO_2. For the dark control, 99.8% was detected in the buffer solution with no volatiles detected. The mean recovery of ^{14}C was 98.5 ± 1.7%. Over 89% of the applied activity from the irradiated pH 9 samples was detected in the buffer solution. Less than 0.7% was detected in the volatile organic traps. On Day 30, CO_2 accounted for 6.9% of the applied activity. For the dark controls, 98.9% was detected in the buffer solution with no volatiles detected. The mean recovery of ^{14}C-activity was 97.8 ± 2.1%	
4.4.2	Quantitation of BIT and photoproducts	The quantitation of BIT and its photoproducts at various sampling intervals is presented in Tables A7.1.1.1.2-5, A7.1.1.1.2-6, and A7.1.1.1.2-7 for pH 5, 7, and 9, respectively. BIT rapidly photolyzed so that by Day 15 there was no parent remaining at any pH tested. At pH 5, after two hours of irradiation there remained 85.7% of the applied activity as BIT while at pH 7 and 9, 13.1% and 20.1% remained. There were seven major photodegradates detected. One cochromatographed with the standard, 2-sulfobenzoic acid (2-SBAH). At pH 5 the major degradates were 2-SBAH, Unknown A and Unknown B. At pH 7 the major degradates were 2-SBAH, Unknown B, and Unknown E. At pH 9 the major degradates were 2-SBAH, Unknown B, Unknown C, Unknown E, and Unknown M. At all pH's Unknown B is transient probably serving as a precursor for a subsequent product. No apparent degradation was observed in the dark control samples. On Day 30 BIT comprised 97.8%, 98.2% and 95.6% of the applied activity at pH 5, 7, and 9, respectively.	
4.4.3	Kinetics	The kinetic results are tabulated below.	

Parameter	pH 5	pH 7	pH 9
k (day^{-1})	1.813	22.879	23.833

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER**

Annex Point IIA7.6.2.2

DT_{50}(h)	9	0.7	0.7
DT_{75} (h)	18	1.4	1.4
DT_{90} (h)	30	2.4	2.4
R^2	0.992445	0.996478	0.988083

Figure A7.1.1.1-4 provides a graphical representation of the natural log decline of BIT at pH 5, 7, and 9.

4.4.4 Confirmation of BIT and Identification of the Degradation Products

Using LC-MS, the presence of BIT in selected samples was confirmed.

Identification of the photodegradation products was undertaken using LC-MS. A summary of the results is presented in Table A7.1.1.1.2-8 providing the structures, names, and maximum percentage of each photodegradate. One photodegradate was initially identified as 2-sulfobenzoic acid (2-SBAH) based on cochromatography with a standard. However LC-MS analysis demonstrated that 2-SBAH was a minor component of this fraction with 2-sulfobenzamide being the major component.

Unknown D has two possible structures; dihydroxylated BIT (hydroxylation of the benzene ring) and the benzene ring mono-hydroxylated sulfoxide. Fragmentation, even from daughter ions (MS/MS), was not sufficient to assign the absolute structure and both photoproducts have the same exact mass. Thus it was not possible to differentiate between these two possibilities.

It was not possible to assign absolute structures to Unknown E and Unknown M.LC-MS did demonstrate that they contained multiple components and probably no single component was greater than 10% of the applied activity.

4.4.5 Photolytic pathway

A photolytic pathway is presented in Figure A7.1.1.1.2-4.

4.4.6 pH and sterility

The solution pH was measured pre and post-irradiation and is provided below.

Interval	Mean Solution pH		
	pH 4	pH 7	pH 9
Pre-irradiation	4.96	7.03	9.02
Post-irradiation	5.08	7.04	8.72
Dark control: post-irradiation	5.32	7.04	9.04

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER**

Annex Point IIA7.6.2.2

	Aliquots of the Day 30 irradiated and dark control solutions were checked for sterility on nutrient agar plates. No colony forming units were detected in any solutions.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The test guidelines employed were OECD Draft Guideline: Phototransformation of Chemicals in Water – Direct and Indirect Photolsysis (August 2000). An initial screen involving an analysis of the UV/VIS spectrum showed that BIT could substantially photodegrade so additional testing was performed. A preliminary kinetic test was performed by adding sterile pH 5, 7, or 9 buffer to a test vessel, dosing at 0.1 µg/mL and 10 µg/mL BIT, and irradiating the sample using a xenon lamp. The solution was analyzed on Days 0, 1, 2, and 7. The results showed that additional testing was warranted. A definitive photolysis study was undertaken by preparing photolysis vessels with either sterile pH 5, 7, or 9 buffer. The vessels were dosed at 10 µg/mL, a series of traps designed to capture volatile organic and evolved CO_2 were attached to each vessel, a stream of sterile moistened air was pulled through the system, and the vessels irradiated with a xenon lamp. pH 5 samples were removed at 0, 2, 4, and 8 hours and 1, 15, and 30 days. pH 7 and 9 samples were removed at 0, 0.5, 1, and 2 hours and 1, 15, and 30 days. Samples and their traps were radioassayed. Aliquots of the buffer solutions were chromatographed (HPLC) to quantitate parent and photodegradates. Photodegradates were identified by LC-MS	
5.2 Results and discussion	BIT rapidly photodegrades and the rate is dependent on pH. The photolytic half-life in pH 5 buffer was 9 hours while in pH 7 and 9, 0.7 hours. Organic volatiles were less than 1% of the applied activity and evolved CO_2 less than 10%. On average, the recovery of applied radioactivity in the definitive study was over 98%. The major photoproducts were: • 2-sulfobenzamide (small quantities of 2-sulfobenzoic acid cochromatographed) • 1,2-benzthiazolin-2-one • hydroxy-1,2-benzisothiazolin-3-one • Saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide) • Dihydroxy-1,2-benzisothiazolin-3-one or hydroxy-1,2-benzisothiazolin-3-one-1-oxide • Unknown E: unable to assign a structure but it contained multiple components • Unknown M: unable to assign a structure but it contained	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER**

Annex Point IIA7.6.2.2

		multiple components	
5.3	**Conclusion**	This study fulfils the requirement for determining the effect of aqueous photolysis on the fate of BIT in the environment. The half-life at pH 5 is 9 hours and at pH 7 and 9, 0.7 hours. Photodegradation of BIT involves cleavage of the isothaizolone ring, hydroxylation of the benzene ring, and/or oxidation of the sulfur.	
5.3.1	Reliability	1-valid without restrictions	
5.3.2	Deficiencies	None	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*November 2010*
Materials and Methods	*Applicant´s version is accepted with the following remarks:* *Samples are duplicated only in Tier 4. In Tier 2, two substanceconcentrations were tested, but only one replicate for each concentration was analysed.* *Test solution: pH 4 should be pH 5* *At the pH and sterility section, Table should read pH 5 and not pH4.* *Testing procedure: Tier 1 screen, Eq 1 is not correct.* *Only an aliquot of 1ml was removed for sampling at day 1 and day 3,instead of using an entire irradiated photolysis cell at each samplinginterval. In addition, dark control was only analysed in day 7, instead ofbeing analysed at each sampling interval.* *Transformation products are identified and quantified, but there is no information about the degradation rate of these products.* *The following sentence should be added in "Testing Procedure-Tier 1Screen" section:* *"The extent of overlap between the absorption bands of the substance and the spectral distribution of the inciden sunlight gave and indication of the potential for photolysis. The result showed that photolysis could account for 100% loss of the substance over the equivalent of 30 days, so further testing was performed".*
Results and discussion	*Accepted.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2 PHOTOTRANSFORMATION IN WATER

Annex Point IIA7.6.2.2

Conclusion	*This study fulfils the requirement for determining the effect of aqueous photolysis on the fate of BIT in the environment. The half-life at pH 5 is 9 hours and at pH 7and 9, 0.7 hours. Photodegradation of BIT involves cleavage of the isothaizolone ring, hydroxylation of the benzene ring, and/or oxidation of the sulfur.*
Reliability	*2*
Acceptability	*Acceptable.*
Remarks	

Table A7.1.1.1.2-1: Chromatographic Reference Standards

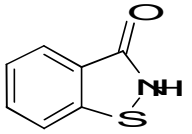
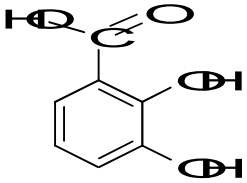
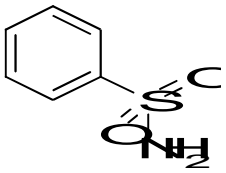
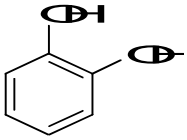
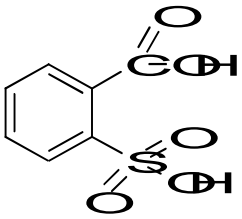
Chemical name	Abbreviation used	Sponsor lot number	Purity (%)	Expiry date	Structure
1,2-Benzisothiazolin-3-one	BIT	[redacted]	[redacted]	18 April 2012	
2,3-Dihydroxybenzoic acid	2,3-DHBA	[redacted]	[redacted]	16 Nov 2008	
Benzene sulphonamide	BS	[redacted]	[redacted]	30 Nov 2008	
Catechol	NA	[redacted]	[redacted]	29 Nov 2008	
2-Sulfobenzoic acid hydrate	2-SBAH	[redacted]	[redacted]	16 Feb 2009	

Table A7.1.1.1.2-2: Distribution and Percent Recovery from Tier 2 (Preliminary Kinetics) Test

Conditions	Sample Day	Percent of Applied Activity			
		Solution[1]	Volatile Organic Traps[2]	NaOH	Recovery
pH 5					
Light	0	103.1	NA[3]	NA	103.1
	1	101.6	ND[3]	MD	101.6
	2	99.1	ND	0.1	99.2
	7	101.3	0.1	0.8	102.1
Dark	7	102.3	NA	ND	102.2
pH 7					
Light	0	102.3	NA	NA	102.3
	1	99.1	ND	0.1	99.2
	2	97.1	ND	0.9	97.9
	7	94.7	0.2	5.4	100.3
Dark	7	101.0	NA	NA	100.9
pH 9					
Light	0	101.6	NA	NA	101.5
	1	101.3	ND	7.3	116.9[4]
	2	96.9	ND	7.4	113.7[4]
	7	99.7	ND	8.9	124.4[4]
Dark	7	99.0	NA	NA	106.0

[1] Buffer solution plus rinse of glass vessel
[2] Combined results of the Ethanediol trap + Paraffin/Xylene trap + polyurethane bung
[3] NA = Not Applicable; ND= Not Detected
[4] The high values may be due to contamination of the first sodium hydroxide trap.

Table A7.1.1.1.2-3: Quantitation of BIT in Tier 2 (Preliminary Kinetic) Test

Conditions	Sample Day	Percent BIT at Dose Rate	
		0.1 µg BIT/mL	10 µg BIT/mL
pH 5			
Light	0	103.4	100.6
	1	6.9	5.8
	2	2.1	ND
	7	ND	ND
Dark	7	102.6	99.6
pH 7			
Light	0	102.9	99.5
	1	3.5	0.4
	2	2.4	0.7
	7	ND	ND
Dark	7	100.6	97.7
pH5			
Light	0	96.5	100.0
	1	8.4	ND
	2	1.8	0.3
	7	2.6	ND
Dark	7	99.5	93.1

Table A7.1.1.1.2-4: Distribution and Percent Recovery from Tier 4 (Advanced) Test

Conditions	Sample Interval	Percent of Applied Activity[1]			
		Solution[2]	Volatile Organic Traps[3]	NaOH	Recovery
pH 5					
Light	0	100.0	NA[4]	NA	99.9
	2 h	98.3	0.2	ND	98.5
	4 h	99.8	0.1	ND	99.9
	8 h	100.4	0.1	ND	100.5
	1 day	98.6	ND[4]	ND	98.6
	15 days	93.2	0.1	2.4	95.5
	30 days	94.2	0.1	3.5	97.7
Dark	30 days	99.6	NA		99.6
pH 7					
Light	0	100.2	NA	NA	100.2
	0.5 h	98.9	0.2	ND	98.8
	1 h	99.0	0.4	ND	99.4
	2 h	98.7	ND	ND	98.7
	1 day	98.5	ND	0.1	98.6
	15 days	90.3	0.1	6.7	97.1
	30 days	86.6	0.1	9.1	95.7
Dark	30 days	99.8	NA	NA	99.8
pH 9					
Light	0	99.6	NA	NA	99.6
	0.5 h	98.6	0.2	ND	98.7
	1 h	96.8	0.6	ND	97.4
	2 h	98.7	ND	ND	98.6
	1 day	98.8	0.1	ND	98.9

Conditions	Sample Interval	Percent of Applied Activity[1]			
		Solution[2]	Volatile Organic Traps[3]	NaOH	Recovery
	15 days	90.9	ND	3.3	94.1
	30 days	89.6	ND	6.9	96.5
Dark	30 days	98.9	NA	NA	98.9

[1] Average of duplicate samples
[2] Buffer solution plus rinse of glass vessel
[3] Combined results of the Ethanediol trap + Paraffin/Xylene trap + polyurethane bung
[4] NA = Not Applicable; ND= Not Detected

Table A7.1.1.1.2-5: Quantitation of BIT and its Photodegradates—pH 5

Conditions	Sample Interval	Quantitation of BIT and Photodegradates as a Percent of Applied Activity[1]									
		BIT	2-SBAH	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Unknown M	Other[2]	Total
Light	0	98.7	ND[3]	ND	ND	ND	ND	ND	ND	0.7	99.5
	2 h	85.7	ND	4.9	6.3	ND	ND	ND	ND	0.6	97.6
	4 h	76.8	0.6	10.1	11.4	ND	ND	ND	ND	0.5	99.4
	8 h	55.1	2.5	19.6	21.7	0.2	ND	0.1	ND	0.7	99.9
	1 day	14.0	7.8	39.9	34.3	ND	ND	ND	1.1	1.0	98.2
	15 days	ND	17.1	46.7	19.4	2.4	1.0	2.5	2.3	1.1	92.7
	30 days	ND	22.7	49.8	9.1	2.5	1.1	4.1	3.3	1.3	93.9
Dark	30 days	97.8	ND	ND	ND	ND	ND	ND	ND	1.3	99.1

[1] Average of duplicate samples
[2] Other = Total Other Unknowns and Unresolved Background
[3] ND = Not Detected

Table A7.1.1.1.2-6: Quantitation of BIT and its Photodegradates—pH 7

Conditions	Sample Interval	Quantitation of BIT and Photodegradates as a Percent of Applied Activity[1]									
		BIT	2-SBAH	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Unknown M	Other[2]	Total
Light	0	98.7	ND	ND	ND	ND	ND	ND	ND	1.1	99.8
	0.5 h	62.2	2.6	0.8	31.3	0.3	ND	ND	0.3	0.7	98.3
	1 h	38.9	4.8	1.6	49.9	0.7	ND	0.8	0.7	1.3	98.6
	2 h	13.1	12.0	2.6	65.4	1.5	0.6	ND	1.8	1.1	98.1
	1 day	0.7	25.2	3.5	51.0	2.8	2.8	2.7	6.9	2.3	98.0
	15days	ND	56.4	4.6	ND	3.0	6.1	11.9	6.8	1.1	89.9
	30 days	ND	53.0	3.7	ND	1.8	5.8	13.8	6.1	1.9	86.2
Dark	30 days	98.2	ND	ND	ND	ND	ND	ND	ND	1.2	99.4

[1] Average of duplicate samples
[2] Other = Total Other Unknowns and Unresolved Background
[3] ND = Not Detected

Table A7.1.1.1.2-7: Quantitation of BIT and its Photodegradates—pH 9

Conditions	Sample Interval	Quantitation of BIT and Photodegradates as a Percent of Applied Activity[1]									
		BIT	2-SBAH	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Unknown M	Other[2]	Total
Light	0	97.7	ND	ND	0.4	ND	ND	ND	ND	1.1	99.1
	0.5 h	54.0	4.6	ND	34.8	1.0	0.7	1.1	1.1	0.7	98.1
	1 h	33.5	7.5	ND	48.4	1.4	1.3	1.8	1.3	1.2	96.4
	2 h	20.1	10.7	ND	59.0	2.2	2.5	2.0	0.9	0.5	98.0
	1 day	0.2	27.2	ND	41.4	9.6	4.8	3.0	10.0	2.1	98.4
	15days	ND	39.5	ND	ND	12.1	8.7	15.7	10.4	2.9	89.2
	30 days	ND	36.9	ND	ND	13.2	7.8	26.1	4.6	0.7	89.3
Dark	30 days	95.6	ND	ND	ND	ND	ND	ND	ND	2.8	98.4

[1] Average of duplicate samples
[2] Other = Total Other Unknowns and Unresolved Background
[3] ND = Not Detected

Table A7.1.1.1.2-8: Major Photodegradates Detected, Their Structures, and Maximum Percentage Detected

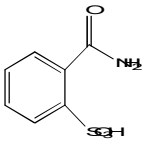
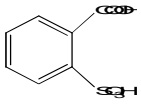
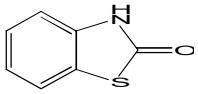
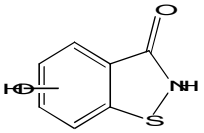
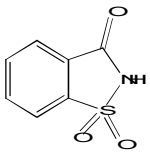
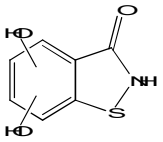
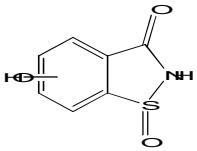
Designation	Structure	Maximum Mean Percent		
		pH 5	pH 7	pH 9
2-SBAH	major component  2-sulfobenzamide minor component  2-sulfobenzoic acid	22.7 (30 days)	56.4 (15 days)	39.5 (15 days)
Unknown A	 1,2-benzthiazolin-2-one	49.8 (30 days)	4.6 (15 days)	ND
Unknown B	 hydroxy-1,2-benzisothiazolin-3-one	34.3 (1 day)	65.4 (2 hours)	59.0 (2 hours)
Unknown C	 Saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide)	2.5 (30 days)	3.0 (15 days)	13.2 (30 days)
Unknown D	 or  dihydroxy-1,2-benzisothiazolin-3-one; hydroxy-1-2-benzisothiazolin-3-one-1-oxide	1.1 (30 days)	6.1 (15 days)	8.7 (15 days)
Unknown E	Multiple components that are chromatographically very polar	4.1 (30 days)	13.8 (30 days)	26.1 (30 days)
Unknown M	Unable to assign structures despite having exact mass information	3.3 (30 days)	6.9 (1 day)	10.4 (15 days)

Figure A7.1.1.1.2-1: UV Absorption Spectrum of BIT in pH 5 Buffer Solution

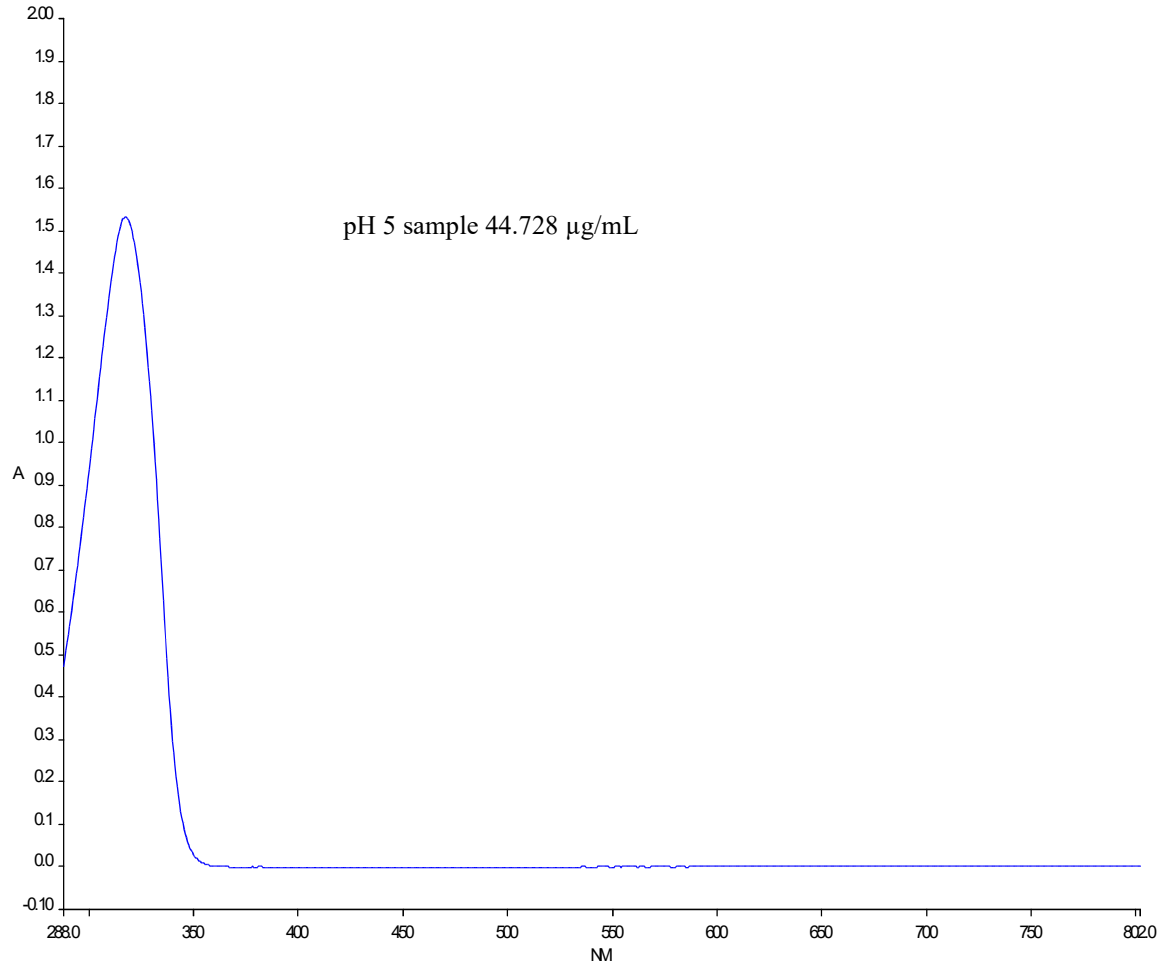


Figure A7.1.1.1.2-2: UV Absorption Spectrum of BIT in pH 7 Buffer Solution

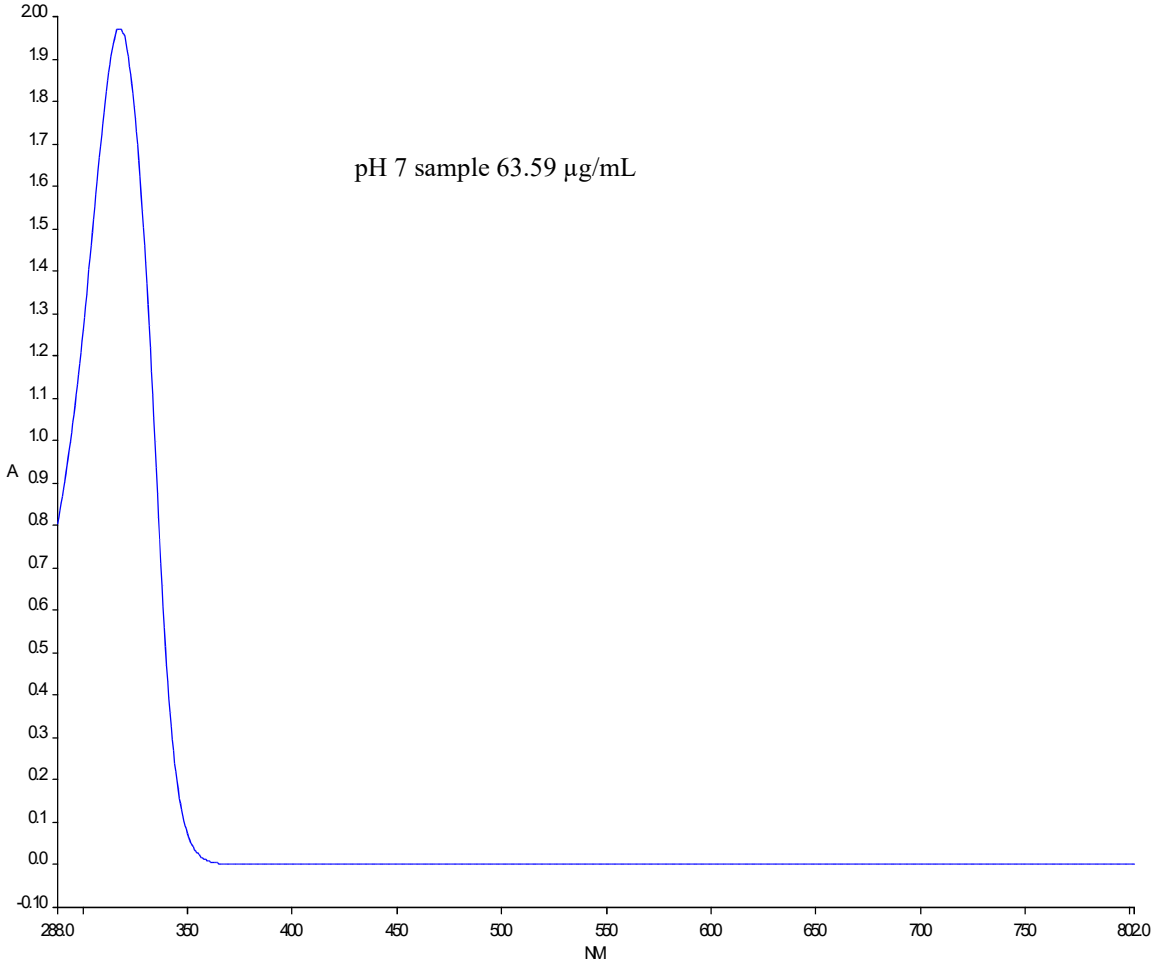


Figure A7.1.1.1.2-3: UV Absorption Spectrum of BIT in pH 9 Buffer Solution

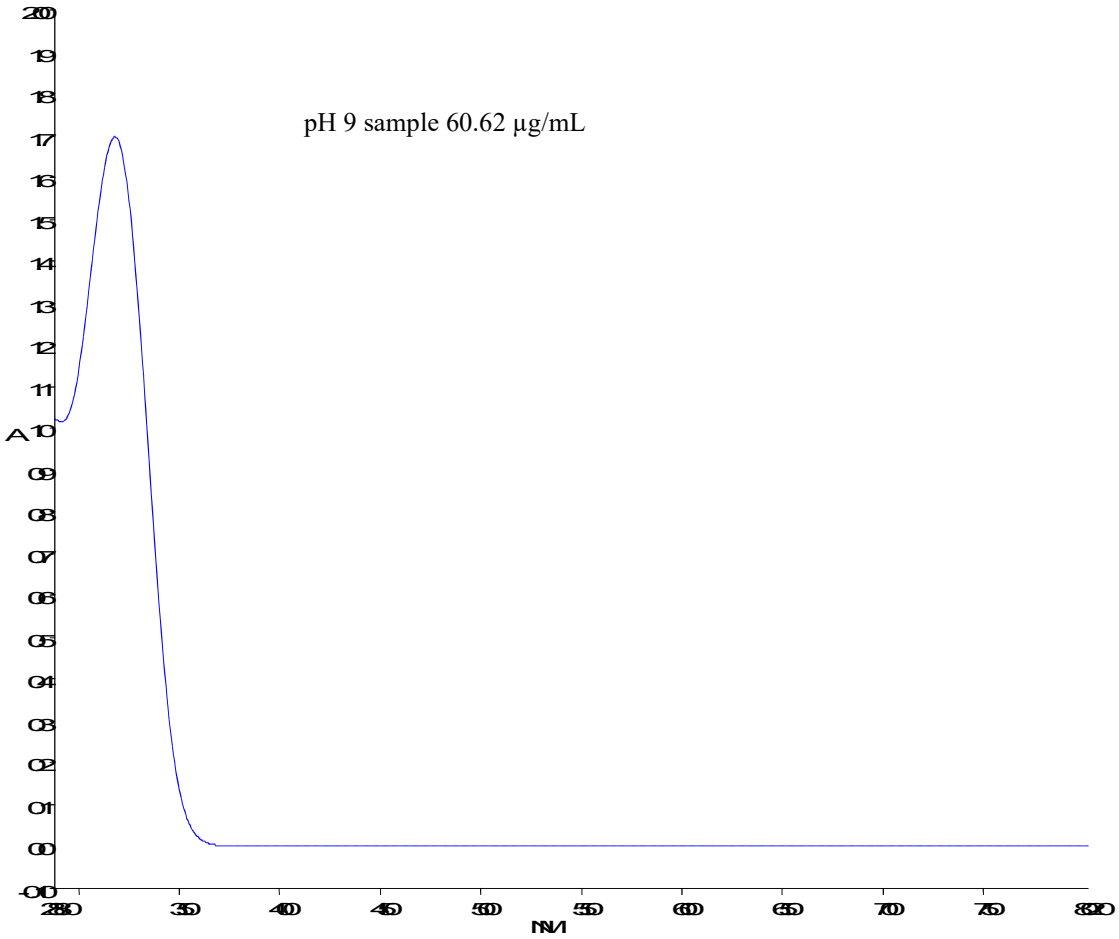


Figure A7.1.1.1.2-4: Dissipation of Parent Compound

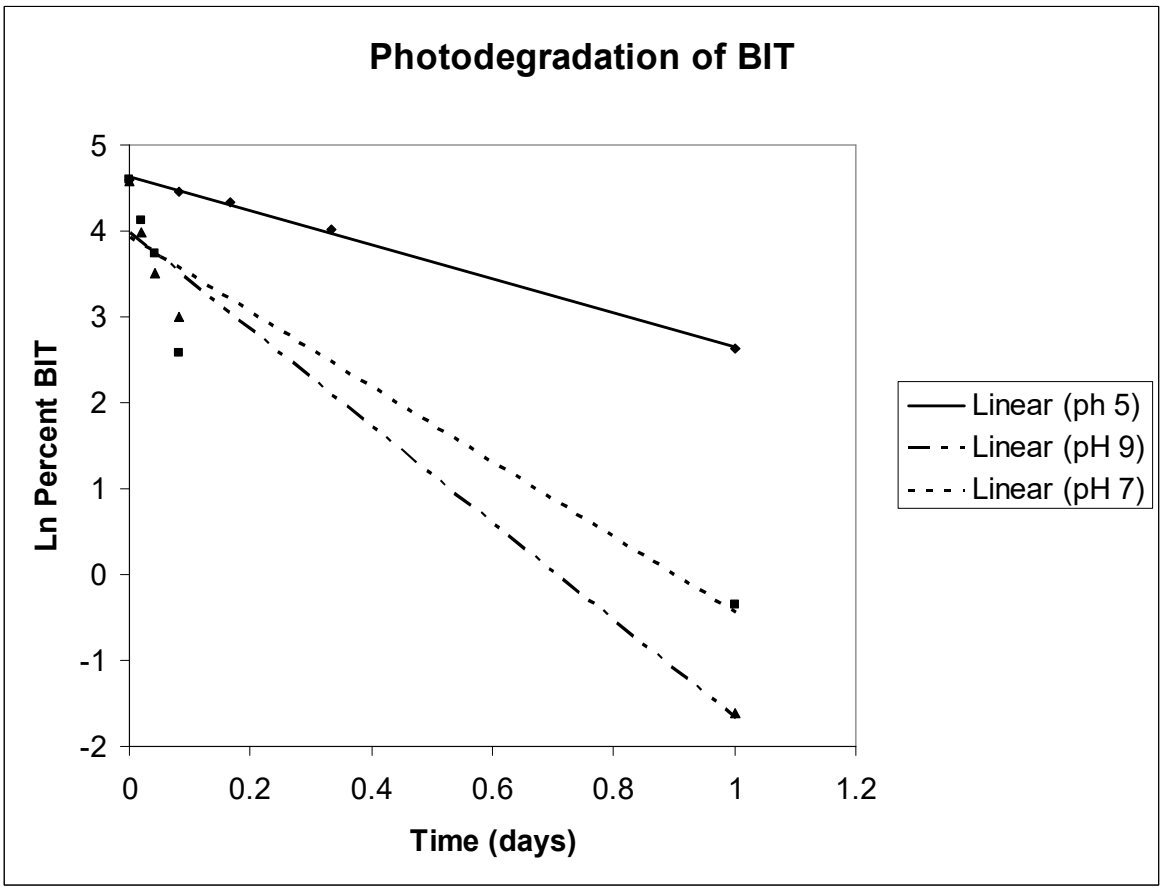
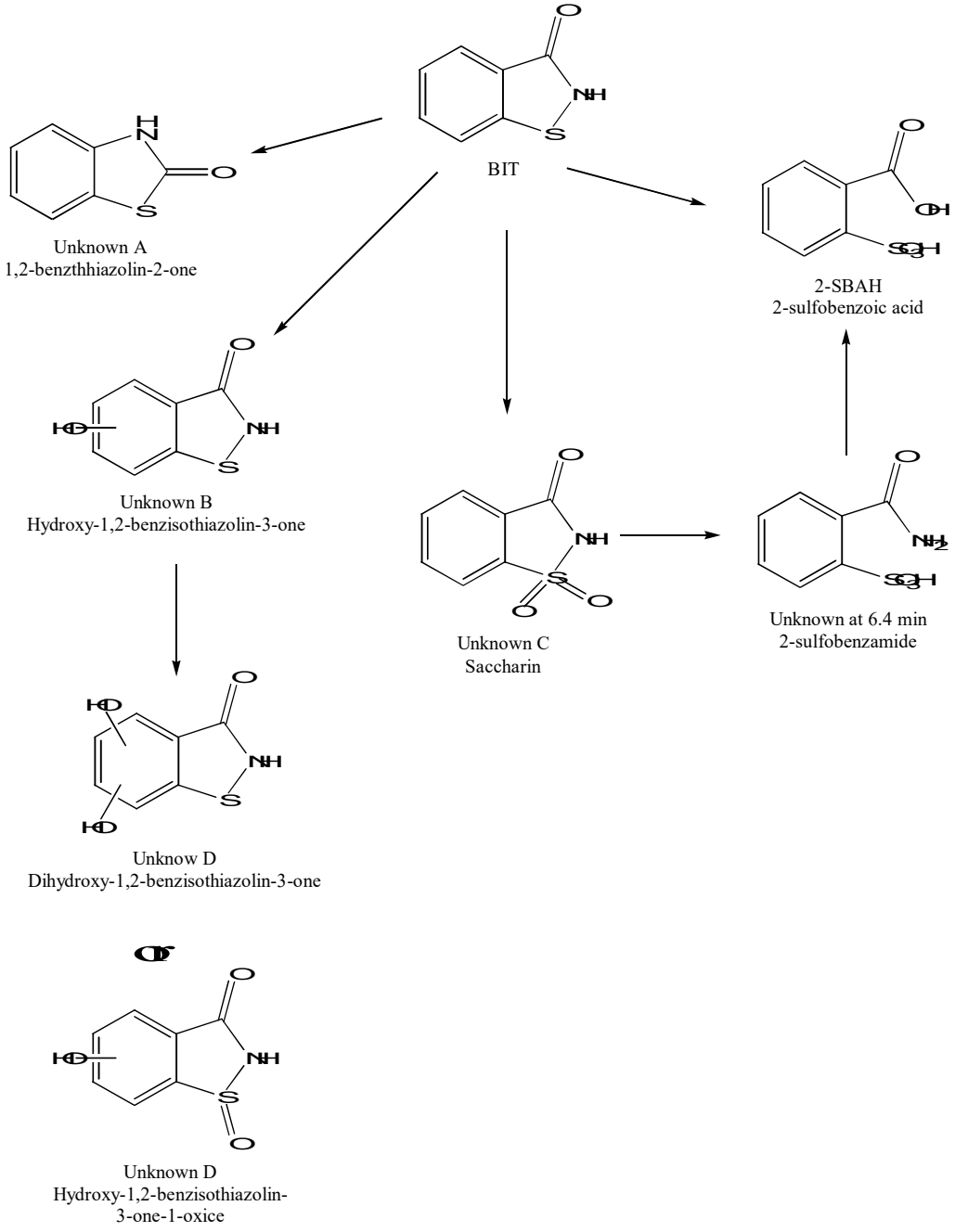


Figure A7.1.1.1.2-5: Aqueous Photolytic Degradation Pathway of BIT



Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.1 BIODEGRADABILITY (READY) (01)

Annex Point IIA7.6.1.1

		1 REFERENCE	Officialuse only
	1.1 Reference	**A7.1.1.2.1/01** [redacted] (April 24, 2006), unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes. OECD No. 301B Ready Biodegradability: CO_2 Evolution (Modified Sturm Test), 1992; 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	1,2-Benzisothiazolin-3-one (BIT)	
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	Solubility in water : > 0.7 g/L Vapor pressure : 2.3 x 10^{-4} Pa at 25°C	
3.1.5	Composition of Product	Not applicable.	

Section A7 Subsection A7.1.1.2.1 Annex Point IIA7.6.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviour BIODEGRADABILITY (READY) (01)	
3.1.6 TS inhibitory to microorganisms	In an activated sludge respiration inhibition test (OECD 209), BIT had an NOEC of 1-3 mg/L (see section A7.4.1.4). BIT is a biocidal active substance and as such, inhibitory to microorganisms (see section A5).	
3.1.7 Specific chemical analysis	Total inorganic carbon was quantitated by a TOC analyzer (Shimadzu TOC-5000A) equipped with an autosampler.	
Reference substance	Yes. Sodium Benzoate.	
3.2.1 Initial concentration of reference substance	25.7 mg/L	
3.2 Testing procedure		
3.3.1 Inoculum / test species	Aerobic activated sludge was obtained from a wastewater treatment facility [redacted] treating primarily domestic wastewater (Table A7.1.1.2.1-1). The sludge was washed twice via centrifugation with tap water and the liquid supernatant phase was decanted. A homogenized aliquot of the final sludge suspension was weighed, thereafter dried and the ratio of wet to dry weight was calculated. Sludge was used at a final concentration of 30 mg dry material per liter.	
3.3.2 Test system	The test system is described in Table A7.1.1.2.1-2.	
3.3.3 Test conditions	Table A7.1.1.2.1-3 describes the test conditions including the composition of the aqueous mineral salts medium, temperature, pH, and aeration. To each of nine 5 L flasks, approximately 2400 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, 17.9-18.2 mg/L of the test item, BIT (10.0-10.1 mg TOC/L), 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 (15 mg OC/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 control blank). The flasks were made up to a volume of three liters with test water. Inoculum was not added to the abiotic control and the abiotic control blank. The test vessels were incubated in a dark room at 20-22 °C. pH of the test flasks solutions was measured on day 0 and again on day 28. The pH measured on Day 0 was between 7.6 and 7.7 and on Day 28 (end	

Section A7 **Subsection A7.1.1.2.1** **Annex Point IIA7.6.1.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **BIODEGRADABILITY (READY) (01)**	
	of exposure) between 7.6 and 7.8.	
3.3.4 Initial TS concentration	17.9 – 18.2 mg/L (10.0 – 10.1 mg total organic carbon/L)	
3.3.5 Duration of test	28 days (exposure period).	
3.3.6 Analytical parameter	CO_2 produced from degradation of test substance measured by TOC analyzer.	
3.3.7 Sampling	On Days 2, 6, 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 off any residual CO_2 into absorber allowing for quantitation of dissolved CO_2.	
3.3.8 Intermediates/ degradation products	Not identified	
3.3.9 Nitrate/nitrite measurement	No.	
3.3.10 Controls	Toxicity control: 18.2 mg/L BIT (Test item) and 25.7 mg/L Sodium Benzoate (Reference item). Procedure control: 25.7 mg/L Sodium Benzoate (Reference item) Abiotic control : 18.2 mg/L BIT (test item) poisoned with 10 mg/L $HgCl_2$ Inoculum control : neither test item nor reference item Abiotic control blank: neither test item nor reference item added. Flasks were poisoned with 10 mg/L $HgCl_2$	
3.3.11 Statistics	IC content in absorber flask : mg IC[1] = IC in absorber x Volume of absorber IC removed in analytical samples : mg IC in sample = IC in absorber x Volume of sample IC produced by Test flask : mg IC produced = mg IC + ∑mg IC in sample $\%deg = \frac{mgICproducedintestflask - mgICproducedinblank}{mgTOC} x100^{l}$ *IC= inorganic carbon*	
	4 RESULTS	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.1 BIODEGRADABILITY (READY) (01)

Annex Point IIA7.6.1.1

	4.1 Degradation of test substance	
4.1.1	Graph	The percent biodegradation for flasks containing BIT (2 replicate flask), sodium benzoate (2 replicate flask), BIT + sodium benzoate, and BIT + $HgCl_2$ is presented in Table A7.1.1.2.1-4 and Figure A7.1.1.2-1. The percent biodegradation of the test item was calculated based on a total carbon content (TOC) of 0.56 mg C/mg BIT. The CO_2 produced in flask containing only BIT was slightly less than that of the inoculum controls (no additions). Consequently BIT was not ready biodegradable under the test conditions within 28 days. In the abiotic control (BIT + $HgCl_2$) no significant degradation was observed at the end of the 28 day test period (i.e. <10% of the TOC). 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 78% by day 14 thus confirming the suitability of the activated sludge (> 60% by Day 14). By Day 28 the sodium benzoate was biodegraded to an average extent of 85%. The extent of biodegradation of sodium benzoate in the presence of BIT was slightly delayed over the course of the experiment compared to sodium benzoate alone.
4.1.2	Degradation	% degradation = $\frac{\text{mg } IC_{prod} \text{ in the test flask - mg } IC_{prod} \text{ in blank}}{\text{mg TOC}} \times 100$

Flask Description	% degradation at the end of incubation (mean)
Test item[1]	-19.0
Procedure control (Sodium Benzoate)[1]	85.4
Toxicity control[1]	35.8
Abiotic control[2]	2.4

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

4.1.3	Degradation of TS in abiotic control	Degradation of BIT in abiotic control corresponds to approximately 3 %.
4.1.4	Degradation of reference substance	See Figure A7.1.1.2-1.

Section A7 Subsection A7.1.1.2.1 Annex Point IIA7.6.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviour BIODEGRADABILITY (READY) (01)	
4.1.5 Intermediates/ degradation products	Not applicable.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	BIT 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: Ready Biodegradation: CO_2 Evolution (Modified Sturm Test), 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, 17.9-18.2 mg/L of the test item, BIT, 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 20-22°C. On Days 2, 6, 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, BIT, was found to be not ready biodegradable under the test conditions within 28 days. In the abiotic control containing BIT and $HgCl_2$, no significant degradation was noted at the end of the 28-day exposure period (<10 %). In the toxicity control containing both BIT 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 78 % by exposure day 14, confirming suitability of the activated sludge. By the end of the test, the reference item was degraded 85%.	
5.3 Conclusion	BIT 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	

Section A7
Subsection A7.1.1.2.1
Annex Point IIA7.6.1.1

Ecotoxicological Profile Including Environmental Fate and Behaviour
BIODEGRADABILITY (READY) (01)

		for further tests.	
5.3.1	Reliability	1-valid without restrictions.	
5.3.2	Deficiencies	No.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2010*
Materials and Methods	*3.3. Testing procedure* *3.3.1. Inoculum test/species: heading Table A7.1.2.3./01-1 should be A7.1.1.2.1-1* *3.3.2. Test system: heading Table A7.1.2.3./01-2 should be A7.1.1.2.1-2* *3.3.3. Test conditions: heading Table A7.1.2.3./01-3 should be A7.1.1.2.1-3*
Results and discussion	*Applicant's version is accepted, but with the following comments:* *The percentage of biodegradation shows a negative biodegradation rate, compared to the inoculum control.*
Conclusion	*BIT was found to be not biodegradable under the tests conditions within 28 days.* *BIT at the concentration used to fulfill the requirements of test OECD 301B seems to be toxic to the inoculum.* *In the toxicity control, containing both 1,2-Benzisothiazolin-3-one and the reference item sodium benzoate, no inhibitory effect on the biodegradation of the reference item was determined. Thus 1,2-Benzisothiazolin-3-one had noinhibitory effect on the activity of activated sludge microorganisms at the testedconcentration of 18 mg/L.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.1.2.3/01-1: Inoculum

Criteria	Details
Nature	Activated sludge

Source	Wastewater treatment plant treating predominantly domestic wastewater
Sampling site	[redacted]
Preparation of inoculum	Sludge was washed twice with tap water by centrifugation and the supernatant liquid phase decanted.
Pretreatment	Sludge was added to mineral salt solution and aerated with CO_2 free air overnight prior to addition of test compound
Concentration	30 mg of washed sludge on a dry weight basis/L

Table 7.1.2.3/01-2: Test System

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Number and Nature of Culture Flask</td><td>Nine 5L flask were dosed as below.
<table>
<tr><th>Identification</th><th>mg/L Test Item</th><th>mg/L Reference Item</th><th>mg/L $HgCl_2$</th><th>Inoculum Added</th></tr>
<tr><td>Test Flask</td><td>18.0</td><td></td><td></td><td>+</td></tr>
<tr><td>Test Flask</td><td>17.9</td><td></td><td></td><td>+</td></tr>
<tr><td>Abiotic Control</td><td>18.2</td><td></td><td>10</td><td>-</td></tr>
<tr><td>Toxicity Control</td><td>18.2</td><td>25.7</td><td></td><td>+</td></tr>
<tr><td>Ref. Control</td><td></td><td>25.7</td><td></td><td>+</td></tr>
<tr><td>Ref. Control</td><td></td><td>25.7</td><td></td><td>+</td></tr>
<tr><td>Inoculum Control</td><td></td><td></td><td></td><td>+</td></tr>
<tr><td>Inoculum Control</td><td></td><td></td><td></td><td>+</td></tr>
<tr><td>Abiotic Blank</td><td></td><td></td><td>10</td><td>-</td></tr>
</table></td></tr>
<tr><td>Aeration Device</td><td>CO_2-free air is passed through the 5 liter flask and into traps at a rate of 30-100 mL/min.</td></tr>
<tr><td>Measuring equipment</td><td>TOC analyzer (Shimadzu TOC-5000A)</td></tr>
<tr><td>Trapping System</td><td>From the exit line of each flask, two 0.05 M NaOH traps were placed in series to capture evolved CO_2. At sampling, 5 mL aliquots were taken from the first trap for assaying.On Day 15 and 28 a 5 mL aliquot was also taken from the second NaOH trap to correct for carry-over.</td></tr>
<tr><td>Test performed in closed vessels due to significant volatility of test substance</td><td>No</td></tr>
</table>

Table A7.1.2.3/01-3: Test Conditions

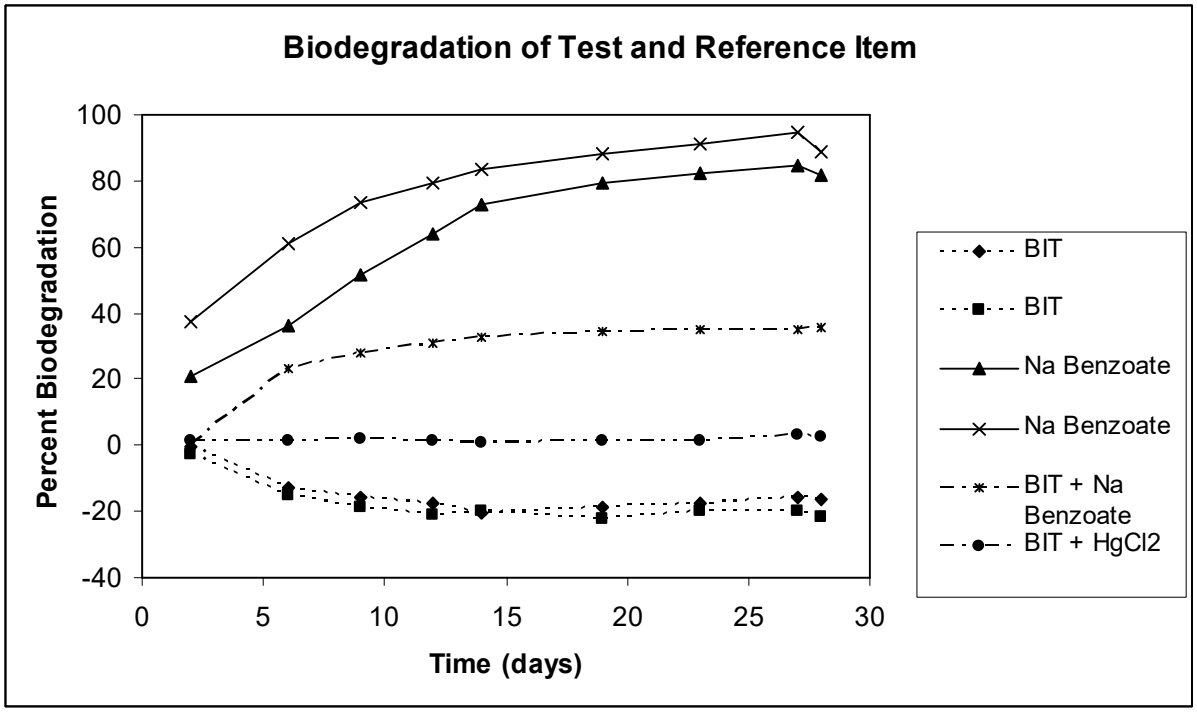
Criteria	Details
Composition of test medium	Stock solutions using analytical grade salts a) KH_2PO_4: 8.50 g/L K_2HPO_4: 21.75 g/L $Na_2HPO_4•2H_2O$ 33.40 g/L NH_4Cl: 0.50 g/L b) $MgSO_4•7H_2O$: 22.50 g/L c) $CaCl_2•2H_2O$: 36.40 g/L d) $FeCl_3•6H_2O$: 0.25 g/L One drop of concentrated HCl was added to solution d) as a preservative. The final testing solution was prepared by adding 10 mL of solution a) and 1 mL of solutions b), c), and d) to 800 mL of purified water. The solution was then made up to 1000 mL with purified water and the pH adjusted to 7.4 with dilute HCl.
Inoculum	The day before the addition of BIT, 90 mL of activated sludge inoculum was added to between 2400-3000 mL of the mineral salt test medium.
Additional substrates	No
Test temperature	20-22°C (temperature controlled room)
pH	At the start the pH in the test samples ranged from 7.6-7.7. At termination, the pH ranged from 7.6-7.8
Aeration of dilution water	The test solutions were aerated through out the study using CO_2-free air

Table A7.1.2.3/01-4: Biodegradation of 1,2-Benzisothiazolin-3-one (BIT, Test Compound) and Sodium Benzoate (Reference Compound)

Time (days)	Percent Biodegradation[a]							
	Test Flask (BIT)			Reference Flask (Sodium Benzoate)			Toxicity Control[b]	Abiotic Control[c]
	1	2	Mean	1	2	Mean		
2	-0.5	-2.8	-1.7	20.7	37.1	28.9	0.0	1.2
6	-12.9	-14.9	-13.9	36.2	61.1	48.6	23.4	1.2
9	-15.5	-18.8	-17.1	51.6	73.2	62.4	27.8	1.7
12	-17.6	-21.3	-19.5	64.0	79.5	71.8	30.6	1.1
14	-20.4	-20.0	-20.2	73.0	83.3	78.2	32.6	0.6
19	-19.0	-22.5	-20.7	79.4	88.0	83.7	34.2	1.3
23	-17.5	-20.0	-18.7	82.4	91.0	86.7	35.3	1.4
27	-16.0	-19.9	-17.9	84.4	94.5	89.5	34.9	3.1
28	-16.4	-21.5	-19.0	81.5	88.9	85.4	35.8	2.4

[a]Values corrected for inoculum control or abiotic blank as appropriate
[b]Toxicity control contains BIT and sodium benzoate.
[c]Abiotic control contains BIT and $HgCl_2$.

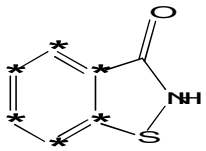
Figure A7.1.1.2.1-1: Biodegradation of the test item and the reference item during incubation period



Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.1 BIODEGRADABILITY (READY) (02)

Annex Point IIA7.6.1.1

		1 REFERENCE	Officialuse only
	1.1 Reference	**A7.1.1.2.1/02** [redacted] (2007) ^{14}C-BIT: Assessment of ultimate biodegradation at a non-biocidal concentration under the conditions of a "ready" biodegradation test, [redacted]	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes. OECD No. 301B, Ready Biodegradability, CO_2 Evolution (Modified Sturm Test)	
	2.2 GLP	Yes	
	2.3 Deviations	No	**X**
		3 MATERIALS AND METHODS	
3.1	**Test material**	^{14}C-BIT  * ^{14}C label position	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Purity	[redacted] specific activity – 163.79 mCi/g	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.1 BIODEGRADABILITY (READY) (02)

Annex Point IIA7.6.1.1

3.1.3	Further relevant properties	Water solubility is > 0.7 ppm Vapor pressure = 2.3 x 10^{-4}Pa at 25°C
3.1.4	TS inhibitory to microorganisms	Yes. That is why ^{14}C label was employed as an attempt to obtain concentrations less than the minimal inhibitory concentration.
3.2	**Reference substance**	
3.2.1	Sodium Benzoate	Sodium benzoate was employed as a reference compound for the test system. The dosing concentration was 15 mg of carbon/L (25.7 mg sodium benzoate/L)
3.2.2	^{12}C-BIT	Non-radiolabeled BIT (^{12}C-BIT) [redacted]
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	The details of the inoculum appear in Table A7.1.1.2.1/01-1.
3.3.2	Preparation of Solutions	<u>BIT</u> For the preliminary tests, an aqueous stock solution of ^{12}C-BIT was prepared at 37.52 mg/L. The required test concentration was achieved by addition of the appropriate volume of this stock solution to the test vessels For the main test an aqueous stock solution of ^{14}C-BITwas prepared. The test vessels were dosed with 9.8 mL (0.971043 mg) of the stock solution resulting in a nominal vessel concentration of 0.3237 mg/L. For the toxicity controls, a ^{12}C-BIT stock solution was prepared at 37.48 mg/L and 6.25 mL added to the appropriate vessel. <u>Sodium Benzoate</u> A stock solution of the reference compound was prepared by adding 3.859 g of sodium benzoate and making up to 1 liter using reverse-osmosis water. The reference and toxicity control vessels were dosed with 20 mL of this solution to give a nominal concentration of 15 mg carbon/L.
3.3.3	Preliminary Test	
3.3.3.1	Preliminary test 1	The purpose of preliminary test 1 was to examine the effect of varying concentrations of ^{12}C-BIT on viable cell counts and on the biodegradation of sodium benzoate. Two treatment vessels were prepared as controls containing only the mineral salt medium (Table A7.1.1.2.1-3) and two were references containing the mineral salt medium and sodium benzoate at 15 mgC/L. There were 5 toxicity controls identical to the references except that ^{12}C-BIT was added at

Section A7 Subsection A7.1.1.2.1 Annex Point IIA7.6.1.1		Ecotoxicological Profile Including Environmental Fate and Behaviour BIODEGRADABILITY (READY) (02)	
		the following concentrations; 0.313 mg/L, 0.625 mg/L, 1.25 mg/L, 2.5 mg/L, and 5 mg/L. All vessels were fitted with three 0.0125M $Ba(OH)_2$ traps and quantitation involved titration of the trap contents. Total viable cell counts was performed on Days 7 and 14. The test duration was 14 days after which the cultures were acidified and purged to remove dissolved CO_2	
3.3.3.2	Preliminary test 2	The purpose of preliminary test 2 was to examine the effect of varying concentrations of ^{12}C-BIT on the respiration of standard cell cultures. Two treatment vessels were prepared as controls containing only the mineral salt medium (Table A7.1.1.2.1-3) and there were 5 test vessels containing the mineral salt medium and ^{12}C-BIT at the following concentrations; 0.313 mg/L, 0.625 mg/L, 1.25 mg/L, 2.5 mg/L and 5 mg/L. All vessels were fitted with three 0.125M $Ba(OH)_2$ traps which were quantitated by titration. The test duration was 9 days after which the cultures were acidified and purged to remove dissolved CO_2.	
3.3.4	Main Test		
3.3.4.1	Test system	The test system is described in Table A7.1.1.2.1/01-2.	
3.3.4.2	Test conditions	Table A7.1.1.2.1/01-3 describes the test conditions including the composition of the aqueous media, inoculum, temperature, pH and aeration.	
3.3.4.3	Initial Test Substance concentration	The initial concentration of ^{14}C-BIT was 0.313 ppm.	
3.3.4.4	Duration of test	The exposure period was 28 days. After sampling on Day 28 1 mL of concentrated HCl was added to every vessel except the two containing ^{14}C-BIT. The vessels were aerated overnight to drive dissolved CO_2 into the alkali traps prior to final analysis. The two test vessels were not acidified to avoid metabolite artifacts as these solutions were being retained for additional chromatographic analysis.	
3.3.4.5	Chemical and biochemical methods	Liquid scintillation spectrometry was employed to quantitate the $^{14}CO_2$ trapped in the NaOH traps. $^{12}CO_2$ in the $Ba(OH)_2$ trapping solutions was quantitated by titration with standard HCl (0.05M) using phenolphthalein as an indicator. Titrations were performed on 20 mL aliquots until two matching (± 0.1 mL) titers were obtained Inorganic carbon concentration of the inoculated salts medium was determined using a carbon analyzer. The sample is acidified with H_3PO_4, sparged with CO_2-free air, and quantitated by a non-dispersive infrared detector. Air flow through the systems was measured weekly, adjusting if necessary, to maintain a flow rate of 50 mL/min. This was accomplished with a bubble flow meter and a stopwatch.	

Section A7 Subsection A7.1.1.2.1 Annex Point IIA7.6.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviour BIODEGRADABILITY (READY) (02)	
	Total viable cell counts were determined by removing duplicate 0.1 mL aliquots from the test vessels and making 10^{-1} and 10^{-2} dilutions with phosphate buffer. The original solution and the dilutions were plated on a nutrient agar plate for 37 h and the subsequently scored manually. Aliquots from the Test Flasks (dosed with ^{14}C-BIT) were analyzed by HPLC using a modified C-18 column and a binary gradient composed of 0.5% aqueous formic acid and 0.5% methanolic formic acid. Detection employed a ^{14}C-flow through monitor and/or UV detector (254 nm).	
3.3.4.6 Sampling	On Days 1, 3, 6, 8, 10, 13, 15, 16, 20, 22, 24, 28, and 29 the trap nearest the test vessel was removed and the detached removed for quantitation. The remaining two bottles in the series were moved up towards the test vessel and a fresh trap placed on the end of the series. Aliquots of the trapping solution were either radioassayed ($^{14}CO_2$)or titrated ($^{12}CO_2$). On Day 28, 1 mL of concentrated HCl was added to each trap and the flask aerated overnight to drive residual CO_2 into the traps thus accounting for dissolved CO_2.	
3.3.4.7 Intermediates/ degradation	The test vessels containing ^{14}C-BIT were chromatographed	
3.3.4.8 Nitrate/nitrite measurement	No	
3.3.4.9 Controls	Toxicity Control: 0.313 mg ^{12}C-BIT/L plus 25.7 mg sodium benzoate/L Reference: 25.7 mg sodium benzoate/L Inoculum Control: no BIT or sodium benzoate Additional details are in Table A7.1.1.2.1/01-2.	
3.3.5 Calculations/ Statistics	The percent biodegradationwas calculated as follows: $\text{Percent Biodegradation} = \frac{\text{cumulative } CO_2 (\text{mg})}{\text{theoretical cumulative } CO_2 \text{ (mg)}} \mathbf{x} 100$ or $\text{Percent Biodegradation} = \frac{\text{cumulative dpm}}{\text{total applied dpm}} \text{ x } 100$ where theoretical CO2 = mg of reference substance added x percent of carbon content of the reference material x 3.667 (the weight of CO_2 produced from 1 mg of carbon)	
	4 RESULTS	
4.1 PRELIMINARY TEST		

Section A7 Subsection A7.1.1.2.1 Annex Point IIA7.6.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviour BIODEGRADABILITY (READY) (02)	
4.2.1 Preliminary Test 1	The purpose of preliminary test 1 was to examine the effect of varying concentrations of ^{12}C-BIT on viable cell counts and on the biodegradation of sodium benzoate. Results from the total viable cell counts appear in Table A7.1.1.2.1-4. These results show that the microbial population was not reduced at any concentration of BIT applied and in fact increased with increasing concentration of BIT. Biodegradation of sodium benzoate in the presence of BIT was only suppressed at the highest concentration, 5 mg BIT/L (Table A7.1.1.2.1-5).	
4.2.2 Preliminary Test 2	The purpose of preliminary test 2 was to examine the effect of varying concentrations of ^{12}C-BIT on the respiration of standard cell cultures. The results in Table A7.1.1.2.1-6 show that at BIT concentrations of 0.313 mg/L, 0.625 mg/L and 0.1.25 mg/L CO_2 evolution was similar to vessels with no added BIT.	
4.2 Main Test		
4.2.1 Test Parameters	Based on the results of the preliminary tests, the main test was dosed at 0.313 mg/L. The inorganic carbon content of the inoculated mineral salts medium was 0.59 mg carbon/L culture solution, or 3.96% of the carbon loading from the addition of sodium benzoate. The pH on Day 0 of the main test ranged from 7.40 – 7.56 and on Day 28, 7.22 -7.40.	
4.2.2 Biodegradation	A summary of the biodegradation results for the test compound ^{14}C-BIT dosed at 0.313 mg/L, for the sodium benzoate reference control, and for the toxicity control (sodium benzoate plus 0.313 mg/L BIT) are presented in Table A7.1.1.2.1-7. Additionally the results are presented in Figure A7.1.1.2.1-1. After an initial lag phase of 8 days, biodegradation of ^{14}C-BIT progressed steadily accounting for 10% by Day 11. From Day 13 onward, the rate slowed reaching 20.1% on Day 16 and 23.7% at the end of the study. The maximum divergence between replicates was 0.5% on Day 20. To be considered readily biodegradable the test substance must achieve 60% biodegradation by the end of the study and that 60% must be reached within 10 days of obtaining 10%. Figure A7.1.1.2.1-2 graphically shows the biodegradation of the test flasks with a 10-day window superimposed. This graphically demonstrates that BIT cannot be considered to be ready biodegradable. The reference controls containing sodium benzoate had rapid and immediate CO_2 generation reaching 64% by Day 8. Thereafter the rate slowed reaching 84% on Day 16 at which time the rate began to plateau. On Day 28, biodegradation level was 88%. The validity requirement is that biodegradation of sodium benzoate exceed 60% by Day 14, which was achieved.	

Section A7 Subsection A7.1.1.2.1 Annex Point IIA7.6.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviour BIODEGRADABILITY (READY) (02)	
	The toxicity control measured the mineralization of sodium benzoate in the presence of BIT. BIT at 0.313 mg/L did not suppress the microbial degradation and thus the mineralization of sodium benzoate. The level of sodium benzoate biodegradation, 88%, was essentially the same as the reference control.	
4.2.3 Abiotic Degradation	Abiotic vessels were not included because they had been examined in an earlier study. Vessels dosed with BIT and $HgCl_2$ showed essentially no biodegradation.	
4.2.4 Material Balance	The distribution of radioactivity and material balance are presented in Table A7.1.1.2.1-8. About 70% of the applied radioactivity was detected in the culture solution and about 24% in the NaOH traps. A wash of the culture vessels collected less than 0.5%. Recovery of applied radioactivity was 95% which is an acceptable result.	
4.2.5 Quantitation of Parent and Characterization of biodegradates	Day 28 aliquots from the Test Flask (containing ^{14}C-BIT) were examined by HPLC. No BIT was present and there were two major metabolites comprising about 22% and 49% of the applied activity. These results indicate that while BIT is not ready biodegradable, it is rapidly biodegraded.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	This study employed OECD 301 B Ready Biodegradability, CO_2 Evolution (Modified Sturm Test). Flasks containing mineral salts solution (KH_2PO_4, K_2HPO_4, Na_2HPO_4, NH_4Cl, $MgSO_4$, $CaCl_2$, and $FeCl_3$) plus activated sludge inoculum were prepared. Preliminary studies were performed to examine the effect of varying concentrations of BIT (0.313 mg/L to 5 mg/L) on microbial cell viability, biodegradation of sodium benzoate, and respiration in mineral salt solution. In the main test, besides control flasks containing just the mineral salt solution there were flasks containing 0.313 mg ^{14}C-BIT, flask containing sodium benzoate, and a flask containing sodium benzoate and BIT. All vessels were aerated and purged with CO_2-free air which was deposited in alkaline traps. Evolved $^{14}CO_2$ from the test flasks and a set of controls was trapped in NaOH while $^{12}CO_2$ from the reference flasks, toxicity flask, and a set of control flasks were trapped in $Ba(OH)_2$. The flasks were incubated in the dark at 22 ± 2°C. On Days 1, 3, 6, 8, 10, 13, 15, 16, 20, 22, 24, 28 and 29 the traps were refreshed and aliquots of the solutions were removed for quantitation by either liquid scintillation spectroscopy or titration. On Day 28, aliquots from the Test Flask containing ^{14}C-BIT were examined by HPLC.	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.1.1.2.1	**BIODEGRADABILITY (READY) (02)**
Annex Point IIA7.6.1.1	

5.2	**Results and discussion**	BIT cannot be considered to be ready biodegradable, as it did not achieve 60% biodegradation to CO_2. Biodegradation plateaued at about 23-24% around Day 20. Sodium benzoate biodegradation was rapid and exceeded 60% by Day 8 demonstrating that the activated sludge culture was viable. BIT had no observable effect on the biodegradation of sodium benzoate since there was no observable difference in the biodegradation of sodium benzoate in the absence or presence of BIT. Chromatography of Day 28 solutions from the Test Flaks demonstrated that no BIT was still present in solution. Thus, while BIT is not ready biodegradable, it does rapidly biodegrade.
5.3	**Conclusion**	This study fulfills the requirements and demonstrates thatBIT cannot be considered to be readily biodegradable.
5.3.1	Reliability	1-valid without restrictions.
5.3.2	Deficiencies	None.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*November 2010*
Materials and Methods	*Applicant's version is accepted with the following comments:* *The test substance was tested a non-biocidal concentration (0,313 mg/L), and it is a non-biocidal concentration. According to the study report, this low concentration is employed because the substance is known to be inhibitory to the test systems routinely employed to assess biodegradation* *3.1.3. Further relevant properties:* *Water solubility should be 0.7> g/L*
Results and discussion	*Applicant's version is accepted with the following comments:* *5.2. Preliminary test 1: Total viable cell count data at day 7 and day 14. The variability between replicates is too high to conclude than the cell density clearly increased with increased concentrations of BIT.*
Conclusion	*This study fulfills the requirements and demonstrates that 14C-BIT cannot be considered to be readily biodegradable. Although 14C-BIT has failed to qualify for classification as readily biodegradable under the conditions employed in this study, based on the chromatography of the test solutions BIT does degrade rapidly.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.1 BIODEGRADABILITY (READY) (02)

Annex Point IIA7.6.1.1

Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.1.1.2.1-1: Inoculum

Criteria	Details
Nature	Activated sludge
Source	Return line of a sewage treatment works treating primarily domestic wastewater
Sampling site	[redacted]
Preparation of inoculum	Sludge was blended and aerated. The suspended solids concentration was determined by filtration, oven drying the filtrate, and the weight of the dry sludge measured.
Pretreatment	The mineral salt medium was inoculated with activated sludge at 90 mg solid/L. The solution was aerated with CO_2 free air overnight prior to addition of test compound
Concentration	30 mg of sludge on a dry weight basis/L

Table 7.1.1.2.1-2: Test System for the Main Biodegradation Test

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Number and Nature of Culture Flask</td><td>Nine 3000 mL flask were dosed as below.
<table>
<tr><th>Identification</th><th>mg/L ^{14}C-BIT</th><th>mg/L ^{12}C Sodium Benzoate</th><th>mg/L ^{12}C-BIT</th></tr>
<tr><td>Control (^{12}C)</td><td></td><td></td><td></td></tr>
<tr><td>Control (^{12}C)</td><td></td><td></td><td></td></tr>
<tr><td>Control (^{14}C)</td><td></td><td></td><td></td></tr>
<tr><td>Control (^{14}C)</td><td></td><td></td><td></td></tr>
<tr><td>Reference</td><td></td><td>25.7</td><td></td></tr>
<tr><td>Reference</td><td></td><td>25.7</td><td></td></tr>
<tr><td>Toxicity Control</td><td></td><td>25.7</td><td>0.313</td></tr>
<tr><td>Test</td><td>0.313</td><td></td><td></td></tr>
<tr><td>Test</td><td>0.313</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>Aeration Device</td><td>CO_2-free air is passed through the flasks and into traps.</td></tr>
<tr><td>Measuring equipment</td><td>Evolved $^{14}CO_2$ measured by liquid scintillation spectrometry and $^{12}CO_2$ by titration with HCl using a phenolphthalein indicator</td></tr>
<tr><td>Trapping System</td><td>From the exit line of each flask dosed with ^{14}C-BIT, three 0.0125M NaOH traps were placed in series to capture evolved $^{14}CO_2$. An identical procedure was employed for vessels dosed with ^{12}C-sodiumbenzoate except that 0.0125 $Ba(OH)_2$ was used instead of NaOH to capture evolved $^{12}CO_2$.</td></tr>
<tr><td>Test performed in closed vessels due to significant volatility of test substance</td><td>No</td></tr>
</table>

Table A7.1.1.2.1-3: Test Conditions

Criteria	Details
Composition of test medium	Stock solutions using analytical grade salts a) KH_2PO_4: 8.50 g/L K_2HPO_4: 21.75 g/L Na_2HPO_4:26.60 g/L NH_4Cl: 0.50 g/L b) $MgSO_4 \cdot 7H_2O$: 22.50 g/L c) $CaCl_2 \cdot 2H_2O$: 36.40 g/L d) $FeCl_3 \cdot 6H_2O$: 0.25 g/L The final testing solution was prepared containing 30 mL/L of solution a) and 3 mL/L of solutions b), c), and d).
Inoculum	The day before the addition of the test and reference substances, mineral salt test medium was inoculated with activated sludge solids at 90 mg suspended solids/L. 1 liter of this mixture was added to each test vessel followed by 1.5L or 1.9 L of ultra pure water. Based on a volume of 3 L in each test volume at Day 0, the activated sludge solid concentration was 30 mg/L.
Additional substrates	No
Test temperature	nominal 21 ± 1°C
pH	At Day 0 the pH ranged from 7.40 – 7.56. At termination (Day 28) the pH ranged from 7.22 – 7.40.
Aeration of dilution water	The test solutions were aerated through out the study using CO_2-free air

Table A7.1.1.2.1-4: Preliminary Test 1—Total Viable Cell Counts

Vessel	Mean Total Viable Cells (cells/mL)	
	Day 7	Day 14
Control 1 Control 2	1,042.5 840	647.5 2,150
Reference 1 Reference 2	720 2,775	2,717.5 1,420
5 mg BIT/L	670,250	100,625
2.5 mg BIT/L	14,225	3,285
1.25 mg BIT/L	3,525	2,482.5
0.625 mg BIT/L	1,752.5	427.5
0.313 mg BIT/L	1,595	505

Table A7.1.1.2.1-5: Preliminary Test 1—Percent Biodegradation of Sodium Benzoate

BIT Concentration (mg/L)	Cumulative Percentage Biodegradation of Sodium Benzoate							
	Day1	Day2	Day3	Day6	Day8	Day10	Day14	Day 15
0 (sodium benzoate only)	8	37	47	62	67	72	79	86
5	0	20	37	61	65	68	72	76
2.5	0	28	43	68	75	80	85	88
1.25	0	32	45	70	77	81	87	91
0.625	3	36	48	68	73	78	84	88
0.313	6	36	46	63	68	73	81	86

Table A7.1.1.2.1-6: Preliminary Test 2—Evolution of $^{12}CO_2$

BIT Concentration (mg/L)	Cumulative CO_2 Evolution in Vessels (mg)					
	Day1	Day3	Day6	Day8	Day9	Day10
0 (control medium)	4.2	13.5	26.2	35.9	42.4	51.6
5	3.8	9.5	17.3	22.7	26.6	32.8
2.5	4.0	11.2	20.5	27.7	33.1	41.8
1.25	4.2	12.4	24.5	34.3	41.1	51.2
0.625	4.3	13.0	25.4	34.8	41.1	50.7
0.313	4.6	14.0	27.1	38.0	44.9	55.9

Table A7.1.1.2.1-7: Main Test—Cumulative Percent Biodegradation

Time (Days)	Cumulative Percent Biodegradation						
	Test Vessels (^{14}C-BIT)			Reference Vessels (Sodium Benzoate)			Toxicity Control[1]
	1	2	Mean	1	2	Mean	
1	0	0	0	7	7	7	3
3	0	0	0	44	44	44	42
6	0.2	0.2	0.2	58	57	58	58
8	0.6	0.6	0.6	65	64	64	67
10	6.6	7.6	7.1	70	68	69	72
13	16.0	16.3	16.2	76	74	75	78
15	18.8	19.2	19.0	79	77	78	81
16	19.9	20.3	20.1	83	81	82	84
20	21.8	22.3	22.1	84	82	83	84
22	22.6	22.8	22.7	86	84	85	85
24	23.0	23.2	23.1	87	85	86	86
28	23.7	23.8	23.8	87	86	87	87
29	*	*	*	89	88	88	88

[*] Samples saved for chromatographic analysis. Thus they were not acidified and purged overnight to prevent the potential for acid catalyzed metabolite artifacts.

Table A7.1.1.2.1-8: Material Balance

Vessel	Percent of Applied Radioactivity			
	Culture Vessel	Vessel Wash	NaOH Traps	Recovery
Test Replicate 1	70.5	0.3	23.7	95
Test Replicate 2	70.3	0.4	23.8	95
			Mean	95

Figure A7.1.1.2.1-1: Overview of Biodegradation of BIT, Sodium Benzoate (Reference Vessels), and Toxicity Control (Sodium Benzoate and BIT)

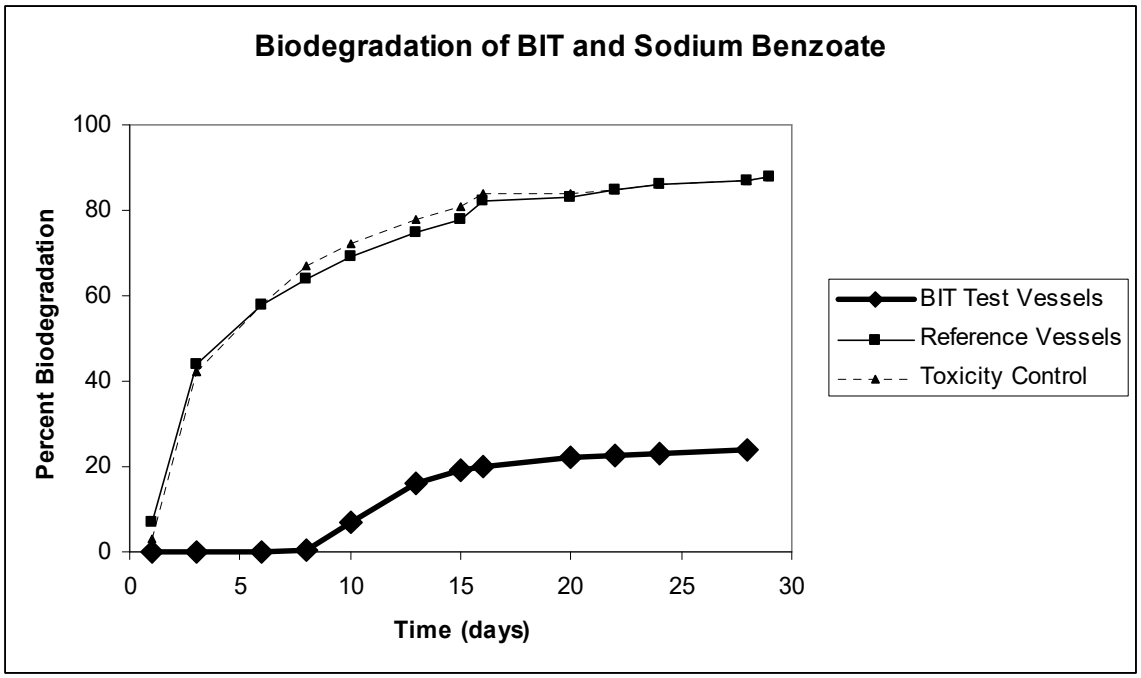
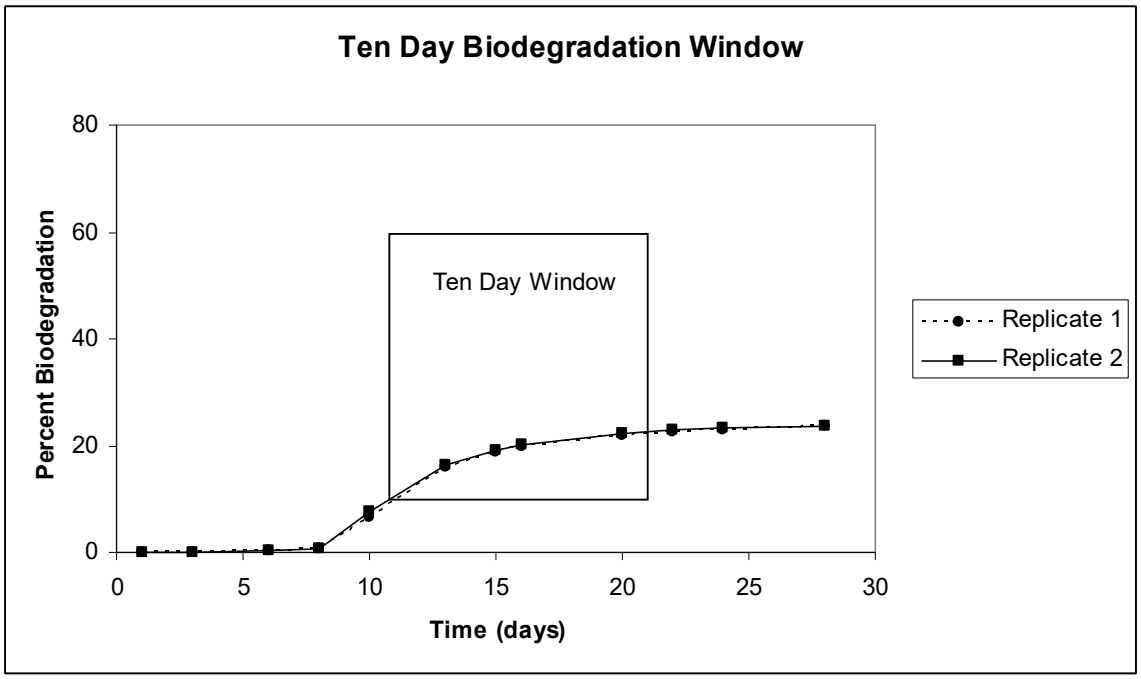


Figure A7.1.1.2.1-2: Ten Day Window for the Biodegradation of ^{14}C-BIT



Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

			Officialuse only
		1 REFERENCE	
	1.1 Reference	**A7.1.1.2.2** [redacted] (2006) 1,2-Benzisothiazolin-3-one: Inherent Biodegradability in a Manometric Respirometry Test; [redacted] October 02, 2006), unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes. OECD No. 302C, Inherent Biodegradability: Modified MITI Test (II) with the following modifications • Activated sludge was from only one source. • Activated sludge was not fed during holding period. • Holding period was maximum seven days. • Test water prepared according to OECD 301F. • Test run at 22°C. • Only BOD monitored. No test specific analysis performed.	
	2.2 GLP	Yes.	
	2.3 Deviations	No.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2.	
3.1.3	Purity	[redacted]	

Section A7	Ecotoxicological Profile Including Environmental Fate and Behaviour	
Subsection A7.1.1.2.2	**BIODEGRADABILITY (INHERENT) (01)**	
Annex Point IIA7.6.1.1		
3.1.4 Further relevant properties	Solubility in water : > 0.7 g/L Vapor pressure : 2.3 x 10^{-4} Pa at 25°C	
3.1.5 Composition of Product	Not applicable.	
3.1.6 TS inhibitory to micro-organisms	In an activated sludge respiration inhibition test (OECD 209), BIT had an NOEC of 1-3 mg/L (see section A7.4.1.4). BIT is a biocidal active substance and as such, inhibitory to microorganisms (see section A5).	
3.1.7 Specific chemical analysis	The biodegradation process consumes dissolved oxygen and subsequently generates CO_2. By adsorbing the CO_2 with soda lime, a pressure drop can be measured using a manometric electrode and this calibrated to oxygen consumption (mg/L)	
3.2 Reference substance	Yes. Sodium Benzoate.	
3.2.1 Initial concentration of reference substance	100 mg/L	
3.3 Testing procedure		
3.3.1 Inoculum / test species	Aerobic activated sludge was obtained from a wastewater treatment facility [redacted] treating primarily domestic wastewater (Table A7.1.1.2.2-1). The sludge was washed twice via centrifugation with tap water and the liquid supernatant phase was decanted. A homogenized aliquot of the final sludge suspension was weighed, thereafter dried and the ratio of wet to dry weight was calculated. Sludge was used at a final concentration of 100 mg dry material per liter.	
3.3.2 Test system	The test system is described in Table A7.1.1.2.2-2	
3.3.3 Test conditions	Table A7.1.1.2.1-3 describes the test conditions including the composition of the aqueous mineral salts medium, temperature, pH, and aeration. Eight 500 mL airtight flasks where filled with 250 mL of mineral salt water (Table A7.1.1.2.2-3) which contained 25 mg of activated sludge inoculum. The reference compound (sodium benzoate) and test compound (BIT) were dissolved in the mineral salt medium and added as described in Table A7.1.1.2.2-2.	
3.3.4 Initial TS concentration	17.9 -18.2 mg/L (10- 10.1 mg total organic carbon/L). See Table A7.1.1.2.2-2	
3.3.5 Duration of test	28 days (exposure period).	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.2.2 **BIODEGRADABILITY (INHERENT) (01)**

Annex Point IIA7.6.1.1

3.3.6	Analytical parameter	Biochemical oxygen demand. Pressure drop due to the consumption of oxygen (Table A7.1.1.2.2-2).
3.3.7	Sampling	Oxygen consumption was measured daily.
3.3.8	Intermediates/ degradation products	Not identified
3.3.9	Nitrate/nitrite measurement	Theoretical oxygen demand for BIT was calculated with and without nitrification..
3.3.10	Controls	Toxicity control: 31 mg/L BIT (Test item) and 100 mg/L Sodium Benzoate (Reference item). Procedure control: 100 mg/L Sodium Benzoate (Reference item) Abiotic control : 30 mg/L BIT(test item) poisoned with 10 mg/L $HgCl_2$ Inoculum control : neither test item nor reference item
3.3.11	Statistics	Percent biodegradation $$\text{Biodegradation (\%)} = \frac{\text{BOD (mg } O_2\text{/mg chemical)}}{\text{ThOD}_{\text{NH4 or NH3}}\text{(mg } O_2\text{/mg chemical)}} \text{ x 100}$$ where: BOD = Biochemical oxygen demand of the test or reference compound $$\frac{\text{(mg } O_2 \text{ uptake/L test or reference cmpd) - (mg } O_2\text{/L inocu}}{\text{mg test and/or reference compound/L}}$$ $ThOD_{NH4 \text{ or } NO3}$ = Theoretical oxygen demand of the test or reference compound without or with nitrification. The theoretical oxygen demand is the total amount of oxygen required to oxidize a chemical completely. It is calculated from the molecular formula, assuming the turnover of H into H_2O, C into CO_2, S into SO_3, Na into Na_2O, and N into NH_3 and/or NO_3. The calculated theoretical oxygen demain is tabulated below.

Theoretical Oxygen Demand in mg O_2/L		
BIT		Sodium Benzoate
$ThOD_{NH4}$	$ThOD_{NO3}$	ThOD
1.80	2.22	1.67

4 RESULTS

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

4.1	**Degradation of test substance**	
4.1.1	Biodegradation of the test compound, BIT	The biodegradation of the test compound, BIT is presented in Tables A7.1.1.2.2-4 and A7.1.1.2.2-5. During the study period of 28 days the biochemical oxygen demand (BOD) of BIT in the test media was less than the normal range found for the inoculum controls. Therefore, BIT was not biodegraded under the test conditions.
4.1.2	Biodegradation of the reference compound, sodium benzoate	Biodegradation in the procedure controls which contained only the reference compound, sodium benzoate is presented in Tables A7.1.1.2.2-4 and A7.1.1.2.2-5 as well as Figures A7.1.1.2.2-1 and A7.1.1.2.2-2. In the procedure controls, sodium benzoate was biodegraded by an average of 72% and 81% on Days 7 and 14, respectively. These results confirm the suitability of the activated sludge used in this study. By the end of the study (Day 28), the reference compound was biodegraded by an average of 86%.
4.1.3	Biodegradation in the toxicity control	The percent biodegradation in the toxicity control which contained both the test compound (BIT) and the reference compound (sodium benzoate) was calculated based on the sum of the theoretical oxygen demand of the test item (with and without nitrification, $ThOD_{NO3}$ and $ThOD_{NH4}$) and the reference compound. The results appear in Tables A7.1.1.2.2-4 and A7.1.1.2.2-5 as well as Figures A7.1.1.2.2-1 and A7.1.1.2.2-2. In the toxicity control, the biochemical oxygen demand (BOD) over the 28 day study period showed a similar course as the BOD of the procedure controls which contained only the reference compound. However, after Day 5 the BOD in the toxicity control was consistently lower than the procedure controls. According to the test guidelines, BIT is assumed to have no relevant inhibitory effect on activated sludge microorganisms at the tested concentration of 32 mg/L because biodegradation in the toxicity control was greater than 25% on Day 14. On Day 14 the biodegradation was 41% and 39% based on the $ThOD_{NH4}$ and $ThOD_{NO3}$, respectively. The percent biodegradation was nearly the same at the end of the exposure period, Day 28.
4.1.4	Percent biodegradation summary	

Percent Biodegradation on Day 28				
BIT		**Sodium Benzoate**	**Toxicity Control (BIT + Sodium Benzoate)**	
$ThOD_{NH4}$	**$ThOD_{NO3}$**		**$ThOD_{NH4}$**	**$ThOD_{NO3}$**
0	0	86	39	37

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.1.2.2 **BIODEGRADABILITY (INHERENT) (01)**

Annex Point IIA7.6.1.1

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	BIT was investigated for its inherent biodegradability in a 28-day Biochemical Oxygen Demand (BOD) test according to a modified version of OECD Guideline for testing of Chemicals N° 302C, Inherent Biodegradability: Modified MITI Test (II). Eight 500 mL airtight flasks were prepared containing 250mL of test water containing mineral salts (KH_2PO_4, K_2HPO_4, Na_2HPO_4, NH_4Cl, $MgSO_4$, $CaCl_2$, and $FeCl_3$) and 25mg of activated sludge inoculum were added. The flask were dosed as follows: • 2 flasks contained 31 mg/L BIT. • 2 flasks contained 100 mg/L sodium benzoate. • 2 flasks were controls (no BIT or sodium benzoate). • 1 flask contained 30 mg/L BIT + 10 mg/L $HgCl_2$. • 1 flask contained 32 mg/L BIT + 100 mg/L sodium benzoate. Biochemcial oxygen demand was measured on Days 0 – 28 using a manometric electrode.	
5.2	**Results and discussion**	The test item, BIT, was found to be not inherently biodegradable under the test conditions within 28 days. In the procedure controls, sodium benzoate was degraded to an average extent of 72% and 81% by Days 7 and 14, respectively, confirming the suitability of the activated sludge. By the end of the test (Day 28) sodium benzoate had biodegraded by 86%. In the toxicity control containing both BIT and the reference item sodium benzoate, biodegradation had a similar course as the BOD of sodium benzoate alone. However, the BOD of the toxicity control was consistently lower from Day 5 onward.	
5.3	**Conclusion**	BIT was not inherently biodegradable under the tests conditions within 28 days. However testing biocides for inherent 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.	
5.3.1	Reliability	1-valid without restrictions.	
5.3.2	Deficiencies	No.	

Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEURMEMBERSTATE	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

	Evaluation by Rapporteur Member State	
Date	**November 2019 .**	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

Materials and Methods

Applicant's version is accepted with the following comments:

2.3 Deviations

1. *The basal culture medium contained different quantities of $Na_2HPO_4 \cdot 2H_2O$, NH_4Cl and $CaCl_2 \cdot 2H_2O$ and the final solution consisted of different volumes of each stock solution to that of the guideline. Culture medium is prepared following OECD guidelines 301F for ready biodegradability (manometric respirometry test).*
2. *The sludge sampling did not take place in at least 10 places throughout the country. According to the study report, only one sample of activated sludge was taken, from a domestic wastewater treatment plant.*
3. *No reference is made to the mixing of old and new activated sludge samples. Only one sample was taken for the test, and the holding period was maximum seven days.*
4. *The number and type of test flasks prepared differed to that of the guideline. The study was performed using 500-mL Erlenmeyer flasks, with a final volume of 250 mL per flask.*

3.1.6. BIT cannot be assumed to be inhibitory on the activity of the sludge following the OECD criteria, because degradation of reference substance in toxicity control is higher than 25% (based on total ThOD) within 14 days. However, the decrease in biodegradation in toxicity control compared to procedure control could indicate a certain inhibitory effect of BIT. This inhibitory effect could also explain the fact that BOD for BIT in the test media was lower than the normal range found for inoculums controls.

3.3.5. Eight 500 mL Airtight flask were dosed as dosed as below. The dosed material was mixed into the Mineral Salt Solution

Identification	***Replicate No.***	***Amount of Test Item (BIT)***		***Amount of Reference Item (Sodium Benzoate)***		***$HgCl_2$ (mg/L)***
		mg/L	***$ThOD_{NH4/NO3}$[a]***	***mg/L***	***ThOD[b]***	
Test Item	*1*	*31*	*56/69*			
Test Item	*2*	*31*	*56/69*			
Inoculum Control	*1*					
Inoculum Control	*2*					
Procedure Control	*1*			*100*	*167*	
Procedure Control	*2*			*100*	*167*	
Abiotic Control	*1*	*30*	*55/68*			*10*
Toxicity Control	*1*	*32*	*57/70*	*100*	*167*	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

Results and discussion	*Adopt applicant's version.*
Conclusion	*BIT was not biodegradable under the test conditions within 28 days.* *Nevertheless, BIT at the concentration used seems to be toxic to the inoculum:* *TS inhibitory to microorganisms: In an activated sludge respiration inhibition test (OECD 209), BIT has a NOEC of 1-3 mg/L.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*There is inhibitory effect in the test medium and BIT can not be assumed to be Inherent biodegradable.*

Table A7.1.1.2.2-1: Inoculum

Criteria	Details
Nature	Activated sludge
Source	Wastewater treatment plant treating predominantly domestic wastewater
Sampling site	[redacted]
Preparation of inoculum	Sludge was washed twice with tap water by centrifugation and the supernatant liquid phase decanted.
Pretreatment	Sludge was added to mineral salt solution and aerated with CO_2 free air overnight prior to addition of test compound
Concentration	100 mg of washed sludge on a dry weight basis/L

Table A7.1.1.2.2-2: Test System Including Flask Composition and Dosing Concentrations

Eight 500 mL Airtight flask were dosed as dosed as below. The dosed material was mixed into the Mineral Salt Solution

Identification	Replicate No.	Amount of Test Item (BIT)		Amount of Reference Item (Sodium Benzoate)		$HgCl_2$ (mg/L)
		mg/L	$ThOD_{NH4/NO3}$[a]	mg/L	ThOD[b]	
Test Item	1	31	56/69			
Test Item	2	31	56/69			
Inoculum Control	1					
Inoculum Control	2					
Procedure Control	1			100	167	
Procedure Control	2			100	167	
Abiotic Control	1	30	55/68			10
Toxicity Control	1	32	57/70	100	167	

Aeration Device	Consumed oxygen was replaced by electrolysis of cupper sulfate
Measuring equipment	manometric electrode
Measurement Principle	The biodegradation process consumes the dissolved oxygen in the test liquid and generates CO_2. The CO_2 is adsorbed by soda lime and the total pressure decreases in the airtight test flask. The pressure drop is detected and converted into an electrical signal by means of an electrode type manometer. The consumed oxygen is replaced by electrolytically generated oxygen from a copper sulfate solution

[a] Theoretical oxygen demand in mg O_2/L (NH_4/NH_3; without/with nitrification)

Table A7.1.1.2.2-3: Test Conditions

Criteria	Details
Composition of test medium	Stock solutions using analytical grade salts a) KH_2PO_4: 8.50 g/L K_2HPO_4: 21.75 g/L $Na_2HPO_4•2H_2O$: 33.40 g/L NH_4Cl: 0.50 g/L b) $MgSO_4•7H_2O$: 22.50 g/L c) $CaCl_2•2H_2O$: 36.40 g/L d) $FeCl_3•6H_2O$: 0.25 g/L One drop of concentrated HCl was added to solution d) as a preservative. The final testing solution was prepared by adding 10 mL of solution a) and 1 mL of solutions b), c), and d) to 800 mL of purified water. The solution was then made up to 1000 mL with purified water and the pH adjusted to 7.4 with dilute HCl.
Additional substrates	HgCl to the abiotic control
Test temperature	22°C (temperature controlled room)
pH	At the start the pH in the test samples was 7.4. At termination, the pH ranged from 7.3-8.0
Aeration of dilution water	Not Applicable

Table A7.1.1.2.2-4: Oxygen Consumption

Time (days)	Cumulative Oxygen Consumption (mg/L)							
	Test Compound (BIT)		Inoculum Control		Reference Compound (Sodium Benzoate)		Abiotic Control	Toxicity Control
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2		
0	0	0	0	0	0	0	0	0
1	0	0	3	2	29	27	0	0
2	--[1]	--	--	--	--	--	--	--
3	0	0	13	12	113	107	0	110
4	0	0	16	15	122	115	0	115
5	0	2	20	18	134	126	0	119
6	0	3	22	20	141	133	0	120
7	0	3	25	23	147	140	0	120
8	1	4	27	25	152	146	0	120
9	3	4	29	27	156	151	0	120
10	6	5	32	29	160	156	0	120
11	6	5	33	30	163	159	0	120
12	7	6	35	32	166	163	0	126
13	8	8	37	34	168	165	0	127
14	8	8	37	34	171	169	0	127
15	8	8	39	35	174	172	0	127
16	8	8	40	36	175	174	0	127
17	8	8	41	37	177	176	0	127
18	8	8	42	37	178	177	0	127
19	8	8	43	38	180	179	0	128
20	8	8	44	39	182	181	0	129
21	8	9	45	40	183	183	0	130
22	8	9	45	40	184	183	0	130
23	8	9	46	40	186	185	0	131

Time (days)	Cumulative Oxygen Consumption (mg/L)							
	Test Compound (BIT)		Inoculum Control		Reference Compound (Sodium Benzoate)		Abiotic Control	Toxicity Control
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2		
24	8	9	46	41	187	185	0	131
25	8	9	47	41	188	186	0	132
26	8	9	47	41	188	186	0	132
27	8	10	48	42	189	187	0	132
28	8	10	48	42	190	188	0	132

[1]No reading taken

Table A7.1.1.2.2.-5: Percent Biodegradation

Time (days)	Percent Biodegradation[1]							
	Test Compound (BIT)				Reference (Sodium Benzoate)		Toxicity Control	
	$ThOD_{NH4}$		$ThOD_{NO3}$		ThOD		$ThOD_{NH4}$	$ThOD_{NO3}$
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2		
0	0	0	0	0	0	0	0	0
1	*[2]	*	*	*	16	15	-1	-1
2	--[3]	--	--	--	--	--	--	--
3	*	*	*	*	60	57	44	41
4	*	*	*	*	64	60	44	42
5	*	*	*	*	69	64	45	42
6	*	*	*	*	72	67	44	42
7	*	*	*	*	74	69	43	40
8	*	*	*	*	75	72	42	40
9	*	*	*	*	77	74	41	39
10	*	*	*	*	78	75	40	38
11	*	*	*	*	79	76	40	37
12	*	*	*	*	79	78	41	39
13	*	*	*	*	79	78	41	39
14	*	*	*	*	81	80	41	39
15	*	*	*	*	82	81	40	38
16	*	*	*	*	82	81	40	38
17	*	*	*	*	83	82	39	37
18	*	*	*	*	83	82	39	37
19	*	*	*	*	84	83	39	37
20	*	*	*	*	84	84	39	37
21	*	*	*	*	84	84	39	37
22	*	*	*	*	85	84	39	37

Time (days)	Percent Biodegradation[1]							
	Test Compound (BIT)				Reference (Sodium Benzoate)		Toxicity Control	
	$ThOD_{NH4}$		$ThOD_{NO3}$		ThOD		$ThOD_{NH4}$	$ThOD_{NO3}$
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2		
23	*	*	*	*	86	85	39	37
24	*	*	*	*	86	85	39	37
25	*	*	*	*	86	85	39	37
26	*	*	*	*	86	85	39	37
27	*	*	*	*	86	85	39	37
28	*	*	*	*	87	86	39	37

[1]Percent Biodegradation corrected for the mean oxygen update in the inoculum controls
[2] * Negative value due to higher oxygen consumption in inoculum controls than in the test compound
[3]-- No readings taken

Figure A7.1.1.2.1-1: Biodegradation in Test Flasks Without Nitrification

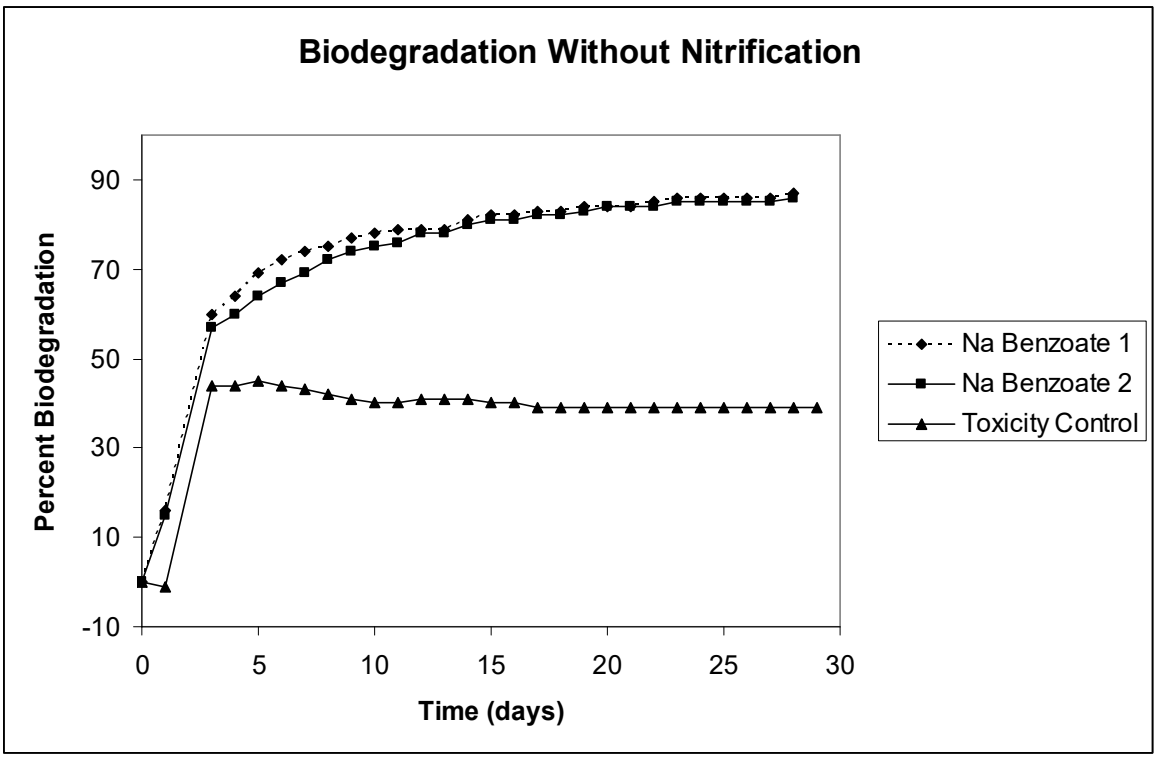
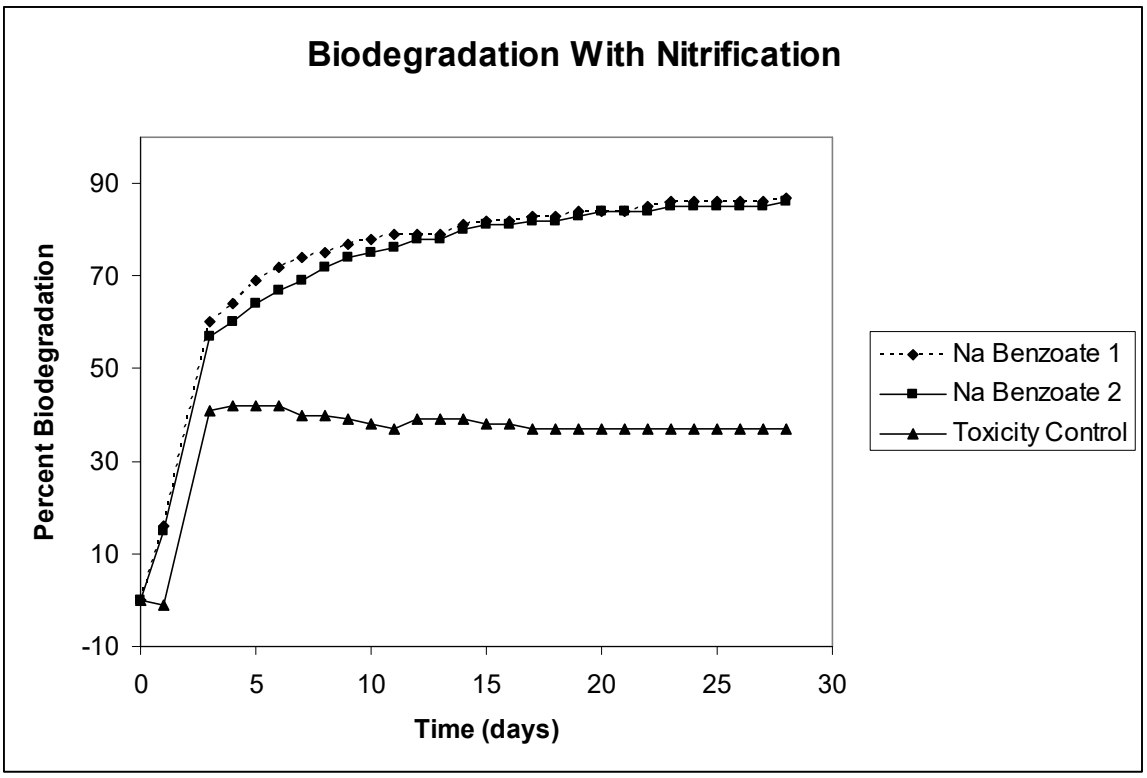


Figure A7.1.1.2.1-2: Biodegradation in Test Flasks With Nitrification



Section A7 **Subsection A7.1.1.2.3** **Annex Point IIIA 12.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **BIODEGRADATION IN SEAWATER**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification […].**	
Detailed justification:	The aqueous photodegradation rate is very rapid as demonstrated by the half-lives at pH 5, 7 and 9 below. **Half-Life in Hours** pH 5 / pH 7 / pH 9 9 / 0.7 / 0.7 Thus in a marine aquatic environment which has a basic pH, BIT will rapidly photodegrade with a half-life of less than 1 hour. The data from the ready biodegradation study employing ^{14}C-BIT also indicates that BIT will rapidly biodegrade. Additionally, based on the use pattern, there should be limited exposure to the aquatic environment. Thus this study will have a negligible impact on the environmental risk assessment.	
Undertaking of intended data submission [ ]	No studies are planned.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	*January 2010*	
Evaluation of applicant's justification	*Applicant's justification is accepted with the following comments:* *Justification regarding the phototransformation of BIT in basic media will only affect the first centimitres of the water column, and it is therefore not accepted as a well-built justification.* *Nonetheless, the study test "Biodegradation in Marine Water" does not need to be performed for PT 6 and 13.*	
Conclusion	*The study test "Biodegradation in Marine Watser" does not need to be performed for PT6 and 13, biodegradability data in freshwater (7.1.1.2.1 and 7.1.1.2.2 documents) are therefore enough for the risk evaluation of the Product-types 6 and 13.*	

Section A7 **Subsection A7.1.1.2.3** **Annex Point IIIA 12.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **BIODEGRADATION IN SEAWATER**	
Remarks		

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.2.1.1 **Biological Sewage Treatment—Aerobic**

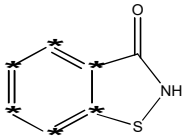
Annex Point

		1 REFERENCE	Official use only
1.1	**Reference**	**A7.1.2.1.1/01** [redacted] (2008) ^{14}C-1,2,Benzisothiazolin-3-one: Porous Pot Test Method for Assessing the Biodegradability of the Test Substance During Wastewater Treatment Simulation. [redacted] September 9, 2008) Unpublished. **A7.1.2.1.1/02** [redacted] (2008) Kinetic Analysis to Determine the Half-Life of BIT in an STP Simulation Study: Supplemental to [redacted] 29 September 2008) Unpublished. **A7.1.2.1.1/03** [redacted] (2009) Metabolite Identification for Samples Generated from BIT Wastewater Treatment Simulation Study ([redacted] (04 August 2009), Unpublished.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA. Data protection claimed in accordance with Article 12.1(c) (ii), as data generated after the entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline Study**	A7.1.2.1.1/01 Yes. OECD Guideline 303A, Simulation Test—Aerobic Sewage Treatment: Activated Sludge and U.S. Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances 835.3220. A7.1.2.1.1/02 and A7.1.2.1.1/03 No applicable guidelines	
2.2	**GLP**	A7.1.2.1.1/01: Yes A7.1.2.1.1/02: Not applicable (calculations only) A7.1.2.1.1/03: Yes	
2.3	**Deviations**	A7.12.1.1/01 Two minor GLP deviations. 1) Characterization and stability of test material under site specific storage conditions were not performed in accordance with GLP guidelines (however chemical characterization	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.2.1.1 **Biological Sewage Treatment—Aerobic**

Annex Point

		was performed under GLP by the sponsor) and 2) analysis of water (purified and municipal) for contaminants were not performed by a GLP certified laboratory (however were performed by a certified laboratory using U.S. EPA analytical methods). A7.1.2.1.1/02: Not Applicable A7.1.2.1.1/03: None
		3 MATERIAL AND METHODS
3.1	**Test Material** (A7.1.2.1.1/01)	^{14}C-BIT  * position of the ^{14}C-label
3.1.1	Lot/Batch number	[redacted]
3.1.2	Purity	[redacted]
3.1.3	Further relevant properties	• Soil adsorption K_f = 55.6 • Water solubility (deionized water) >0.7 g/L • Half-life in aerobic soil simulation study is 5.6 hours (20°C) • Half-life in aerobic surface water simulation study is 31 hours (20°C)
3.2	**Reference substances**	No reference substances were employed to validate the STP system.
3.3	**Sludge**	
3.3.1	Test inoculum	Fresh settled sewage was collected from the [redacted] Wastewater Facility [redacted] and sieved through a 2 mm sieve. This facility treats sewage of predominantly domestic origin. The total suspended solids concentration was measured and adjusted to approximately 2500 mg/L
3.3.2	Domestic sewage	Domestic sewage was collected weekly from [redacted] the Wastewater Facility [redacted] and sieved through a 1mm sieve. This sewage provides nutrients for the bacterial metabolism. The sewage was maintained refrigerator and continuously stirred.
3.4	**Test procedures**	
3.4.1	Test system	A bioreactor was comprised of a “porous pot”; a glass vessel

		containing a porous polyethylene membrane that retains the solids but allows the liquid to flow through the system. The test contained two bioreactors that were continuously dosed with ^{14}C-BIT and a single control reactor that was not exposed to the test substance but allowed measurements of the operational parameters. Approximately 1.13 L of test inoculum (adjusted to 2500 mg/L of total suspended solids) was added to each bioreactor. During the Stabilization Period, 2.4 mL/min of domestic sewage was added plus 0.3 mL/min water while during the Acclimation Period and Steady State Period 2.4 mL/min of domestic sewage and 0.3 mL/min of 0.25 ppm ^{14}C-BIT solution (or 0.3 mL/min water to the control reactor) were added to each bioreactor. The resulting hydrolytic retention time (HRT) was approximately 7 hours. Approximately 113 mL/day of the activated sludge/domestic sewage was removed from each bioreactor per day yielding a sludge retention time (SRT) of approximately 10 days. Test temperature, measured daily was maintained at 20°C – 22°C. The pH was measured at least twice a week and if necessary adjusted to 7.5 ± 0.5. Dissolved oxygen was also measured at least twice a week and aeration rates were adjusted so that the dissolved oxygen concentration was greater than 2 mg/L. A stabilization period during which the sludge becomes adjusted to the test system lasted 8 days. During this period all three bioreactors received 0.3 mL/min of water (instead of ^{14}C-BIT in the two test reactors). The stabilization period ended once the DOC and/or COD removal was greater than or equal to 80% (actually achieved in 4 days). After the stabilization period the two test bioreactors were dosed continuously at nominal 0.25 mg/L ^{14}C-BIT (the BIT was substituted with water in the control bioreactor). The acclimation period lasted 12 days. DOC and COD concentration were measured twice weekly and the influent, effluent and mixed liquor samples were radioassayed periodically. At the termination of the acclimation period a steady state period was initiated lasting 22 days. During this period the effluent from each bioreactor was collected in a sealed container. The effluent gases from the containers were passed through a 1.5N KOH trap. The dosing solution, the combined influent, effluent, mixed liquor, and KOH traps were collected three times each week and radioassayed.	
3.4.2	Preparation of test solution	A7.1.2.1.1/01 A stock solution was prepared containing 103.37 mg of ^{14}C-BIT dissolved in 10 mL of ethanol. The stock solution was stored frozen. A dosing solution was prepared using 1.58 ml of the stock solution and diluting with 7L of nitrogen purged water to obtain a final concentration of approximately 2.3 mg ^{14}C-BIT/L. Concentration was verified by radioassay and the percentage of BIT in the solution analyzed by HPLC. The results are in Table A7.1.2.1.1-1. Average concentration was 2.35 mg/L (102% of nominal value) and the solutions averaged 97% BIT. Dosing solutions were prepared at least	

Section A7 / Subsection A7.1.2.1.1 / Annex Point		Ecotoxicological Profile Including Environmental Fate and Behaviour Biological Sewage Treatment—Aerobic	
		weekly and were continuously refrigerated and mixed. Additionally they were maintained in the dark and in a nitrogen atmosphere to prevent oxidation. Water was administered to the control bioreactor under the same conditions as the BIT dosed bioreactors. A7.1.2.1.1/03 A stock solution was prepared by dissolving 10.34 mg ^{14}C-BIT into 2 ml of methanol. A dosing solution was prepared by combining 40 µl of this stock solution with 3.960 mL of methanol. The final concentration based on radioassay was 94.1 ppm. Both the stock solution and dosing solution were stored in the freezer until needed.	
3.4.3	Dosing of test unit	The 2.35 mg/L ^{14}C-BIT dosing solution was delivered by volumetric addition at a rate of 0.3 mL/min and this was combined with domestic sludge at a rate of 2.4 mL/min. The resulting nominal dosing concentration was 0.25 mg ^{14}C-BIT/L. The flow rates for both the ^{14}C-BIT and the domestic sewage was measured each working day and adjusted if necessary. In the control units, 0.3 mL/min of water was substituted for the ^{14}C-BIT.	
3.4.4	Duration of test	The unit was operated for 8 days (stabilization period) before dosing. Dosing with ^{14}C-BIT continued for a period of 33 days; 12 days acclimation and 22 days steady state.	
3.4.5	DOC/COD analysis	DOC was measured using a carbon analyzer. COD was measured using Hach Method 8000 and a Hach DR/890 colorimeter with preprogrammed calibrations.	
3.4.6	Sampling analysis: dosing solution and influent	The dosing solution was analyzed periodically by removing triplicate aliquots and radioassaying. Additionally, aliquots were diluted for HPLC quantitation of percent parent. Periodically replicate aliquots of the influent were obtained and radioasssayed.	
3.4.7	Sample analysis: effluent	A7.1.2.1.1/01 The effluent was analyzed on Days 10, 13, 14, and 16 during the stabilization period and all on non-weekend days throughout the steady test period. Aliquots were radioassayed to determine total ^{14}C-activity. An additional 10 mL aliquot was removed and 1 mL of acetonitrile added. The sample was filtered and chromatographically analyzed by HPLC to quantitate the amount of BIT remaining in the effluent. The KOH traps were radioassayed three times a week. A7.1.2.1.1/03 (Metabolite Identification) Six ml effluent samples from Days 1 through 41of the original simulation study (A7.1.2.1.1/01) were sent frozen to [redacted] TechnicalCenter where they were temporarily stored in a freezer.	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.2.1.1 **Biological Sewage Treatment—Aerobic**

Annex Point

Samples as listed below were selected for metabolite identification:

Porous Pot Reactor #2: Days 13, 31, 36, 38, and 41

Porous Pot Reactor #3: Days 13, 20 , 28, 36, 37, and 38

Samples to be analyzed were removed from the freezer, radioassayed and preserved with $HgCl_2$. The sample was concentrated to about 1 mL, filtered, and analyzed by either HPLC (for metabolite profiling/quantitation) or LC-MS (metabolite identification).

As part of the metabolite identification, the storage stability of BIT in effluent was examined. Control effluent (150 mL) was mixed with 15 ml of acetonitrile mimicking the procedure done in the initial study. ^{14}C-BIT was added to give a concentration of 0.25 µg/L, the sample mixed, and 4 mL aliquots transferred into vials and stored either in a refrigerator (~4°C) or a freezer (~18°C). Periodically over 89 days, duplicate vials were removed from the refrigerator and freezer, 1 mL aliquots transferred to autosampler vials, and the analyzed by HPLC.

3.4.8 Sample analysis: Mixed liquor

A7.1.2.1.1/01

A mixed liquor sample was taken every workday during the steady test period. A 40 mL aliquot of mixed liquor was centrifuged and the supernatant radioassayed. To a 10 mL aliquot of the supernatant, 1 mL of acetonitrile was added, the sample filtered, and chromatographed (HPLC).

The solids resulting from centrifugation were extracted 3 times with acetonitrile and the combined volume determined and aliquots radioassayed. Aliquots of the remaining solids were combusted prior to radioassay. A 25 mL portion of the acetonitrile extract was concentrated to dryness, redissolved in 0.2 – 0.5 mL of acetonitrile followed by 1.8 to 4.5 mL of 0.1% aqueous H_3PO_4. The resulting samples were chromatographed (HPLC).

A7.1.2.1.1/03

Six ml aliquots of the supernatant that was produced by centrifugation of the mixed liquor sludge from Days 1 through 41of the original simulation study (A7.1.2.1.1/01) were sent frozen to [redacted] Technical Center where they were temporarily stored in a freezer. A number samples as listed below were selected for metabolite identification:

Porous Pot Reactor #2: Days 21 and 37

Porous Pot Reactor #3: Day 24

Samples to be analyzed were removed from the freezer, radioassayed and preserved with $HgCl_2$. The sample was concentrated to about 1 mL, filtered, and analyzed by either HPLC (for metabolite profiling/quantitation) or LC-MS (metabolite identification).

Even though ACN extracts were provided, it was decided to analyze only the supernatant from the mixed liquor sludge. The activities in the ACN extracts were too low for metabolite identification.

To examine the stability of 14C-BIT, control sludge was centrifuged

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.1.2.1.1	**Biological Sewage Treatment—Aerobic**
Annex Point	

	and the 150 mL of the supernatant was mixed with 15 mL of acetonitrile, again mimicking the procedure employed in the original study. ^{14}C-BIT was added to give a final concentration of 0.26 µg/L, the sample mixed, placed into a plastic bottle, and stored in a refrigerator. Periodically over 89 days, 1 mL aliquots were removed, transferred to autosampler vials, and duplicate analysis by HPLC was performed. The sludge remaining after centrifugation was transferred to a centrifuge tube, 10 mL of acetonitrile added, mixed, centrifuged, and the acetonitrile supernatant removed. The sludge was extracted two more times with acetonitrile and placed in the refrigerator. The next day the extract was dosed with ^{14}C-BIT for a concentration of 0.24µg/L and returned to the refrigerator. Duplicate samples were analyzed periodically over 89 days	
3.4.9 Analytical methods	A7.1.2.1.1/01 Radioassay of solutions was performed using liquid scintillation counters. Solid samples were first combusted in a sample oxidizer to yield $^{14}CO_2$ which was trapped in a liquid adsorbent. The resulting sample was then quantitated by liquid scintillation spectrometry. HPLC employed a modified C-18 column and a binary gradient consisting of 0.1% aqueous H_3PO_4 and acetonitrile. Detection employed a UV detector at 313 nm and a radioactive flow through monitor using a 1000 µl cell. A7.1.2.1.1/03 Radioassay of solutions was performed using liquid scintillation counters. Metabolite profiling/quantitation was performed by HPLC using a radioactivity flow through detector with a 100 µL cell. HPLC employed a modified C-18 column and a binary gradient consisting of acetic acid in water and acetic acid in methanol. Liquid Chromatography-Mass Spectroscopy (LC-MS) was performed with a modified C-18 column and a binary gradient consisting of acetic acid in water and acetic acid in methanol. The mass spectrometer was an ion trap employing an electrospray interface.	
3.4.10 Half-Life Calculations (A7.1.2.1.1/02)	[redacted] The half-life of BIT was calculated in the simulated STP study using the data in Reference 1 (A7.1.2.1.1/01). Kinetics were calculated using the data in the steady test period only and assuming first order degradation. The calculations were based on the previous published work: Nyholm et al., Water Research 26(3): 339-353 (1992).	
	4 RESULTS	
	Note: Section 4.1 to 4.5 refers to Reference A7.1.2.1.1/01 Section 4.6 refers to Reference A7.1.2.1.1/02 Section 4.7 refers to Reference A7.1.2.1.1/03	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.2.1.1 Biological Sewage Treatment—Aerobic

Annex Point

4.1 Temperature, pH, dissolved oxygen, and operational parameters

The temperature range recorded during the test was 20°C to 22°C which is within the specified limits of 20 ± 2°C for the duration of the study.

The average pH, dissolved oxygen, mixed liquor total suspended solids, DOC, and COD for the control and two ^{14}C-BIT dosed bioreactors is presented in Table A7.1.2.1.1-2. The mean pH in the two dosed bioreactors was 7.4- 7.5 and the mean dissolved oxygen, 3.4 mg O_2/L. .

For both reactors dosed with ^{14}C-BIT, the average sludge retention time was 10 days and the hydraulic retention time, 6.8 hours. These observed parameters were acceptable for good operational performance of the test system.

4.2 Organic carbon removal

As shown in Table A7.1.2.1.1-2 mean COD as a percent removal averaged greater than 90% for the two dosed bioreactors. This demonstrates that the microbial activity in the test system was operating satisfactorily.

4.3 Distribution and recovery of radioactivity

The sampled daily distribution of radioactivity between the effluent, mixed liquor, and evolved $^{14}CO_2$ for the two test reactors are presented in Tables A7.1.2.1.1-3 and A7.1.2.1.1-4. The mean distribution during the steady test period is tabulated below.

Reactor	Percent Distribution of Applied Radioactivity Mean ± Standard Deviation			
	Effluent	Mixed Liquor	$^{14}CO_2$	Mass Balance
2	74.7 ± 5.5	17.7 ± 1.6	0.3 ± 0.2	92.7 ± 4.9
3	82.9 ± 7.0	15.0 ± 1.2	0.2 ± 0.1	98.1 ± 6.3

Thus most of the applied activity was present in the effluent and very little as evolved CO_2. The cumulative $^{14}CO_2$ during the steady test period (Days 20-41) in reactor 2 was 3.4% of the applied activity and in reactor 3, 2.4%.

The mixed liquor fraction was centrifuged to remove the supernatant and the resulting solids extracted with acetonitrile. The sampled daily distribution of ^{14}C-activity in the mixed liquor fractions is presented in Table A7.1.2.1.1-5. Most of the ^{14}C-activity remained associated with the sludge solids after centrifugation and ACN extraction. Approxiamtely 2% of the applied activity was in the sludge solution after centrifugation and about 0.7% was extractable with ACN.

4.3.1 Recovery of ^{14}C-activity

The mean recovery of applied ^{14}C-activity during the steady test period for Reactor 2 was 92.7 ± 4.9% and for reactor 3, 98.1 ± 6.3%. The average recovery from the two reactors was 95.4 ± 6.2%.

4.5 Chromatographic

The effluent, supernatant resulting form centrifugation of the mixed liquor, and the acetonitrile extract of the mixed liquor solids were

Section A7	Ecotoxicological Profile Including Environmental Fate and Behaviour
Subsection A7.1.2.1.1	Biological Sewage Treatment—Aerobic
Annex Point	

	analysis	chromatographed (HPLC). A summary of the chromatographic results are presented in Table A7.1.2.1.1-6. There were 5 chromatographic regions detected. BIT had a retention time of about 7.5 minutes (Region 4).
4.5.1	Effluent	There were two major peak regions in the effluent. Parent (Region 4) was present a about 22-25% of the applied activity (NOTE: subsequent analysis described below demonstrated that parent percentage was actually about 3.3% of applied activity). There was a major polar metabolite with a retention time of 4.4-5.2 minutes (Region 2) that represented about 32-33% of applied activity. The metabolite (or metabolites) was not identified. About 10% of the applied activity was present in the system void volume (ca. 2 minutes; Region 1) and this polar and poorly retentive fraction generally contains multiple compounds. The other two Regions, 3 and 5, accounted for less than 10% each.
4.5.2	Mixed liquor supernatant	The total activity in the mixed liquor was significantly less than in the effluent. Thus the supernatant from the mixed liquor had less than 3% of the total applied activity. About 0.7% of the applied activity was parent and the polar Region 2 contained about 1%.
4.5.3	ACN Extract of sludge solids	Similar to the mixed liquor supernatant, the acetonitrile extract of the sludge solids accounted for much less than the effluent; less than 1% of the total applied activity (Table A7.1.2.1.1-5). BIT in this extracted accounted for 0.3% of the applied activity (Table A7.1.2.1.1-6) (NOTE: subsequent analysis described below demonstrated that parent percentage was actually less than 0.1% of the applied activity). The remaining regions contained less than 0.3% of the applied activity.
4.6	**Degradation kinetics** **(A7.1.2.1.1/02)**	A summary of the degradation kinetics calculations for ^{14}C-BIT are presented in Tables A7.1.2.1.1-7. The kinetics were calculated assuming the steady state kinetics accounting for the direct dissipation in the aqueous, solids, and volatile phases. The kinetic results are summarized below.

Half-life (hours)	
Test Reactor #2	Test Reactor #3
1.9	2.4

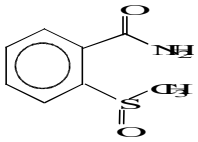
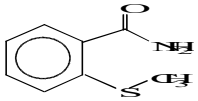
These results show that there is a very fast turnover of parent and total ^{14}C-activity in the system having a half-life of less than 3 hours.

4.7	**Metabolite Identification**	As described above, In the initial study two major chromatography peaks were observed; one assigned as a metabolite and the other as BIT. Using the HPLC conditions described in the initial report (A7.1.2.1.1/01) the chromatography was essentially replicated for metabolite identification. However, instead of using a 1000 µL radioactivity detector flow cell a 100 µL cell was employed. This resulted in the two major peaks, a metabolite and BIT, being split into multiple peaks due to the increased resolution caused by a narrower

peak width. Thus the samples were reanalyzed using the smaller flow cell and an enhanced gradient to assist with separation. The BIT results of this analysis, as well as the previous results, are presented in Table A7.1.2.1.1-08. In the initial study, BIT accounted for about 24% (Table A7.1.2.1.1-06) of the applied activity in the effluent, however, with reanalysis using improved HPLC conditions, the average BIT concentration was 3.3% (also see Table A7.1.2.1.1-09). Originally in the ACN extract of the sludge solids BIT comprised 0.3% (Table A7.1.2.1.1-06) of the applied activity but with reanalysis this was less than 0.1% (Table A7.1.2.1.1-09).

In the effluent, reanalysis of the original BIT peak with improved HPLC conditions showed that besides BIT this peak also contained several metabolites of which one was greater than 10% (M3 = 11.6%, Table A7.1.2.1.1-09). Reanalysis of the metabolite that originally had a retention time of 4.1 – 5.2 minutes (Table A7.12.1.1-06) had primarily on major metabolite, M2, at an average of 45.5% of applied activity. Similar results were seen in the ACN extract of the sludge solids but the percent of applied was significantly small due to less activity residing in the solids (Table A7.1.2.1.1-09).

Major metabolites M2 and M3 were identified by LC-MS as noted below.

Structure/Name	Average Percent of Applied Activity	
	Effluent	Supernatant
 2-methylsulfinyl-benzamide	45.53	1.39
 2-methylthio-benzamide	11.57	0.61

Since metabolite identification did not commence immediately storage stability was examined.[14]C-BIT was spiked into effluent, mixed liquor sludge supernatant, and an acetonitrile extract of the mixed liquor sludge solids. The results from the HPLC analysis are presented in Table A7.1.2.1.1-10. In all situations examined BIT was stable for up to 89 days.

The results from the storage stability study show that under the storage conditions examined, BIT was stable in the effluent, mixed liquor sludge supernatant, and an acetonitrile extract of the mixed liquor sludge solids. Thus the reduction of BIT observed in the metabolite

	identification is due to improved chromatography and not due to degradation of BIT in the samples.
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	The test guideline was OECD 303, Simulation Test-Aerobic Sewage Treatment: Activated Sludge Units. The test unit was a porous pot bioreactor which consists of a glass vessel housing a polyethylene membrane that retains the sludge solids but allows the liquid to flow through. Three reactors were prepared; a control dosed with water and two test reactors dosed with ^{14}C-BIT. About 1.13L of activated sludge was added to the reactors and domestic sewage was pumped into the system at 2.4 mL/min. A 2.35 mg/L solution of ^{14}C-BIT was added to the porous pot system at a flow rate of 0.3 mL/min for a resulting concentration in the porous pot of 0.25 mg/L. About 113 mL of activated sludge was removed per day. The hydraulic retention time in the aeration vessel was 6.8 hours and the sludge retention time, 10 days. The effluent was collected in a refrigerated container. The unit was allowed to equilibrate (stabilization period) for 8 days prior to dosing with ^{14}C-BIT during which the DOC/COD became greater than 80%. A 12 day acclimation period followed the stabilization period and during this time the systems were dosed with BIT (the control with a similar volume of water). The effluent, mixed liquor and dosing solution were radioassayed. After 12 days the system had reached equilibrium and a 22 day steady test period was commenced. During the steady test period, the effluent, mixed liquor, mixed liquor supernatant, acetonitrile extract of the mixed liquor solids, and dosing solution were radioassayed. The system temperature was maintained between 20°C and 22°C. Dissolved organic carbon, pH, temperature, and oxygen content were monitored throughout the study. During the steady test period volatile traps consisting of NaOH were connected to the effluent to collect evolved $^{14}CO_2$. Aliquots of the NaOH were taken periodically for radioassay. The effluent, the supernatant result from centrifugation of the mixed liquor, and an acetonitrile extract of the sludge solids were chromatographed using HPLC. Effluent and an acetonitrile extract of the mixed liquor sludge solids were analyzed a second time using an enhanced HPLC method and LC-MS to check the initial quantitation of BIT and to quantitate and identify metabolites greater than 10% of applied activity. Additionally, effluent, supernatant from mixed liquor sludge, and the acetonitrile extract of mixed liquor sludge solids were fortified with ^{14}C-BIT and the storage stability examined.
5.2 Results and Discussion	
5.2.1 Distribution and	Average recovery of applied radioactivity from the two reactors dosed

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**	
Subsection A7.1.2.1.1	**Biological Sewage Treatment—Aerobic**	
Annex Point		
	recovery of applied ^{14}C-acitivity	with BIT was 95.4 ± 6.2%. Over 74% of the applied activity was in the effluent and 15% to 18% was in the mixed liquor continuously removed from the porous pot system. Volatiles averaged about 0.2-0.3% of the applied activity per steady test period study day and the total accumulated during this period was less than 3.5%.
5.2.2	Quantitation of parent	Based on improved chromatography and the use of a smaller flow cell in the radioactivity detector, about 3.3% of the applied activity detected in the effluent was BIT and less than 0.1% in the acetonitrile extract of mixed liquor sludge. Parent was shown to be stable in effluent (stored refrigerated and frozen), supernatant from mixed liquor sludge, and an acetonitrile extract of mixed liquor sludge.
5.2.3	Metabolites	Initially there was only one major metabolite identified by HPLC. However, an using enhanced HPLC method it was detected that the majority of what was originally thought to be parent, was actually a metabolite. Thus there were two metabolites detected at greater than 10% and they were identified by LC-MS as 2-methylsulfinyl-benzamide and 2-methylthio-benzamide.
5.2.4	Half-life	The half-life of total applied radioactivity (parent and metabolites) in the sewage treatment system studied was calculated in the two test reactors to be less than 3 hours.
5.2.5	Organic carbon turnover	The average COD was 90.1% which satisfies the OECD guideline requirement
5.3	**Conclusion**	In a sewage treatment plant simulation system dosed with ^{14}C-BIT over 74% of the applied activity was in the effluent and 15%-18% in the mixed liquor. Evolved CO_2 totaled less than 3.5% of the total applied radioactivity. The half-life of BIT in the simulated STP systems was less than 3 hours. About 3.3% of the total applied activity in the effluent was parent. In the acetonitrile extraction of the sludge solids, BIT accounted for less than 0.1% of the applied activity. Two metabolites present at greater than 10% of the applied activity were detected and identified; 2-methylsulfinyl-benzamide (average 45.5%) and 2-methylthio-benzamide (average 11.6%).
5.3.1	Reliability	1-valid without restrictions
5.3.2	Deficiencies	None

Evaluation by Competent Authorities	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.2.1.1 Biological Sewage Treatment—Aerobic

Annex Point

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*December 2010*
Materials and Methods	*Applicant's version is accepted with the following remarks:* *Deviations from GLP:* *1) Characterization and stability of test material under site specific storage conditions were not performed in accordance with GLP guideline, however chemical characterization was performed under GLP by the sponsor.* *2) Analysis of water (purified and municipal) for contaminants were not performed by a GLP certified laboratory, however RMS accepts that this was performed by a certified laboratory using U.S. EPA analytical method).* *3.2. No reference substances were employed to validate the STP system.* *3.3.1. Test inoculum is collected from one single source. However, to get as many different species of bacteria as possible, it is adviseable to add inocula from various other sources, for example surface water.* *3.4.3. Concentration of BIT is lower than recommended in OECD guidelines. However, the choice of this low concentration may be justified to avoid possible toxicity effects in the inoculum. In addition, the test substance is radiolabelled.*
Results and discussion	*Applicant's version is adopted*
Conclusion	*Applicant's version with the following remarks:* *A typing error was detected in 5.1. "During the steady test period volatile traps consisting of NaOH were connected to the effluent to collect evolved $^{14}CO_2$. Aliquots of the NaOH were taken periodically for radioassay." The traps are actually consisting in KOH, not in NaOH.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.1.2.1.1-1: Dosing Concentration of ^{14}C-BIT

Study Day	mg ^{14}C-Activity/mL[1]	Percent Recovery	^{14}C-BIT Peak Area Percent
8[2]	2.36	103	97.3
15A[3]	2.34	102	98.2
15B	2.33	101	98.6
22A	2.34	102	99.3
22B	2.37	103	92.8
29A	2.34	102	98.9
29B	2.38	103	95.9
36A	2.36	103	96.7
36B	2.34	102	98.4
41	2.33	101	97.2
Average	2.35 ± 0.02	102 ± 0.74	97.3 ± 1.91

[1] Average of three replicate LSC analysis

[2] Day 8 was the start of dosing with ^{14}C-BIT

[3] A = analysis before changing dosing solution. B = analysis on freshly prepared dosing solution.

Table A7.1.2.1.1-2: Summary of Test Reactor Operational Paramters

Test Unit	Mean ± Standard Deviation				
	pH	Dissolved Oxygen (mg O_2/L	Mixed Liquor Total Suspended Solids (mg/L)	DOC (% Removal)	COD (% Removal)
Control (Bioreactor #1)	7.3 ± 0.1	3.6 ± 1.2	3586 ± 791	68.1 ±14.8	90.1 ± 5.2
Treatment Replicate #1	7.4 ± 0.1	3.3 ± 1.0	3655 ± 589	68.3 ± 11.6	90.4 ± 5.9
Treatment Replicate #2	7.5 ± 0.2	3.5 ± 1.0	3681 ± 435	67.7 ± 11.0	90.1 ±5.1

Table A7.1.2.1.1-3: Distribution of Radioactivity—Test Reactor #2

Day	Percent of Applied Activity				
	Effluent	Percent Removal	Mixed Liquor	NaOH Trap[2]	Mass Balance
Acclimation Period					
10	68.3	31.7	6.5		74.8
13	69.5	30.5	12.2		81.7
14	72.8	27.2	12.6		85.3
16	76.9	23.1	13.2		90.2
Steady Test Period					
20	69.9	30.1	17.6	0.0	87.5
22	70.9	29.1	19.5	0.1	90.5
24	67.1	32.9	19.3	0.2	86.5
27	69.4	30.6	17.8	0.4	87.7
29	80.4	19.6	18.0	0.6	98.9
31	82.6	17.4	14.2	0.3	97.1
34	72.4	27.6	16.7	0.5	89.6
36	76.9	23.1	16.4	0.4	93.7
38	80.6	19.4	17.8	0.3	98.7
41	76.6	23.4	19.3	0.6	96.5
Mean	74.7 ± 5.5[1]	25.3 ± 5.5[1]	17.7 ± 1.6[1]	0.3 ± 0.2[1,2]	92.7 ± 4.9[1]

[1] Mean and Standard Deviation during Study Test Period.
[2] Values presented are the daily $^{14}CO_2$ determinations. Cumulative $^{14}CO_2$ was 3.4 at study termination.

Table A7.1.2.1.1-4: Distribution of Radioactivity—Test Reactor #3

Day	Percent of Applied Activity				
	Effluent	Percent Removal	Mixed Liquor	NaOH Trap[2]	Mass Balance
Acclimation Period					
10	75.8	24.2	6.1		81.9
13	82.1	17.9	10.6		92.7
14	89.3	10.7	11.3		100.6
16	83.7	16.3	11.6		95.2
Steady Test Period					
20	76.8	23.2	15.5	0.0	92.3
22	76.9	23.1	17.1	0.2	94.1
24	75.1	24.9	14.9	0.3	90.4
27	75.9	24.1	16.5	0.4	92.8
29	83.5	16.5	14.4	0.3	98.2
31	85.3	14.7	13.1	0.1	98.6
34	82.8	17.2	15.8	0.2	98.8
36	85.2	14.8	14.1	0.3	99.6
38	91.1	8.9	13.8	0.3	105.2
41	96.2	3.8	14.7	0.2	111.1
Mean	82.9 ± 7.0[1]	17.1 ± 7.0[1]	15.0 ± 1.2[1]	0.2 ± 0.1[1,2]	98.1 ± 6.3[1]

[1] Mean and Standard Deviation during Study Test Period.
[2] Values presented are the daily $^{14}CO_2$ determinations. Cumulative $^{14}CO_2$ was 2.4% of applied activity at study termination

Table A7.1.2.1.1-5: Distribution of Applied Radioactivity in Mixed Liquor Fractions During Steady Test Period

Day	Percent of Applied Radioactivity					
	Supernatant		Acetonitrile Extract		Sludge Solids	
	Reactor #2	Reactor #3	Reactor #2	Reactor #3	Reactor #2	Reactor #3
20	2.6	2.2	0.7	0.5	25.1	17.7
21	2.8	2.6	0.7	0.6	26.8	22.5
22	2.7	2.2	0.9	0.4	24.9	18.6
23	2.8	2.3	1.0	0.6	26.3	20.1
24	2.6	2.3	1.0	0.7	27.3	21.4
27	2.6	2.0	0.8	0.6	26.5	19.2
28	2.1	1.9	0.8	1.5	21.0	17.0
29	1.7	1.3	0.5	0.5	15.7	12.2
30	1.6	1.4	0.6	1.0	11.9	10.0
31	1.5	1.3	0.4	0.3	12.3	9.4
34	2.4	1.7	0.7	0.4	22.1	13.5
35	2.4	1.6	0.9	1.0	23.3	12.9
36	2.1	1.5	0.5	0.3	19.0	11.3
37	1.7	0.8	0.4	0.1	13.5	5.9
38	1.8	1.0	0.4	0.2	15.2	6.2
41	2.2	0.8	0.6	0.2	20.4	5.5
Mean	2.2 ± 0.5	1.7 ± 0.6	0.7 ± 0.2	0.6 ± 0.4	20.7 ± 5.5	14.0 ± 5.7

Table A7.1.2.1.1-06: BIT as a Percent of Applied in the Effluent and the Supernatant andAcetonitirile Mixed Liquor Fractions

Reactor	TLC Regions—Mean Percent of Applied Radioactivity During Steady Test Period				
	Region 1 (Rt 2.0 – 4.4)	**Region 2 (Rt 4.4 – 5.2)**	**Region 3 (Rt 5.2 – 7.3)**	**Region 4 BIT (Rt 7.3 – 8.0)**	**Region 5 (Rt 8.0 – 10.0)**
Effluent					
2	7.7 ± 2.1	31.9 ± 5.6	6.6 ± 2.8	22.4 ± 2.8	7.7 ± 0.9
3	11.6 ± 2.9	32.9 ± 2.7	8.5 ± 3.3	24.5 ± 3.1	6.3 ± 1.3
Mixed Liquor Supernatant					
2	0.3 ± 0.1	1.0 ± 0.2	0.2 ± 0.1	0.7 ± 0.2	0.2 ±0.1
3	0.3 ± 0.1	0.8 ± 0.3	0.2 ± 0.1	0.7 ± 0.3	0.1 ± 0.1
Acetonitrile Extract of Mixed Liquor Solids					
2	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0
3	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.3	0.1 ± 0.1

Table A7.1.2.1.1-7: Steady State Degradation Kinetics

	Unit 2		Unit 3	
	Value	**Unit**	**Value**	**Units**
Reactor Volume (V)	1.13	L	1.13	L
Influent flow rate (Qi)	3.888	L/day	3.888	L/day
Effluent flow rate (Qo)	3.775	L/day	3.775	L/day
Volume of wasted sludge (Qw)	113.0	ml/day	113.0	ml/day
Concentration of Suspended Solids in Wasted Sludge (Xw)	4320.6	mg dry wt/L	4178.4	mg dry wt/L
Concentration of Suspended Solids in Effluent (Xo)	112.0	mg dry wt/L	112.0	mg dry wt/L
Test Substance Concentration in Influent (Ci)	261.1	µg/L	261.1	µg/L
Total BIT Concentration in Effluent (Co)	45.0	µg/L	54.6	µg/L
Test Substance Concentration in Sludge Solids (Wss)	422.3	µg/g	418.5	µg/g
Mineralization Rate (Mo)	34.5	µg/day	24.4	µg/day
Kd (=Wss/Co)	9384.9		7662.0	
Fi (=Qi x Ci)	1015.2	µg/day	1015.2	µg/day
Fo,diss (=Qi x Co)	175.0	µg/day	212.4	µg/day
Fo, part (=Kd x Co x (Qo x Xo + Qw x Xw)	384.7	µg/day	374.5	µg/day
Rbio (=Fi-Fo,diss-Fo,part/V)	403.1	µg/L/day	379.0	µg/L/day
k (=Rbio/Co)	9.0	day^{-1}	6.9	day^{-1}
Half-life	0.1	Days	0.1	days
Half-life	1.9	Hours	2.4	hours

Note: Kinetic calculations incorporate steady test period only.

Table A7.1.2.1.1-08: Quantiation of BIT in STP Effluent and Mixed Liquor Supernatant: Initial Quantitation (A7.1.2.1.1/01) and Revised based on Metabolite Identification (A7.1.2.1.1/03).

Day	BIT as a Percent of Applied Radioactivity[1,2]							
	Effluent				Supernatant			
	Reactor 2		Reactor 3		Reactor 2		Reactor 3	
	Initial	Current	Initial	Current	Initial	Current	Initial	Current
13	24.9	3.0	18.5	2.4				
20			23.3	2.5				
21					1.1	0.1		
24							1.2	0.1
27	19.3	3.6	20.2	3.3				
28			25.0	4.9				
31	23.1	2.5						
37			29.9	3.8	0.5	0.1		
38	19.2	3.6	23.1	4.0				
41	19.6	2.6						

[1] Initial = Reference 2; Current refers to this report
[2] Reactor 2 effluent contained an average of 74.7% of the applied radioactivity; Reactor 3, 82.9%; Reactor 2 Supernatant contained an average of 2.2%, and Reactor 3 Supernatant 1.7%

Table A7.1.2.1.1-09: Metabolite Profile of STP Samples (expressed as a percent of total applied radioactivity)

Sample	Day	Reactor	Compound (Percent of Applied Activity)[1]				
			M1	Mx	M2	M3	BIT
Effluent							
R2-E-091707	13	2	3.218	6.260	38.240	10.183	2.992
R3-E-091707	13	3	1.235	2.984	48.049	8.638	2.371
R3-E-092407	20	3	1.260	0.738	46.424	9.144	2.479
R3-E-100207	28	3	0.000	0.000	48.331	16.157	4.924
R2-E-100507	31	2	2.032	0.000	43.169	11.586	2.465
R2-E-101007	36	2	4.153	4.990	45.126	13.693	3.623
R3-E-101007	36	3	4.361	5.726	50.384	15.393	3.319
R3-E-101107	37	3	1.945	4.887	46.877	12.003	3.848
R2-E-101207	38	2	5.231	4.656	48.093	7.731	3.613
R3-E-101207	38	3	6.185	5.811	47.410	10.350	3.963
R2-E-101507	41	2	0.896	4.764	38.716	12.389	2.633
Average			2.774	3.711	45.529	11.570	3.294
Supernatant							
R2-SF-092507	21	2	0.041	0.000	1.229	0.654	0.098
R3-SF-092807	24	3	0.059	0.000	0.931	0.503	0.061
R2-SF-101107	37	2	0.067	0.021	1.528	0.448	0.101
Average			0.056	0.007	1.229	0.535	0.086

[1]Total activity in the effluent of reactor 2 was 74.7% and reactor 3, 82.9%. Total activity in the supernatant of reactor 2 was 2.2% and reactor 3, 1.7% (Reference 2).

Table A7.1.2.1.1-10:Results of Storage Stability Study

Date (t = day)	Effluent (Frozen)	Effluent (Refrigerated)	Supernatant	ACN Extract
03/24-25/09 (t=0)				
Parent BIT, %	100	100	100	100
Degradates, %	0	0	0	0
04/15-16/09 (t=21-22 day)				
Parent BIT, %	100	100	94.2	100
Degradates, %	0	0	5.8	0
05/27-28/09 (t=64 day)				
Parent BIT, %	100	100	100	100
Degradates, %	0	0	0	0
06/22-23/09 (t=88-89 day)				
Parent BIT, %	92.4	100	100	100
Degradates, %	7.6	0	0	0

Overall BIT Recovery = 99.2±2.3%

Section A7 **Subsection A7.1.2.1.2** **Annex Point IIIA, 6.2.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **BIOLOGICAL SEWAGE TREATMENT—ANAEROBIC**	
	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:	7.1.2.1.2 Anaerobic Biological Sewage Treatment A waiver from performing an anaerobic biological sewage treatment simulation study for BIT in Product Type 12 is requested. As noted in the Chapter 3, Section 7.1.2.1.2 for the Guidance on Data Requirements in the Technical Guidance Document, an Anaerobic study is only required if exposure to anaerobic conditions is likely. For the Product Types PT6 and 13 in question, this exposure is unlikely.	
Undertaking of intended data submission [ ]	No further studies planned	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*December 2010*	
Evaluation of applicant's justification	*Applicant's justification is accepted due to the unlikely anaerobic exposure of BIT*	
Conclusion	*Accepted*	
Remarks		

Section A7 **Subsection A7.1.2.2.1** **Annex Point IIIA, 7.2.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AEROBIC AQUATIC DEGRADATION STUDY**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [...]**	
Detailed justification:	The aqueous photodegradation rate is very rapid as demonstrated by the half-lives at pH 5, 7 and 9 below. **Half-Life in Hours** pH 5 / pH 7 / pH 9 9 / 0.7 / 0.7 Thus in an aquatic environment, BIT will rapidly photodegrade. The data from the ready biodegradation study employing ^{14}C-BIT also indicates that BIT will rapidly biodegrade. Additionally, based on the use pattern, there should be limited exposure to the aquatic environment. Thus this study will have a negligible impact on the environmental risk assessment.	
Undertaking of intended data submission [ ]	No studies are planned.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*January 2010*	
Evaluation of applicant's justification	*Applicant's justification is accepted with the following comments:* *Justification regarding the phototransformation of BIT in the different pH media will only affect the first centimitres of the water column, and it is therefore not accepted as a well-built justification.* *Nonetheless, the study test "Biodegradation in Marine Watser" does not need to be performed for PT6 and 13.*	
Conclusion	*The study test "Biodegradation in Marine Water" does not need to be performed for PT6 and 13, biodegradability data in freshwater (7.1.1.2.1 and 7.1.1.2.2 documents) are therefore enough for the risk evaluation of the product types 6 and 13.*	

Section A7 **Subsection A7.1.2.2.1** **Annex Point IIIA, 7.2.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AEROBIC AQUATIC DEGRADATION STUDY**	
Remarks		

Section A7 **Subsection A7.1.2.2.2** **Annex Point IIIA, 7.2.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **WATER:SEDIMENT DEGRADATION STUDIES—AEROBIC AND ANAEROBIC**	
	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:	A waiver is requested from performing aerobic and anaerobic water:sediment studies (A7.1.2.2.2). As noted in Chapter 3, Section 7.0.2.3.2 (and Figure 1 in section 7) a sediment:water study is only required when the K_p> 2000. For BIT, the maximum measured K_{oc}, in a sediment is 35 (K = 0.67). In 4 soils the K_{oc} ranged from 58 – 144. Therefore the K_p will be significantly less than 2000.	
Undertaking of intended data submission [ ]	No studies are planned.	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*December 2010*	
Evaluation of applicant's justification	*Accept the applicant's version.*	
Conclusion	*Accept the applicant's version.*	
Remarks		

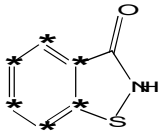
Section A7

Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.3b

ADSORPTION / DESORPTION SCREENING TEST (01)

Annex Point IIA.7.7

		1 REFERENCE	**Officialuse only**
	1.1 Reference	**A7.1.3.b/01** [redacted] (2007). [^{14}C] BIT: Adsorption/Desorption in Soil and Sediment, [redacted]	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes. OECD Guideline for testing chemicals 106: Adsorption-desorption using a batch equilibration method, adopted January 2006 and US EPA OPPTS 855.2210: Sediment and Soil Adsorption/Desorption Isotherm (January 1998)	
	2.2 GLP	Yes	
	2.3 Deviations	No claim of GLP compliance is made for soil sterilization or sterility testing. However these procedures were conducted in accordance with current GLP requirements.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	^{14}C-BIT  *** site of ^{14}C label**	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As specified in the study guidelines, ^{14}C-material was employed. Specifications for the ^{14}C-material are listed below.	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.3b ADSORPTION / DESORPTION SCREENING TEST (01)

Annex Point IIA.7.7

3.1.3	Purity	[redacted]
3.1.4	Specific Activity	Specific activity: 53.57 mCi/g
3.1.5	Further relevant properties	Water solubility is greater 0.7 g/L.
3.1.6	Method of analysis	Adsorption and desorption was determined by radioassay of the two phases, soil and aqueous solution. Confirmation of parent stability examined by HPLC and LC-MS.
3.2	**Degradation products**	Degradation products were not tested in this study. Only the adsorption and desorption of parent was measured in this study.
3.2.1	Method of analysis for degradation products	Not applicable
3.3	**Reference substance**	No system reference substance was employed. A BIT reference standard for chromatography was employed.
3.3.1	Method of analysis for reference substance	The chromatography reference standard employed was: ^{12}C-BIT [redacted]
3.4	**Soil types**	Four soils and one sediment were employed. The sample location, soil type, and physiochemical characteristics of the soils and sediment used in this study are presented in Table A7.1.3-1. Soils were obtained from the top 25 cm of agricultural land, were air dried, passed through a 2 mm sieve, and sterilized by gamma irradiated prior to use.
3.5	**Test Solutions**	
3.5.1	BIT Test Solutions	The preparation of each dosing solution is described within the appropriate test performance section
3.5.2	0.01M $CaCl_2$	0.01M $CaCl_2$ was prepared by dissolving either 1.11 g or 2.22 g of anhydrous $CaCl_2$ in 1 L or 2 L or water. Additionally it was prepared by dissolving 2.94 g of hydrated $CaCl_2$ in 2 L of water. The solutions were sterilized by autoclaving
3.6	**Preliminary Investigations**	
3.6.1	Solubility	Stock solutions were made by dissolving 4.560 mg ^{14}C-BIT in 10 mL acetonitile and 138.272 mg ^{12}C-BIT in 50 mL acetonitrile. 250 µL of the ^{14}C-stock solution and 360 µL of the ^{12}C stock solution were added to a centrifuge tube and taken to dryness. The BIT was reconstituted in 10 ml 0.01M $CaCl_2$ with the resulting concentration being 110 µg/mL. This is greater than twice the proposed highest

	application concentration. The solution was sonicated for 1 h, centrifuged and the supernatant radioassayed. The mean recovery was 97.4%. A second solubility check was performed by adding 168µl of the ^{14}C-BIT stock solution and 244 µL of the ^{12}C-BIT stock solution to a centrifuge tube and taking the sample to dryness. The was reconstituted in 15 mL $CaCl_2$ and the resulting 5 µg/mL solution sonicated for 10 minutes, centrifuged, and the supernatant radioassayed. The mean recovery was 100.8%.	
3.6.2Adsorption to containers	16 µL ^{14}C-BIT stock solution and 24 µL ^{12}C BIT stock solutions (stock solutions from solubility test) were added to a Teflon® centrifuge tube and taken to dryness. The sample was reconstituted with 15 mL $CaCl_2$, shaken for 24 hours, and radioassayed. The mean recover was 102.6% demonstrating that there was no adherence of the test substance to the tube walls.	
3.6.3Ratio of soil to solution	An application solution was prepared from the solubility test stock solutions. 340 µL ^{14}C-BIT and 1750 µL ^{12}C-BIT were added to a container, the acetonitrile evaporated, 100 mL of 0.01M $CaCl_2$ added, and the solution sonicated. The testing scheme is tabulated below.	

Soil:Solution Ratio	BIT (mL)	Soil (g)	0.01M $CaCl_2$ (mL)
1:1	1.0	10	9.0
1:2	2.0	10	18.0
1:5	2.5	5	22.5

	The final BIT concentration was 5 µg/mL. The tubes were mixed for 24 hours, centrifuged, and the supernatant radioassayed.	
3.6.4Equilibration time determination	An application solution was prepared by dissolving 5.718 mg ^{14}C BIT in 114 mL of 0.01M $CaCl_2$. 10 g of the four soils or one sediment were added to centrifuge tubes. Eight tubes per soil/sediment were prepared. To each tube, 18 mL of 0.01M $CaCl_2$ was added and the tubes shaken overnight. The next morning 2 mL of the BIT solution was added to give a concentration of 5 µg/mL and a soil:solution ratio of 1:2. At Hours 1, 3, 6, and 24 duplicate tubes were removed for each soil/sediment, centrifuged, and the supernatant radioassayed.	
3.6.5Stability test	The supernatants from the above equilibration determination were analyzed by HPLC. The soils were extracted three times by shaking (20 min) with methanol (20 mL) and centrifuged. They were further extracted an additional three times by shaking (20 min) with 0.1M NaOH:methanol (80:20; 20 mL) and centrifuged. The supernatant was radioassayed and then analyzed by HPLC.	

	The sterility of each soil was checked by plating aliquots on nutrient agar plates. An additional test was performed using only the clay loam soil. Four samples were prepared as above except that that hydrogen peroxide was added at 1% and 3%. After shaking for 1 hour, the tubes were centrifuged, and the supernatant analyzed by HPLC.	
3.7 Definitive test (isotherm)	A stock solution was prepared by dissolving 10.983 mg ^{14}C BIT in 2 mL of acetonitrile. Three application solutions were prepared directly from the stock solution by taking to dryness 370 µL, 110 µL, and 36 µL and reconstituting in 40 mL of 0.01M $CaCl_2$ resulting in concentrations of 52µg/mL, 15 µg/mL, and 5 µg/mL. Two additional application concentrations were prepared by diluting 1240 µL and 400 µL of the 52 µg/mL solution in 40 mL $CaCl_2$ resulting in concentrations of 1.4 µg/mL and 0.5 µg/mL. Ten samples were prepared for each soil/sediment so that five concentrations could be investigated in duplicate. 10 g of soil/sediment were added to a Teflon® centrifuge tube and mixed overnight with 18 mL of $CaCl_2$. The next day 2 mL of each application solution was added to duplicate tubes for each of the soil/sediment types. The resulting BIT concentration was 0.05, 0.15, 0.5, 1.5, and 5 µg/mL. After 1 h of mixing, the tubes were centrifuged, and the supernatants radioassayed and the pH measured. The supernatants were also analyzed by HPLC. The soils dosed at 5 µg/mL were radioassayed. They were subsequently extracted as per the stability test (section 3.6.5) in the preliminary investigations.	
3.7.1 Analytical Procedures	Radioassay of liquid samples was performed using Packard liquid scintillation counter. Radiopurity and aliquots from the buffer solutions were analyzed by HPLC using a modified C-18 column and a binary gradient composed of 0.5% aqueous formic acid and 0.5% methanolic formic acid. Detection employed a ^{14}C-flow through monitor and/or UV detector (254 nm). Thin layer chromatography (TLC) was used for radiopurity determination. Silica gel plates (250 µm thick) were developed with ethyl acetate:methanol:acetonitrile:acetic acid (90:5:5:1). Solutions were cochromatographed with non-radiolabeled BIT. Radiolabeled compounds were detected using a phophorimager while non-labeled compounds visualized with a UV lamp (254 nm). Representative samples were analyzed by LC-MS (ion trap) to confirm the presence of parent. Analysis employed a modified C-18 column and a binary gradient composed of 0.5% aqueous formic acid and 0.5% methanolic formic acid. Detection was by a radioactivity flow monitor and the mass spectrometer. The LC effluent was split between the two detectors and introduction in to the MS via an API interface and positive and negative ionization was employed.	
	4 RESULTS	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.3b **ADSORPTION / DESORPTION SCREENING TEST (01)**

Annex Point IIA.7.7

4.1 Preliminary Investigations

Solubility

The solubility of BIT in 0.01M $CaCl_2$ was examined initially at 110 µg/mL, which was at least double the expected study concentration. Over 97% of the BIT was found soluble at this concentration. A second experiment was performed at the proposed final test concentration, 5 µg BIT/mL, and the solubility was 100%.

Adsorption to containers

BIT in 0.01M $CaCl_2$ was added to Teflon® centrifuge tubes without soil and shaken for 24 hours. The mean recovery of ^{14}C-BIT was 103% demonstrating no adherence of the test compound to the test vessels.

Ratio of soil to solution

Soil:0.01M $CaCl_2$ ratios of 1:1, 1:2, and 1:5 and dosed at 0.5 µg ^{14}C-BIT were examined. The results are summarized below.

Soil	Percent ^{14}C-BIT in Supernatant (0.01M $CaCl_2$)		
	1:1 Ratio	1:2 Ratio	1:5 Ratio
Clay Loam	28.2	34.8	55.4
Silt Loam	12.1	22.2	44.1
Loam/Silt Loam	19.9	27.8	46.4
Loamy Sand	30.9	48.9	72.5
Loamy Sand (sediment)	14.8	25.7	53.1

Based on difference, this indicates that the following percentage ranges were adsorbed to the soil with the highest adsorption to the silt loam and the lowest to the loamy sand soil:

1:1—61.8% to 87.9%

1:2—51.1% to 74.3%

1:5—27.5% to 46.9%

The 1:2 soil:0.01M $CaCl_2$ ratio was chosen since the percent adsorption to soil and sediment was between 50% and 80%.

Equilibrium Time and Stability Tests

Distribution of radioactivity between soil and sediment is presented in Table A7.1.3-2. The average recovery of ^{14}C-activity was 90.7 ± 10.6%. A graphical presentation of the equilibration results can be seen in Figure A7.1.3-1. The percent of applied radioactivity recovered as BIT is presented in Table A7.1.3-3. The average recovery of BIT for the 1 and 3 hour equilibration time intervals was

55.0 ± 11.0%.

The results indicate that the adsorption of BIT to soil did not fully reach equilibration in the 24 hour period. This is probably due to the dissipation of BIT during the study period. Examination of the soil sterility showed that it was sterile (no colony forming units observed on agar plates) and degradation was the result of abiotic activity.

Nucleophiles are known to cleave the isothiazolone ring. To examine if this was the cause for degradation, hydrogen peroxide was added to the soil:$CaCl_2$ mixture. The results showed that degradation was greater in the presence of peroxide than in its absence.

4.2 Definitive test (isotherm)

Based on the preliminary test a soil:0.01M $CaCl_2$ ratio of 1:2 and a 1 hour equilibration time were used for the isotherm test.

The pH of adsorption supernatants are presented in Table A7.1.3-4.

The mean percent of adsorption for the four soils and 1 sediment are presented in Table A7.1.3-4. For clay loam, silt loam, loam/silt loam, loamy sand soil, and loamy sand sediment the adsorption ranged from 44.5% to 65.7%, 63.2% to 77.4%, 49.7% to 65.8%, 23.1% to 37.8%, and 24.0 to 48.5%, respectively. The adsorption coefficients (K_d, K_{doc}, and K_{dom}) determined at each dosing concentration is presented in Table A7.1.3-5.

Fruedlich adsorption coefficients and linearity values, 1/n and r^2, are presented in Table A7.1.3-6. The K_{oc} values range from 35-144 mL/g. A summary of these results are presented below.

Soil	Adsorption Range (%)	K_d	K_{oc}	r^2
Clay Loam	45 - 66	1.98	41	0.9966
Silt Loam	63 - 77	3.88	144	0.9985
Loam/Silt Loam	50 – 66	2.27	58	0.9987
Loamy Sand Soil	23 – 38	0.75	94	0.9958
Loamy Sand Sediment	24 - 49	0.67	35	0.9764

The r^2 values demonstrate there is a good correlation between the log of the concentration adsorbed and the log of the dosing concentrations.

The mobility class of BIT in soil is high mobility.

4.3 Desorption test

No desorption test was performed due to the degradation of BIT during the adsorption phase.

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.3b **ADSORPTION / DESORPTION SCREENING TEST (01)**

Annex Point IIA.7.7

4.4	**Mass balance**	Material balance was determined for the 4 soils and 1 sediment from the isotherm test at an application rate of 5 µg/mL. The results are presented in Table A7.1.3-7. Recoveries ranged from 96.8% to 98.4% with a mean of 97.5 ± 0.9%.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The test guideline followed was OECD 106 and US EPA OPPTS 855.2210. There were no deviations from this test guideline. The four soils and 1 sediment were gamma irradiated prior to dosing to enhance sterility and prevent biodegradation of BIT during the course of the experiment. Initially the solubility of BIT in 0.01M $CaCl_2$ and the potential to adsorb to the test vessel were examined. Both tests were performed in the absence of soil. ^{14}C-BIT was added to Teflon® centrifuge tubes and the supernatant radioassayed. The effect of the ratio of soil to 0.01M $CaCl_2$ solution was examined. Soil:$CaCl_2$ solutions ratios of 1:1, 1:2, and 1:5 were examined. Soil and $CaCl_2$ were equilibrated by shaking overnight and the next morning ^{14}C-BIT was added. The mixture was shaken for 24 hours, centrifuged, and the supernatant radioassayed. A study to determine the time necessary to reach equilibration was performed by adding soil and 0.01M$CaCl_2$ in a 1:2 ratio and mixing overnight. ^{14}C-BIT was added at 5 µg/ml and duplicate tubes removed and radioassayed at 1, 3, 6, and 24 hours. The supernatants were also chromatographed (HPLC). The soils from the 1 and 3 hours intervals were extracted with methanol and NaOH:methanol and the extracts chromatographed (HPLC). The definitive adsorption isotherm study was performed with a soil:0.01M $CaCl_2$ solution ratio of 1:2 and ^{14}C-BIT concentrations of 0, 0.05, 0.15, 0.5, 1.5, and 5 µg/mL. The soil and $CaCl_2$ solution were added to Teflon® centrifuged tubes, mixed overnight, and then the ^{14}C-BIT added. Tubes were shaken for 1 hour, centrifuged, and the supernatant radioassayed and chromatographed. The soils dosed at 5 µg/mL were extracted with methanol and NaOH:methanol in order to obtain a material balance.
5.2	**Results and discussion**	BIT showed a small adsorption to the 5 soils/sediment examined. There was abiotic degradation of BIT observed during the preliminary investigations and thus a 1 hour equilibration time was chosen for the isotherm test. Due to the degradation of BIT no desorption study was performed. Where examined, the recovery of applied ^{14}C-activity was greater than 96%.
5.3	**Conclusion**	Based on classifications of Briggs (Proc. 7th British Insecticide and Fungicide Conference, Nottingham, UK, 83-86, 1973) and Verdam et al. ([redacted] 1988) for the estimation of the mobility of plant protectants in soil based on K_d and/or K_{oc}-values, OPP can be classified as a moderately strong adsorbed substance.
5.3.1	Adsorbed a.s. [%]	The percent of ^{14}C-adsorption for the 5 soils/sediment after a 1hour

equilibrium is tabulated below.

Clay Loam	Silt Loam	Loam/Silt Loam	Loamy Sand Soil	Loamy Sand Sediment
45-66	63- 77	50-66	23-38	24-49

5.3.2 K_d (adsorption)

The adsorption coefficients (K_d) from the isotherm test are tabulated below.

Clay Loam	Silt Loam	Loam/Silt Loam	Loamy Sand Soil	Loamy Sand Sediment
1.98	3.88	2.27	0.94	0.67

5.3.3 K_{oc} (adsorption)

The adsorption constants (K_{oc}) are tabulated below.

Clay Loam	Silt Loam	Loam/Silt Loam	Loamy Sand Soil	Loamy Sand Sediment
41	144	58	94	35

5.3.4 Degradation products

BIT degraded in the test system. Degradation was abiotic as the system was found to be sterile after a 24 hour equilibration period. The identity of the degradate(s) was not determined, however, it is probably an oxidation product such as hydroxylation of the benzene ring or oxidation of the sulfur moiety.

5.4 Conclusion

The study provided is satisfactory to describe the mobility of BIT in soil. According to the US EPA classification scheme, BIT is considered high to very highly mobile. While the compound did degrade during testing, the adsorption values obtained here are similar to those reported in the US. EPA Registration Eligibility Document (RED) and thus are probably representative of BIT adsorption. It is highly likely that BIT and its oxidized products are similar in adsorption/mobility. Additionally, a ready biodegradation study (A7.1.1.2.1) demonstrated that BIT rapidly biodegrades. In soil BIT is probably biodegraded before it can leach and be an environmental concern.

5.4.1 Reliability

1-The study was conducted in full compliance with the OECD guidelines and in good agreement with the current US EPA guidelines.

5.4.2 Deficiencies

None

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.1.3b **ADSORPTION / DESORPTION SCREENING TEST (01)**

Annex Point IIA.7.7

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	*December 2010*
Materials and Methods	*Applicant's version is accepted with the following remarks:* *3.2. Degradation products are not tested.* *The pH of the aqueous phase should be measured before and after contact with the soil, since it plays an important role in the adsorption process, especially for ionisable substances such as BIT. Nevertheless, in this report, applicant only provides the value of pH of the supernatant after the performance of the test.* *According to OECD guidelines, the detection limits of the analytical method should be at least two orders of magnitude below the nominal concentration. In this test, the applicant does not provide the limit of detection of BIT with the analytical method.*
Results and discussion	*Applicant's version is accepted with the following remarks:* *Table A7.1.3-6: Freundlich Coefficients for ^{14}C-BIT 1/n (linearity term of theequation) and K_d values Table A7.1.3- show that the sorption of BIT isconcentration dependent. Therefore the freundlich K parameter is underestimatingthe sorption of BIT at environmentally relevant concentrations (corresponding tothe low part of the isotherm). In the absence of a risk exposure assessmentdepending on the adsorbed concentration, an average value of the single $K_d$measure is more representative than the $K_{Feundlich}$ value.* *The final K_d and K_{oc} table should be:*

Soil	Percent AS Adsorbed	K_d	K_{oc}
Clay Loam	45 - 66	2.85	59
Silt Loam	63 - 77	5.41	200
Loam/Silt Loam	50 – 66	3.01	79
Loamy Sand Soil	23 – 38	0.94	117
Loamy Sand Sediment	24 - 49	1.22	64

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

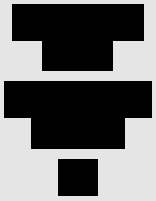
Subsection A7.1.3b

Annex Point IIA.7.7

ADSORPTION / DESORPTION SCREENING TEST (01)

Conclusion	*Applicant's version is accepted with minor changes:* *While determining the equilibration time it was discovered that BIT was degrading. This degradation was due to an abiotic process (oxidation) because the soils were sterile. It was necessary to use a short equilibration time (1 h) to reduce the effect of degradation on the study even though BIT had not come to acomplete equilibrium. Yet, according to the water solubility and K_d values published in the US EPA Registration Eligibility Document (RED) for BIT, K_d values ranged between 1.24 and 9.56. Therefore the adsorption values obtained in this study are reasonable and BIT can be considered as a highly mobile compound.*
Reliability	*2*
Acceptability	*acceptable*
Remarks	

Table A7.1.3-1: Classification and Physiochemical Characteristics of Soils and Sediment Used as Absorbents

Parameter	Soil Type				
	Clay Loam	Silt Loam	Loam/Silt Loam	Loamy Sand Soil	Loamy Sand Sediment
Sampling Location					
Percent Sand[1]	40	23	32	87	76
Percent Silt[1]	32	61	50	4	20
Percent Clay[1]	28	16	18	9	4
Organic Matter (%)	8.3	4.7	6.7	1.4	3.3
Organic Carbon (%)	4.8	2.7	3.9	0.8	1.9
pH	8.0	7.0	5.3	5.1	7.3
CEC[2] (meq/100g)	41.6	26.7	23.3	11.4	12.1
Water Holding Capacity (0.33 bar)	31.7	26.3	15.5	7.1	11.2
Nitrogen content (%)	0.50	0.34	0.30	0.12	0.17

[1]USDA particle size distribution
[2]CEC = Cation Exchange Capacity

Table A7.1.3-2: Distribution and Recovery of ^{14}C-Activity During Equilibration Time Determination

Soil	Sampling Interval (h)	Supernatant	Methanol Soil Extract	NaOH/Methanol Soil Extract	Recovery
Clay Loam	1	33.5	30.9	24.4	88.8
	3	31.7	28.3	24.9	84.9
	6	29.2	27.1	24.2	80.5
	24	26.1	16.2	19.4	61.7
Silt Loam	1	24.7	40.9	33.9	99.5
	3	21.6	40.4	35.9	97.9
	6	19.1	34.6	39.0	92.7
	24	16.3	34.5	38.8	89.6
Loam/Silt Loam	1	27.2	36.9	27.1	91.2
	3	24.7	35.5	27.8	88.0
	6	22.0	34.7	28.5	85.2
	24	19.4	23.0	26.1	68.5
Loamy Sand Soil	1	57.2	40.3	5.0	102.5
	3	54.6	41.5	6.3	102.4
	6	52.8	43.6	6.4	102.8
	24	45.0	44.6	9.6	99.2
Loamy Sand Sediment	1	36.2	34.7	27.5	98.4
	3	27.2	35.0	33.9	96.1
	6	26.9	34.5	33.9	95.3
	24	20.3	30.5	38.2	89.0

Table A7.1.3-3: Distribution and Recovery of ^{14}C-BIT

Soil	Sampling Interval (h)	BIT as a Percent of Applied Radioactivity		
		Supernatant	Total Soil Extract	Recovery
Clay Loam	1	44.5	7.7	52.2
	3	37.5	8.3	45.8
Silt Loam	1	33.0	30.7	63.7
	3	28.5	27.7	56.2
Loam/Silt Loam	1	35.9	14.7	50.6
	3	29.9	22.1	52.0
Loamy Sand Soil	1	68.6	3.3	71.9
	3	65.6	7.5	73.1
Loamy Sand Sediment	1	46.2	1.2	47.4
	3	36.6	0.1	36.7

Table A7.1.3-4: Adsorption of ^{14}C BIT to Soil During the Isotherm Test

Soil	Nominal Dose (µg/mL)	Percent of ^{14}C BIT Applied[1]		pH
		Adsorbed to Soil	Supernatant	
Clay Loam	5	44.5	55.5	7.37
	1.5	52.3	47.7	7.49
	0.5	59.5	40.5	7.65
	0.15	65.7	34.3	7.69
	0.05	64.5	35.5	7.77
Silt Loam	5	63.2	36.8	6.49
	1.5	70.2	29.8	6.87
	0.5	73.0	27.0	6.71
	0.15	77.3	22.7	6.66
	0.05	77.4	22.6	6.68
Loam/Silt Loam	5	49.7	50.3	4.27
	1.5	56.7	43.3	5.25
	0.5	60.3	39.7	5.22
	0.15	65.4	34.6	5.22
	0.05	65.8	34.2	5.21
Loamy Sand Soil	5	23.1	76.9	4.33
	1.5	29.0	71.0	4.36
	0.5	31.3	68.7	4.33
	0.15	37.5	62.5	4.33
	0.05	37.8	62.2	4.32
Loamy Sand Sediment	5	24.4	75.6	6.85
	1.5	24.0	76.0	6.58
	0.5	36.8	63.2	7.09
	0.15	48.4	51.6	7.22
	0.05	48.5	51.5	7.65

[1]Average of duplicate samples

Table A7.1.3-5: Adsorption Coefficients Resulting from the Isotherm Test

Soil	Nominal Dose (µg/mL)	Adsorption Coefficients (mL/g)[1]		
		K_d	K_{doc}	K_{dom}
Clay Loam	5	1.63	34	20
	1.5	2.19	46	26
	0.5	2.97	62	36
	0.15	3.81	79	46
	0.05	3.65	76	44
Silt Loam	5	3.41	126	72
	1.5	4.64	172	99
	0.5	5.39	200	115
	0.15	6.79	251	144
	0.05	6.84	253	146
Loam/Silt Loam	5	1.98	51	30
	1.5	2.64	68	39
	0.5	3.02	78	45
	0.15	3.81	98	57
	0.05	3.87	99	58
Loamy Sand Soil	5	0.60	74	43
	1.5	0.81	101	58
	0.5	0.89	111	64
	0.15	1.18	147	84
	0.05	1.20	150	85
Loamy Sand Sediment	5	0.65	34	20
	1.5	0.62	33	19
	0.5	1.14	60	35
	0.15	1.83	96	55
	0.05	1.86	98	56

[1]Average of duplicate samples

Table A7.1.3-6: Frundlich Coefficients for ^{14}C-BIT

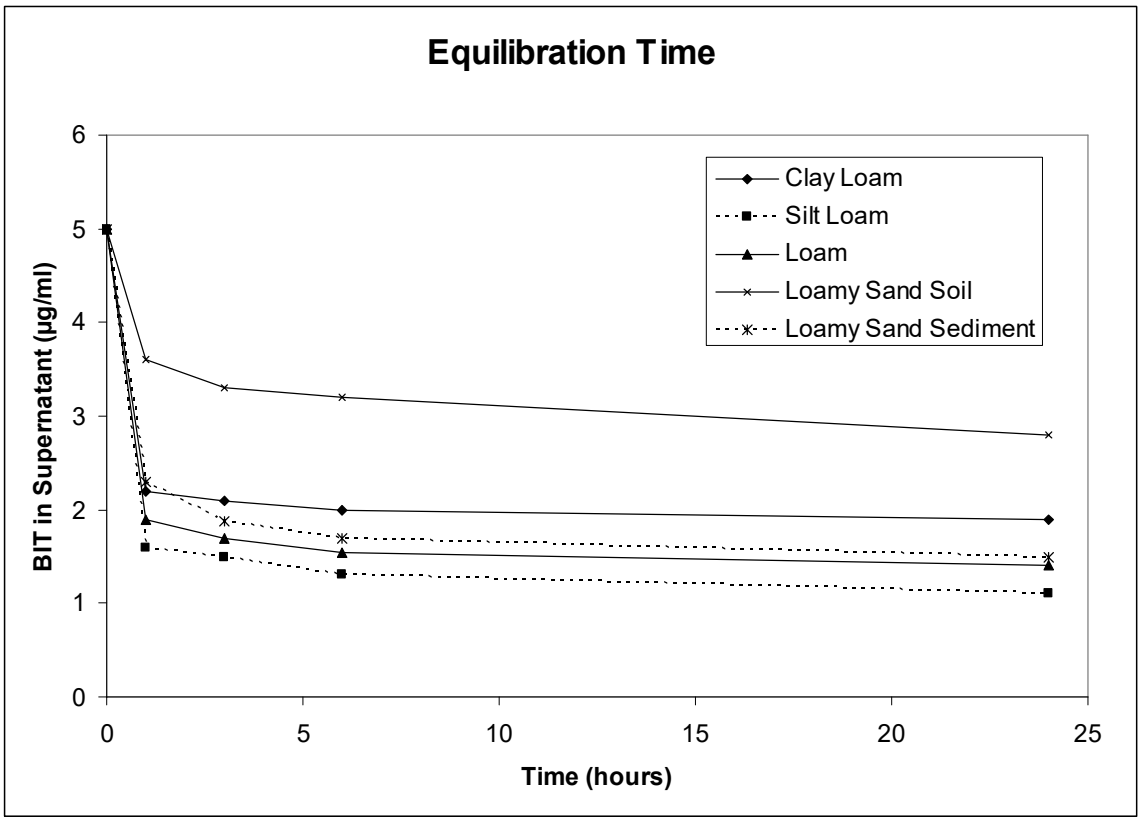
Soil	Adsorption Coefficients (mL/g)			1/n	r^2
	K	K_{oc}	K_{om}		
Clay Loam	1.98	41	24	0.8319	0.9966
Silt Loam	3.88	144	83	0.8629	0.9985
Loam/Silt Loam	2.27	58	34	0.8654	0.9987
Loamy Sand Soil	0.75	94	54	0.8538	0.9958
Loamy Sand Sediment	0.67	35	20	0.7463	0.9794

Table A7.1.3-7: Material Balance of Applied Radioactivity from Soils Treated at 5 µg/mL

Soil	Percent of Applied ^{14}C-Activity[1]					
	Supernatant	Methanol Soil Extract	NaOH/Methanol Soil Extract	Acetone Soil Wash	Combusted Residues	Recovery
Clay Loam	31.0	31.6	23.7	0.6	10.1	96.8
Silt Loam	23.1	39.9	31.9	0.7	2.9	98.4
Silt/Silt Loam	26.2	38.8	25.0	1.0	6.5	97.4
Loamy Sand Soil	56.0	36.5	3.9	0.1	0.5	96.9
Loamy Sand Sediment	27.3	33.7	30.7	0.9	5.4	97.9
Mean						97.5 ± 0.9

[1]Average of duplicate samples

Table A7.1.3.b-1: Adsorption Equilibration



Section A7 **Subsection A7.1.4.1** **Annex Point IIIA 12.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **FIELD STUDY ON ACCUMULATION IN THE SEDIMENT**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X].**	
Detailed justification:	A waiver for performing Field Studies on the Accumulation of BIT in sediment is requested. A waiver has been requested for performing water:sediment studies (A7.1.2.2.2) based on the limited adsorption of BIT to sediment. According to Chapter 3, Section 7.0.2.3.2 (and Figure 1 in section 7) a sediment:water study is only required when the K_p> 2000. As this is not the case, field studies on sediment are not applicable. Additionally, based on the use pattern, there should be limited exposure to sediment. Thus this study will have no impact on the environmental risk assessment.	
Undertaking of intended data submission [ ]	No studies are planned.	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	*January 2011*	
Evaluation of applicant's justification	*Adopt applicant's version.*	
Conclusion	*Adopt applicant's version.*	
Remarks		

Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

Official use only

- **REFERENCE**

• **Reference**	[redacted] (2020a) 1,2-Benzisothiazol-3(2H)-one – Route and Rate of Degradation of [14C]-1,2-Benzisothiazol-3(2H)-one in Four Soils under Aerobic Conditions [redacted] 20 December 2018, Amended 28 January 2020.
• **Data protection**	Yes
Data owner	[redacted]
Criteria for data protection	Data on existing a.s. submitted for first entry into the European list of approved biocidal active substance

- **GUIDELINES AND QUALITY ASSURANCE**

• **Guideline study**	Yes (OECD Guideline 307 (Adopted 24th April 2002) OPPTS 835.4100, US EPA, October 2008)
• **GLP**	Yes
• **Deviations**	No

- **MATERIALS AND METHODS**

• **Test material**	Test substance details are summarised below
General information	1,2-Benzisothiazol-3(2H)-one; CAS number: 2634-33-5; Molecular formula: C_7H_5NOS; Molecular weight: 151.19 g/mol
Labelled test material (Lot/Batch number; purity)	1,2-[ring-U-^{14}C]Benzisothiazol-3(2H)-one (thereafter referred to as [^{14}C]Benzisothiazolone) [redacted]
Unlabelled test material (Lot/Batch number; purity, description)	1,2-Benzisothiazol-3(2H)-one; [redacted] white to yellow and faint beige to beige powder)
Reference items	MET1 (R1): Hydroxy-1,2-benzisothiazolin-3-one MET2 (R2): 1,2-Benzisothiazolin-3-one-1-oxide MET3 (R3): Dihydroxy-1,2-benzisothiazolin-3-one MET4 (R4): o-Sulphobenzamide (sodium salt) MET7 (R7): N-(4-amino-4-hydroxy-buta-1,3-dienyl)-benzamide Saccharin (R8): 1,2-Benzisothiazolin-3-one-1-dioxide 2-Sulphanylbenzamide (R9) 2-Sulphobenzoic acid hydrate (R11)

Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

Stability	Stability was determined before and after application. Test substance was stable during the application procedure.
Composition of Product	Not relevant as active substance was tested
• **Test system**	Laboratory test
Selection of test system	Four field fresh soil types were selected to evaluate the route and rate of degradation of the test substance in the environment.
Soil type and preparation	Four standard representative fresh field soils with a wide range of soil properties were used: Soil I: Speyer 2.4 (loam), Soil II: Speyer 5M (sandy loam), Soil III: RefeSol 02-A (silt loam) and Soil IV: RefeSol 04-A (loamy sand). Soils were characterised for particle size distribution, moisture content at water holding capacity and pF 2, pH, % organic matter and cation exchange capacity. Details are given in Table A7.2.1/01-1. Bioactive soils were conditioned to room temperature for approx. 6-8 days prior to application. Sterile soils were sterilised by gamma radiation. Moisture content was adjusted to pF 2, controlled during incubation and adjusted if necessary.
Determination microbial biomass	For bioactive soil the microbial biomass was determined before during and at the end of incubation according to the fumigation extraction method by Vance, Brookes and Jenkinson.
Experimental conditions	The test was performed under aerobic conditions in the dark in an air-conditioned room at a temperature of 20.8 ± 0.2°C and 20.9 ± 0.2°C and a soil moisture content of pF 2. Samples are equipped with a trapping system including a safety trap and two absorption traps for organic volatiles and CO_2.
• **Treatment and sampling**	Soil samples of 100 g (equivalent dry weight) were treated with 50 µg test substance which is equivalent to an initial concentration of 0.5 mg per kg dry soil equivalent. Duplicate samples were taken for extraction and analysed after 0.00, 0.04, 0.08, 0.17, 0.33, 1.0, 2.1, 4, 7, 14, 28, 56, 91 (Soils I-III only) and 120 (Soil IV only) days of incubation for bioactive soils and after 0.00, ~1, 13, 28, 91 (Soils I-III only) and 120 (Soil IV only) days of incubation for sterile soils.
• **Extraction**	Soils were extracted four times with acetonitrile, acetonitrile/water (4:1, v:v), acetonitrile/water (1:1, v:v) and acetonitrile/0.1 hydrochloric acid (1:1, v:v). Soxhlet extraction using acetonitrile/water/32% hydrochloric acid (80:20:0.1, v:v:v) was performed if >10% AR remained non-extracted in the samples after the first four extraction steps. If non-extractable radioactivity is still > 10% AR harsh extraction under reflux conditions followed by organic matter fractionation was performed.
• **Analytical method**	Radioactivity contained in solutions was measured by liquid scintillation counting (LSC). Volumes of extracts were determined and dispensed aliquots were assayed in duplicate. The quantity of radioactivity was determined using a calibrated Packard liquid scintillation counter equipped with DPM and luminescence options. Non-extractable radioactivity remaining within the soils was determined after combustion by LSC and volatile radioactivity in the trapping solutions were also analysed by LSC. For identification radioactive components were compared with reference standards by co-chromatography. Aliquots from extracts were mixed with solutions of reference items and the mixtures injected to the HPLC system. Mass spectrometry (MS) was

used to confirm the identification of major metabolites performed by co-chromatography with reference standards and to identify metabolite(s) for which no reference standard was available.

- **RESULTS**

- **Analytical results**

Total mean recovery of radioactivity during the incubation period accounted for 97.6 ± 2.9, 96.8 ± 3.4, 96.7 ± 3.4 and 94.9 ± 3.0% AR for four bioactive soils respectively. The corresponding values for the sterile soils were 98.0 ± 0.8, 97.7 ± 1.0, 97.4 ± 1.1 and 97.1 ± 3.4% AR. The mean amount of extractable radioactivity at room temperature at 0.00 DAT was 66.7, 83.1, 79.6 and 90.3% AR in the bioactive soils respectively, and 70.9, 91.6, 88.0 and 91.9% AR in the sterile soils, respectively. Thereafter, it decreased to 2.8, 2.4, 5.7 and 11.4% AR in the bioactive soils, respectively, and to 59.1, 62.5, 61.3 and 43.4% AR in the sterile soils, respectively. Soxhlet extraction was performed for all soil samples except 3 samples where the extractable radioactivity was below >90% AR after extractions at room temperature. The mean amount of radioactivity extractable with Soxhlet extraction reached a maximum of 5.6, 5.9, 7.5 and 6.0% AR for bioactive soils, and a maximum of 7.2, 5.6, 5.5 and 7.8% AR for sterile soils, respectively. Non-extractable residues increased from 27.6, 10.3, 13.0 and 6.9% AR on 0.00 DAT to maximum levels of 52.0, 42.9, 44.6 and 45.6% AR on 56 DAT respectively for bioactive soils tested, and from 20.3, 5.8, 8.1 and 4.8% AR on 0.00 DAT to maximum levels of 40.7, 37.7, 36.4 and 47.0% AR on 13-28 DAT, respectively, for the four sterile soils tested. At the end of incubation, amounts were 48.6, 39.9, 43.2 and 41.9% AR respectively for the four bioactive soils and 36.2, 33.1, 31.3 and 41.8% AR, respectively, for the four sterile soils tested. The mineralisation of [14C]Benzisothiazolone was extensive and carbon dioxide reached a maximum of 47.9, 56.2, 46.1 and 39.9% AR at the end of incubation in bioactive soils. Harsh extraction of bioactive soil samples from 56 DAT under reflux conditions further released 5.7, 3.7, 7.3 and 5.7 % AR, proving that only small amounts might become bioavailable in addition. Mineralisation in sterile soils was negligible. No other organic volatiles exceed 0.1% AR over the study duration. Determination of the microbial biomass showed that the soils were viable throughout the incubation period.

- **Degradation and transformations**

In the bioactive soils, up to six major degradation products were detected with maximum occurrences of 29.4 (MET2), 8.2 (M5), 16.9 (M8), 45.0 (M6 and M6b; could not sufficiently separated by HPLC), and 21.1% (M9) AR. MET 2, M5, M8 and M6b were confirmed to be 1,2-Benzisothiazolin-3-one-1-oxide, Saccharin, 2-Sulphanyl benzamide and 2-Sulphobenzoic acid. M6 was proposed to be 2-Sulpha-moylbenzoic acid and M9 to be 2-Aminosulphinylbenzoic acid. [^{14}C]Benzisothiazolone degraded in the bioactive soils with DT_{50} values between 0.02 and 0.24 days, and DT_{90} values ≤0.80 days based on the SFO kinetic model (please refer to Table A7.2.1/01-1). In the sterile soils, the degradation was only slightly slower with DT_{50} values of 0.4 to 0.7 days, and DT_{90} values ≤2.45 days.

Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

- **APPLICANT'S SUMMARY AND CONCLUSION**

• **Materials and methods**	The degradation of [^{14}C]Benzisothiazolone was performed according to the Regulation (EU) No 528/2012 and the OECD Guideline 307 (2002) and the US EPA Guideline OPPTS 835.4100 (2008). [^{14}C]Benzisothiazolone was applied to four soils and incubated under aerobic conditions at a temperature of 20.8 ± 0.2°C and 20.9 ± 0.2°C and a soil moisture content of pF2 in the dark for up to 120 days.
• **Results and discussion**	Mineralization of [^{14}C]Benzisothiazolone was extensive in bioactive soils and carbon dioxide released reached a maximum of 47.9, 56.2, 46.1 and 39.9% AR at the end of incubation in four soils tested, respectively. Mineralization of [^{14}C]Benzisothiazolone in sterile soils was negligible and did not exceed 0.4% AR. [^{14}C]Benzisothiazolone degraded via oxidation to 1,2-Benzisothiazolin-3-one-1-oxide (MET2) and further to Saccharin (M5). Two other degradation products M6 and M9 were observed, which were proposed to be 2-Sulphamoylbenzoic acid and 2-Aminosulphinyl-benzoic acid. M6 and M9 were presumably formed by opening of the thiazolinone ring. Further oxidation or hydrolysis formed 2-Sulphobenzoic acid (M6b). Additionally, the transient metabolite 2-Sulphanyl benzamide (M8) was quickly oxidised to 2-Sulphobenzoic acid. Non-extractable residues increased to maximum levels of 52.0, 42.9, 44.6 and 45.6% AR on 56 DAT respectively for four soils tested.
• **Conclusion**	[^{14}C]Benzisothiazolone degraded in soil with half-lives ranging from 0.02 to 0.24 days, and DT_{90} values ≤ 0.80 days. [^{14}C]Benzisothiazolone degrades under formation of 1,2-Benzisothiazolin-3-one-1-oxide (MET2), Saccharin (M5), M6, M9, 2-Sulphobenzoic acid (M6b), and the transient metabolite 2-Sulphanyl benzamide (M8) with ultimate formation of bound residues and CO_2.
Reliability	1
Deficiencies	No

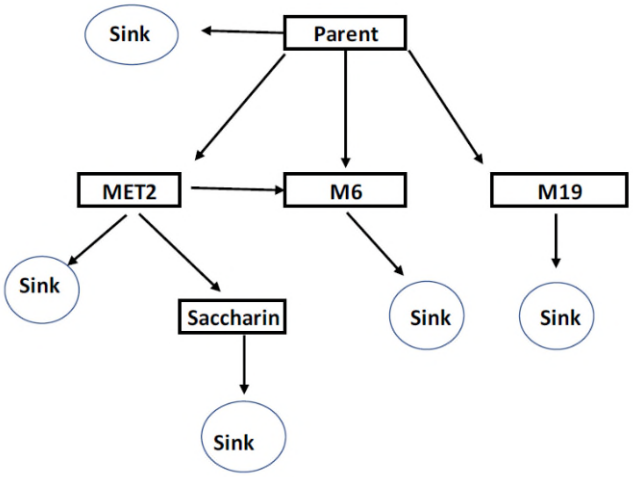
EVALUATION BY COMPETENT AUTHORITIES	
Date	*19/08/2021*
Materials and Methods	Applicant's version is adopted. The degradation of [14C]Benzisothiazolone was performed according to the Regulation (EU) No 528/2012 and the OECD Guideline 307 (2002) and the US EPA Guideline OPPTS 835.4100 (2008). Four standard representative fresh field soils with a wide range of soil properties were used: Soil I: Speyer 2.4 (loam), Soil II: Speyer 5M (sandy loam), Soil III: RefeSol 02-A (silt loam) and Soil IV: RefeSol 04-A (loamy sand). Sampling was done after 0.00, 0.04, 0.08, 0.17, 0.33, 1.0, 2.1, 4, 7, 14, 28, 56, 91 (Soils I-III only) and 120 (Soil IV only) days of incubation for bioactive soils and after 0.00, ~1, 13, 28, 91 (Soils I-III only) and 120 (Soil IV only) days of incubation for sterile soils. The soils were applied at three time-points with application solution #1, #2 and #3 on March 20, 2018, March 22, 2018 and April 26, 2018 respectively. On each application day, prior to, during and after application, identical aliquots (i.e. 1000 µl) of the used application solution were diluted to 20 mL with water. The required recovery of radioactivity (90-110% AR) was achieved for all samples with an exception of four replicates from bioactive soils (intervals of 7, 14 and 28 DAT; Table 3 to Table 6). For these four replicates, it can be assumed that the loss of radioactivity occurred in trapping of radiolabelled carbon dioxide, as might be noted from the lower levels of 14CO2 found in these samples in comparison to corresponding other replicates, and intervals before and after. Therefore, the results obtained from HPLC analysis of these replicates are considered acceptable, and have not been excluded from the kinetic evaluation.

Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

Results and discussion	The applicant's version is acceptable with the following remarks: Total mean recovery of radioactivity during the incubation period accounted for 97.6 ± 2.9, 96.8 ± 3.4, 96.7 ± 3.4 and 94.9 ± 3.0% of applied radioactivity (AR) for four bioactive soils respectively. The corresponding values for the sterile soils were 98.0 ± 0.8, 97.7 ± 1.0, 97.4 ± 1.1 and 97.1 ± 3.4% AR. The required recovery of radioactivity (90-110% AR) was achieved for all samples with an exception of four replicates from bioactive soils (intervals of 7, 14 and 28 DAT. For these four replicates, it can be assumed that the loss of radioactivity occurred in trapping of radiolabelled carbon dioxide, as might be noted from the lower levels of 14CO2 found in these samples in comparison to corresponding other replicates, and intervals before and after. BIT disappears very fast in every soil and the number of data points before the DT50 is limited. In addition DT50 values presented in this summary are not adequate because: • Values presented in table Table A7.2.1/01- 18 correspond only to parent. Metabolites were not considered in the parent's DT50 calculation and they should be considered as indicated Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (FOCUS Kinetics Guidance) • Data issues such as time zero samples or values below the quantification and detection limit were not adequately considered for DT50 calculations. Nevertheless, the applicants have presented a document: "Determination of rates of decline for 1,2-Benzisothiazol-3(2H)-one and its metabolites in soil according to FOCUS Kinetics Guidance" written by Dr. A. Mamouni, Dr. T. Jarvis & V. Montesano where all these aspects were adequately considered. The procedure followed for kinetic assessment has been the following: The data were fitted directly using CAKE v. 3.3 using the Application Preferences FOCUS Guideline and the Iteratively Reweighted Least Squares (IRLS) fitting option. The optimisation was conducted as follows: • First, the parent compartment was fitted, without any reference to the metabolite. • Then the metabolite compartment was fitted, with the parameters for the parent calculated in the first step fixed (and therefore not increasing the complexity of the optimisation). • Finally, both compartments were fitted, using the results of step 2 as a starting point. This step is complex (with all parameters free) but started from near the optimum. Metabolites were fitted in the stepwise procedure indicated by the guidance (FOCUS, 2014). Parent data were fitted with the parent best-fit model, the parameters were fixed for the metabolite fitting step and, finally, the parameters were un-fixed for a re-fit. For the kinetic fit, parent BIT was assumed to degrade according to the metabolism scheme as presented in Figure 1 and 2, next. This pathway showed to give the best fit for the metabolites in all soils.

Figure 2. Simplified metabolic pathway used for metabolite kinetics



The first step of degradation of the parent compound was observed to be the oxidation of sulphur to form the 1,2-benzisothiazolin-3-one-1-oxide metabolite (MET2), followed by a further oxidation step to form saccharin (M5) and/or opening of the thiazolinone ring leading to several metabolites such as the 2-sulphamoylbenzoic acid metabolite (M6), and the transient 2-aminosulphinyl-benzoic acid metabolite (M9). The ultimate oxidation/hydrolysis products were identified as 2-sulphobenzoic acid (M6b), which is rapidly mineralized, and the minor metabolite o-sulphobenzamide MET4 (detected in sterile soils only). Additionally, the transient 2-sulphanyl benzamide metabolite (M8) was observed, and it was quickly oxidised under the incubation conditions to 2-sulphobenzoic acid.

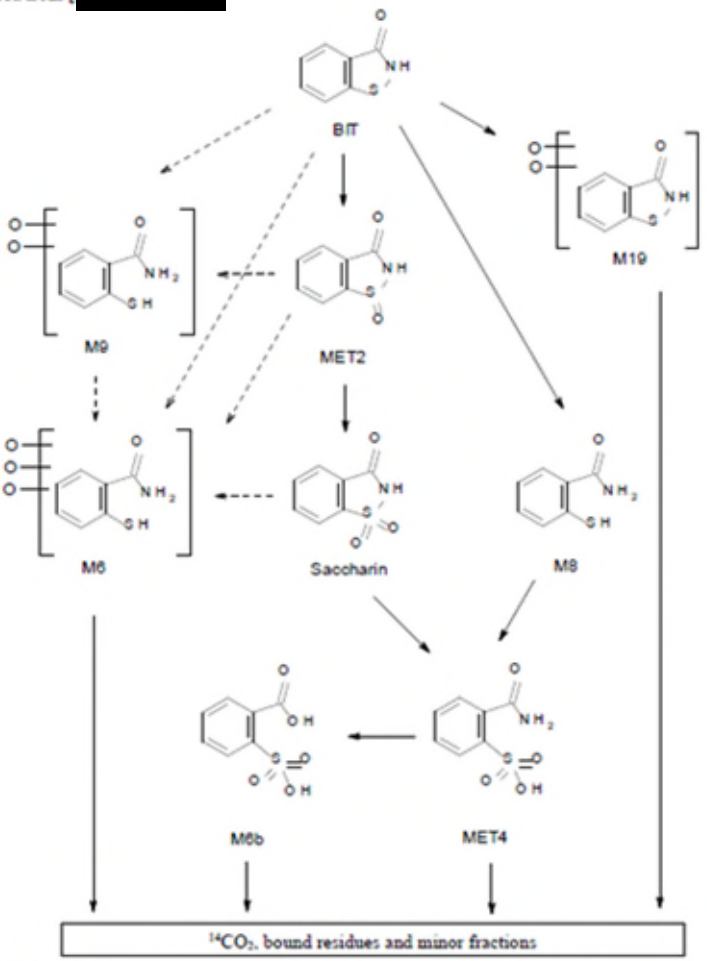
Major degradants include 1,2-benzisothiazolin-3-one-1-oxide (met 2, max average 23.1% of AR across the 4 soils). MET-2 is an intermediate metabolite with unclear structure, but it degrades rapidly to saccharin. Saccharin (7.8%AR across the three soils were it was found)%), 2-sulphanyl benzamide (M8) (10.52%), 2-aminosulphinylbenzoic acid (M9) (14.1%), Metabolite 6 (whose chemical structure could not be identified, 40.55% including M6b). Metabolite M19 did not exceed 5% in the non-sterile soils and reached the maximum of 4.9% AR. M9 is a transient metabolite which is further rapidly degraded to M6. M8 also degraded very fast, as well as saccharin and 1,2-benzisothiazolin-3-one-1-oxide.

Formation fractions of the different metabolites were: 0.31 for metabolite 2 (from parent), 0.88 for metabolite 6 (including M6b) (from parent and from met 2), 0.366 for met 5 or saccharin (from met 2) and 0.046 for M19 (see also the transformation pathway above).

Several other unidentified metabolites were found in bioactive soils, but none of them at levels >10% AR at a single sampling event, or ≥5% AR at two consecutive sampling intervals

Figure 1. **Proposed metabolic pathway (**[redacted]

Revised from [[redacted]



All metabolites but MET4 were found in bioactive and sterile soils; MET4 was found in sterile soils only [Piskorski, 2020a].
Structures of metabolites M6, M9 and M19 were tentatively proposed based on the LC-MS structure elucidation and chromatographic behaviour only [Piskorski, 2020a]; likely structures of M6 and M9 are given on page 9.

For determining the best model aspects such as visual fit, chi square and t-test were considered for goodness of fit.

The values reported for parent alone are:

Table 6: Summary of BIT kinetics in soil calculated with parent only under aerobic soil conditions

Laboratory study: Parent (non-sterile conditions) / Trigger (T) and modelling (M) endpoints

Soil	Kinetic model	Mo	Parameter (K, K1, k2, g, tb, α, β)	χ2 %-error & visual fit	Prob>t	Lower CI	Upper CI	DT50 [days]	DT90 [days]
Soil I	**SFO (T & M)**	94.3	k=63.97	5.2 Very good	1.8E-09	56.5	71.5	0.01	0.04
	FOMC	94.3	α=1.192 β=0.004875	3.5 Very good	n.r. n.r.	0.48 -0.003	1.91 0.013	0.004/0.009** not reliable	0.029 not reliable
	DFOP	94.3	K1=70.9 K2=0.3004 g =0.9823	1.1 Very good	1.8E-09 0.27 n.r.	64.9 -0.80 0.97	76.97 1.4 0.99	nd not reliable	nd not reliable
Soil II	**SFO (M)**	93.8	k=32.12	9.9 Very good	1.4E-10	28.4	35.8	0.02	0.07
	FOMC (T)	94.1	α=1.545 β=0.02729	3.2 Very good	n.r. n.r.	1.09 0.014	2.0 0.04	0.02/0.03**	0.09
	DFOP	94.1	K1=45.44 K2=6.311 g =0.8532	4.3 Very good	1.9E-05 0.039 n.r.	30.96 -0.86 0.69	59.9 13.48 1.02	0.02/0.11* not reliable	0.09 not reliable
Soil III	**SFO (M)**	92.4	k=45.75	8.1 Very good	3.1E-09	40.07	51.44	0.02	0.05
	FOMC (T)	92.5	α=1.315 β=0.01197	3.6 Very good	n.r. n.r.	0.84 0.003	1.79 0.02	0.01/0.02**	0.06
	DFOP	92.5	K1=53.64 K2=1.344 g =0.9588	3.2 Very good	6.6E-09 0.06 n.r.	48.28 -0.48 0.94	59.0 3.17 0.98	0.01/0.52* not reliable	nd not reliable
Soil IV	SFO	84.5	k=6.67	17.3 Acceptable	1.5E-05	4.43	8.91	0.10	0.35
	FOMC	93.5	α=0.7476 β=0.04234	6.3 Very good	n.r. n.r.	0.51 0.02	0.98 0.07	0.06/0.27**	0.88
	DFOP (T&M)	94.2	K1=42.53 K2=2.731 g =0.4576	3.5 Very good	0.004 1.1E-04 n.r.	13.39 1.65 0.33	71.66 3.81 0.59	0.05/0.25*	0.02

* slow phase
** DT90/3.32
n.r. = not relevant
nd = not determined
Bold: optimum fit/ T= Trigger / M = Modelling
Prob >t: P value from the t-test (acceptability criteria P ≤ 0.05)
CI: confidence interval (95%)

Once the best model for parent was determined, metabolites fitting was done starting from the best parent fit. SFO was considered enough for metabolites fitting.

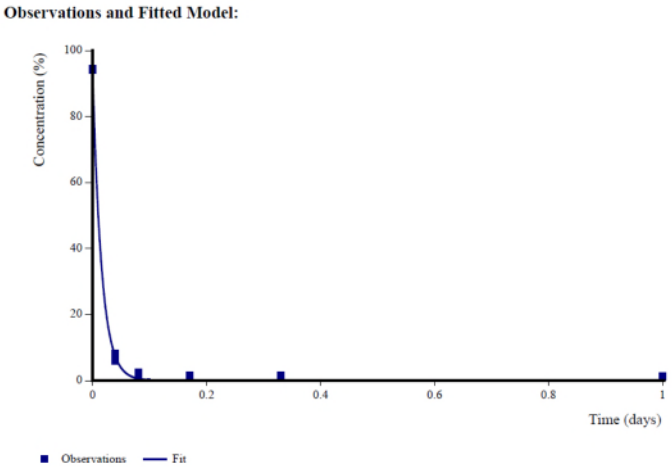
The next table shows parent results when all metabolites are included in Cake iteration process.

Soil	Kinetic model for parent	Parameter (k, k1,k2, k3, g	Chi-square	T test	DT50	DT90
I	SFO	62.89	5.26	1.38E-29	0.01	0.004
II	FOMC	Alpha =1.452 Beta: 0.025	3.65	N/A	0.0157 0.0993/3.32 = 0.03	0.09
III	FOMC	Alpha: 1.308 Beta: 0.01178	3.56	N/A	0.00823 0.0567/3.32 = 0.017	0.06
IV	DFOP	K1: 41.23 K2: 2.5	3.64	8.93E-6 8.1E-10	Overall: 0.056 DT50k1: 0.0168 DT50k2: 0.27	0.656

The results are similar to the DT50s obtained with parent alone, eCA considers this is a good indication of good adjustment.

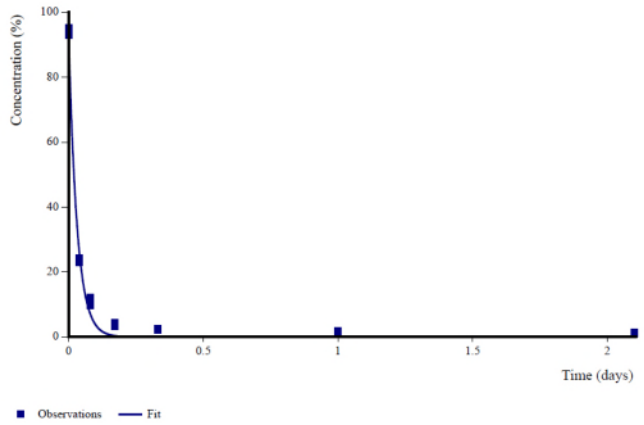
eCA notes that that due to the rapid disappearance of BIT, the number of data points before the DT50 occurs is limited in three of the soils, in fact only the initial value was measured as the following graphs show.

Soil I



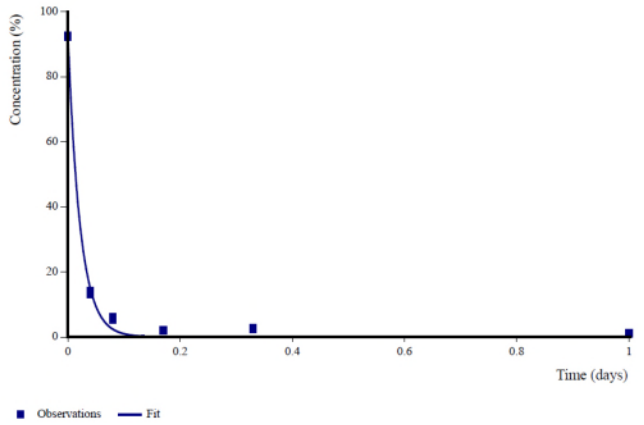
Soil II

Observations and Fitted Model:



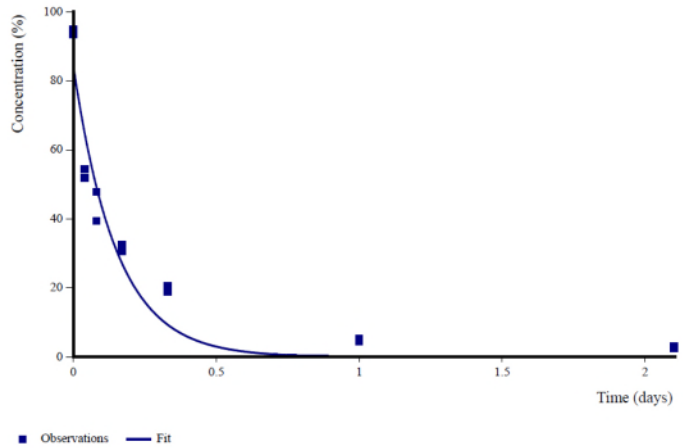
Soil III

Observations and Fitted Model:



Soil IV

Observations and Fitted Model:



Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

	This was considered as an uncertainty to the calculated DT50s for soils I, II and III. For this reason, eCA considered it adequate to use the worst case (loamy sand) DT50 = 0.27 days or 0.54 at 12ºC for risk assessment, also because soil IV is the case where more data points (3) exist before the DT50. This DT50 is the result of modelling the best parent fit for soil IV Refe Sol 04-A (loamy sand) which is DFOP, with the metabolites found in this soil. In soil, among the relevant metabolites, the highest DT50 corresponds to metabolite M6. The rate of degradation of M6 metabolite (including M6b fraction and the transient metabolite M9) was much slower when compared to the parent compound. DT50 values ranging from 21.5 to 46.3 days were calculated (43.8 and 94 days at 12ºC and 62.14 geomean at 12ºC). MET2 metabolite, which was shown to be rapidly formed from the parent compound, was very rapidly degraded in all soils with DT50 values ranging from 0.3 to maximum 2.3 (slow phase) days. Saccharin and M19 metabolites showed also acceptable fits and were degraded with DT50 values ranging from 6.3 (12.6) to 10.3 (20.6), and 2.0 (4) to 23.2 (46.4 at 12ºC) days, respectively. Due to the rapid degradation and the lack of sufficient data points, no kinetics can be calculated for metabolites M8 and M9. For metabolites risk assessment eCA considers it relevant to assess metabolite 6. This metabolite has a DT50 in soil of 62.14 at 12ºC (geomean) and a predicted koc = 10 L/kg and is a concern in case of direct releases to soil, which occur in the paint and coatings scenario. The other metabolites of BIT are less toxic than the parent substance and show a potential for rapid degradation in the environment. In addition, they do not show a potential for bioaccumulation. Mineralization of [14]Benzisothiazolinone was extensive and carbon dioxide released reached a maximum of 47.9, 56.2, 46.1 and 39.9% AR at the end of incubation in four soils tested, respectively. In the sterile soils, the mineralization of BIT was negligible and did not exceed 0.4% AR in all soils tested. For the bioactive soils, the mean amount of non-extractable residues increased from 27.6, 10.3, 13.0 and 6.9% AR on 0 DAT to maximum levels of 52.0, 42.9, 44.6 and 45.6% AR on 56 DAT respectively for four soils tested. At the end of incubation, the amounts were 48.6, 39.9, 43.2 and 41.9% AR respectively for four soils tested.
Conclusion	eCA considers the study and analysis provided by the applicant valid. The test was done according to Guidelines. The required recovery of radioactivity (90-110% AR) was achieved for all samples with an exception of four replicates from bioactive soils (intervals of 7, 14 and 28 DAT). For these four replicates, it can be assumed that the loss of radioactivity occurred in trapping of radiolabelled carbon dioxide, as might be noted from the lower levels of 14CO2 found in these samples in comparison to corresponding other replicates, and intervals before and after. Due to the rapid disappearance of BIT, the number of data points before the DT50 occurs is limited in three of the soils, in fact only the initial value was measured. This adds uncertainty to the calculated DT50s for these three soils (soil I, II and III). For this reason, eCAs considers it adequate to use the worst case (loamy sand) DT50 = 0.27 days or 0.54 at 12ºC for risk assessment also because soil IV is the case where more data points (3) exist before the DT50. A DT50 = 62.14 d will be considered for metabolite 6.
Reliability	*1*

Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

Acceptability	acceptable

Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

Table A7.2.1/01- 2: Test soils used

Parameters	Soil I	Soil II	Soil III	Soil IV
	Speyer 2.4	Speyer 5M	RefeSol 02-A	RefeSol 04-A
Site location	[redacted]	[redacted]	[redacted]	[redacted]
Batch	[redacted]	[redacted]	[redacted]	[redacted]
Sampling date	19.01.2018	19.01.2018	11.01.2018	11.01.2018
Sampling depth (cm)	Approx. 0-20	Approx. 0-20	0-25	0-25
Soil characteristics*				
- pH (0.01 M $CaCl_2$)	7.4 ± 0.1	7.3 ± 0.1	6.54	5.11
- Organic carbon (%)	2.04 ± 0.17	1.01 ± 0.09	1.04	3.04
- Nitrogen content (%)	0.22 ± 0.01	0.13 ± 0.01	1.20	1.76
- Cation exchange capacity (meq/100 g soil)	26.5 ± 15.5	15.7 ± 5.3	40.60	41.20
- C/N Ratio**	9.3	7.77	0.87	1.73
- Organic matter (OM %)***	3.52	1.74	1.79	5.24
- Weight per volume (g/l)*	1251 ± 39	1221 ± 72	Not available	Not available
Soil type (USDA [7])*	Loam	Sandy loam	Silt loam	Loamy sand
Particle size analysis (mm)*				
< 0.002 (clay) %	26.6 ± 0.7	11.2 ± 0.8	15.8	6.5
0.002-0.05 (silt) %	41.2 ± 1.3	29.8 ± 1.2	80.1	12.2
> 0.05 (sand) %	32.3 ± 1.4	59.0 ± 1.6	4.1	81.2
Soil water content (g water/100 g soil)				
at pF 1.0 (WHC)*	44.6 ± 2.2	41.6 ± 2.6	47.1	34.6
at pF 2.0****	28.1	19.6	35.8	7.7
Biomass				
Start of incubation (mg C/100 g dry soil)	74.28	22.52	26.57	17.69
Start of incubation (% OC)	3.6	2.2	2.6	0.6
During incubation (mg C/100 g dry soil)	71.20	30.17	20.27	10.92
During incubation (% OC)	3.5	3.0	1.9	0.4

Parameters	Soil I	Soil II	Soil III	Soil IV
	Speyer 2.4	Speyer 5M	RefeSol 02-A	RefeSol 04-A
End of incubation (mg C/100 g dry soil)	60.46	20.94	15.68	14.39
End of incubation (% OC)	3.0	2.1	1.5	0.5

* Mean values of different batch analyses ± standard deviations given by [redacted] Germany (Soil I and II; GLP) or by the [redacted] (Soil III and IV; GLP)
** C/N ratio = % organic carbon / % nitrogen content
*** %OM = 1.724 x % organic carbon
**** Determined under GLP by [redacted]
OC: Organic carbon
WHC: water holding capacity

Table A7.2.1/01- 3: Material balance in Soil I (Speyer 2.4); bioactive soil incubated at 20°C

Sampling times (days)	Extractables (RT)	Extractables (Soxhlet)	Total Extractables	$^{14}CO_2$	Other organic volatiles	Bound residues	Material balance
	[% applied radioactivity]						
0.00	66.7	3.0	69.7	na	na	27.6	97.3
0.04	54.2	5.6	59.8	<0.1	<0.1	37.6	97.4
0.08	53.0	4.4	57.4	<0.1	<0.1	38.8	96.2
0.17	53.4	3.4	56.8	<0.1	<0.1	41.1	97.9
0.33	53.6	3.2	56.8	0.2	<0.1	37.3	94.3
1.0	53.3	3.5	56.8	1.8	<0.1	40.2	98.8
2.1	49.3	2.3	51.6	4.0	<0.1	43.9	99.4
4	47.6	2.4	50.0	6.7	<0.1	41.4	98.1
7	42.9	2.3	45.2	9.2	<0.1	42.9	97.4
14	34.2	2.3	36.6	16.7	<0.1	45.3	98.6
28	19.5	1.3	20.8	23.0	<0.1	48.7	92.5
56	5.2	1.4	6.6	42.8	<0.1	52.0	101.4
91	2.8	0.8	3.6	47.9	<0.1	48.6	100.1

na: not analysed

Table A7.2.1/01- 4: Material balance in Soil II (Speyer 5M); bioactive soil incubated at 20°C

Sampling times (days)	Extractables (RT)	Extractables (Soxhlet)	Total Extractables	$^{14}CO_2$	Other organic volatiles	Bound residues	Material balance
	[% applied radioactivity]						
0.00	83.0	3.8	86.8	na	na	10.3	97.1
0.04	61.6	5.6	67.2	<0.1	<0.1	28.4	95.6
0.08	57.6	5.9	63.6	<0.1	<0.1	33.4	97.0
0.17	55.8	4.6	60.4	<0.1	<0.1	37.4	97.9
0.33	57.5	4.1	61.6	0.4	<0.1	34.7	96.7
1.0	59.0	3.3	62.3	1.1	<0.1	31.6	94.9
2.1	57.7	2.7	60.4	5.7	<0.1	34.2	100.3
4	53.9	2.2	56.1	7.6	<0.1	31.8	95.5
7	49.7	2.4	52.1	5.6	<0.1	33.7	91.4
14	37.4	2.2	39.6	18.9	<0.1	36.9	95.4
28	24.7	1.6	26.3	34.9	<0.1	39.3	100.5
56	7.5	1.6	9.0	44.7	<0.1	42.9	96.7
91	2.4	1.2	3.6	56.2	<0.1	39.9	99.6

na: not analysed

Table A7.2.1/01- 5: Material balance in Soil III (RefeSol 02-A); bioactive soil incubated at 20°C

Sampling times (days)	Extractables (RT)	Extractables (Soxhlet)	Total Extractables	$^{14}CO_2$	Other organic volatiles	Bound residues	Material balance
	[% applied radioactivity]						
0.00	79.6	2.9	82.5	na	na	13.0	95.5
0.04	59.5	6.4	66.0	<0.1	<0.1	32.0	98.0
0.08	56.5	7.5	64.1	<0.1	<0.1	32.9	97.0
0.17	56.7	4.9	61.6	<0.1	<0.1	36.4	98.0
0.33	56.3	6.3	62.6	0.4	<0.1	34.1	97.1
1.0	53.8	4.4	58.2	2.4	<0.1	37.0	97.7
2.1	51.0	4.0	55.0	4.5	<0.1	38.9	98.4
4	48.6	4.1	52.7	5.9	<0.1	38.4	97.0
7	45.7	4.0	49.6	8.1	<0.1	40.1	97.8
14	37.7	4.3	42.0	11.6	<0.1	40.3	93.9
28	26.7	4.6	31.3	19.9	<0.1	37.2	88.4
56	12.6	3.6	16.2	39.2	<0.1	44.6	100.0
91	5.7	3.0	8.7	46.1	<0.1	43.2	98.0

na: not analysed

Table A7.2.1/01- 6: Material balance in Soil IV (RefeSol 04-A); bioactive soil incubated at 20°C

Sampling times (days)	Extractables (RT)	Extractables (Soxhlet)	Total Extractables	$^{14}CO_2$	Other organic volatiles	Bound residues	Material balance
	[% applied radioactivity]						
0.00	90.3	na	90.3	na	na	6.9	97.2
0.04	78.6	2.2	80.8	<0.1	<0.1	16.0	96.9
0.08	74.3	4.7	79.0	<0.1	<0.1	16.7	95.7
0.17	68.4	5.8	74.2	<0.1	<0.1	22.2	96.5
0.33	59.2	6.0	65.2	<0.1	<0.1	28.5	93.8
1.0	54.8	4.3	59.2	0.4	<0.1	34.2	93.9
2.1	53.7	5.0	58.7	1.0	<0.1	35.0	94.7
4	52.5	5.8	58.3	1.8	<0.1	31.0	91.1
7	48.3	2.7	51.1	3.4	<0.1	40.2	94.6
14	45.6	5.5	51.2	4.2	<0.1	35.5	90.8
28	37.3	4.9	42.3	13.8	<0.1	34.9	91.0
56	25.1	5.5	30.6	24.1	<0.1	45.6	100.2
91	11.4	4.6	16.0	39.9	<0.1	41.9	97.7

na: not analysed

Table A7.2.1/01- 7: Material balance in Soil I (Speyer 2.4); sterile soil incubated at 20°C

Sampling times (days)	Extractables (RT)	Extractables (Soxhlet)	Total Extractables	$^{14}CO_2$	Other organic volatiles	Bound residues	Material balance
	[% applied radioactivity]						
0.00	90.3	na	90.3	na	na	6.9	97.2
0.91	78.6	2.2	80.8	<0.1	<0.1	16.0	96.9
13	74.3	4.7	79.0	<0.1	<0.1	16.7	95.7
28	68.4	5.8	74.2	<0.1	<0.1	22.2	96.5
91	59.2	6.0	65.2	<0.1	<0.1	28.5	93.8

na: not analysed

Table A7.2.1/01- 8: Material balance in Soil II (Speyer 5M); sterile soil incubated at 20°C

Sampling times (days)	Extractables (RT)	Extractables (Soxhlet)	Total Extractables	$^{14}CO_2$	Other organic volatiles	Bound residues	Material balance
	[% applied radioactivity]						
0.00	91.6	na	91.6	na	na	5.8	97.4
0.88	61.3	5.6	66.8	<0.1	<0.1	29.8	96.6
13	55.0	4.2	59.3	<0.1	<0.1	37.7	97.1
28	58.4	3.3	61.7	0.2	<0.1	36.8	98.6
91	62.5	2.6	65.1	0.4	<0.1	33.1	98.6

na: not analysed

Table A7.2.1/01- 9: Material balance in Soil III (RefeSol 02-A) sterile soil incubated at 20°C

Sampling times (days)	Extractables (RT)	Extractables (Soxhlet)	Total Extractables	$^{14}CO_2$	Other organic volatiles	Bound residues	Material balance
	[% applied radioactivity]						
0.00	88.0	1.4	89.4	na	na	8.1	97.5
0.83	62.5	5.5	68.0	<0.1	<0.1	28.6	96.6
13	55.6	5.2	60.9	<0.1	<0.1	35.9	96.8
28	57.4	4.7	62.1	0.2	<0.1	36.4	98.7
91	61.3	4.5	65.8	0.4	<0.1	31.3	97.5

na: not analysed

Table A7.2.1/01- 10: Material balance in Soil IV (RefeSol 04-A); sterile soil incubated at 20°C

Sampling times (days)	Extractables (RT)	Extractables (Soxhlet)	Total Extractables	$^{14}CO_2$	Other organic volatiles	Bound residues	Material balance
	[% applied radioactivity]						
0.00	91.9	na	91.9	na	na	4.8	96.7
0.83	67.5	3.1	70.5	<0.1	<0.1	24.5	95.1
13	47.2	6.4	53.7	<0.1	<0.1	46.5	100.3
28	45.8	7.5	53.3	<0.1	<0.1	47.0	100.4
91	43.4	7.8	51.3	0.2	<0.1	41.8	93.3

na: not analysed

Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

Table A7.2.1/01- 11: Degradation of [^{14}C]Benzisothiazolone and formation of major metabolites in extracts of bioactive soil samples (Soil I; Speyer 2.4) incubated at 20°C

Sampling times (days)	Benzisothiazolone	MET2	M5	M6 (incl. M6b)	M8	M9
	[% applied radioactivity]					
0.00	46.5	14.9	nd	3.7	4.5	nd
0.04	7.0	19.3	nd	28.1	2.0	nd
0.08	2.1	8.7	nd	17.4	1.7	21.1
0.17	1.3	12.9	nd	35.1	1.4	nd
0.33	1.6	11.6	2.3	33.3	1.5	nd
1.0	1.2	2.8	4.9	38.3	2.1	nd
2.1	0.4	1.3	7.3	36.9	2.0	nd
4	nd	nd	6.8	39.0	nd	nd
7	0.3	0.4	4.8	37.5	nd	nd
14	nd	nd	2.1	30.0	nd	nd
28	nd	nd	nd	17.9	nd	nd
56	0.3	0.2	nd	3.0	nd	nd
91	0.2	<LOD	nd	0.4	<LOD	nd

nd: not detected

Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

Table A7.2.1/01- 12: Degradation of [^{14}C]Benzisothiazolone and formation of major metabolites in extracts of bioactive soil samples (Soil II; Speyer 5M) incubated at 20°C

Sampling times (days)	Benzisothiazolone	MET2	M5	M6 (incl. M6b)	M8	M9
	[% applied radioactivity]					
0.00	54.6	18.9	nd	2.4	10.9	nd
0.04	23.6	22.7	nd	14.0	2.0	nd
0.08	11.0	17.9	nd	18.8	2.9	7.1
0.17	3.9	21.0	nd	26.7	2.7	nd
0.33	2.3	16.4	2.1	31.8	2.1	nd
1.0	1.7	6.9	3.3	40.9	2.0	nd
2.1	1.2	2.1	7.6	41.5	nd	nd
4	nd	nd	8.2	41.4	nd	nd
7	0.6	nd	6.4	42.0	0.6	nd
14	nd	nd	2.0	35.1	nd	nd
28	nd	nd	nd	25.6	nd	nd
56	0.4	nd	nd	7.3	nd	nd
91	0.4	0.3	nd	0.4	0.3	nd

nd: not detected

Table A7.2.1/01- 13: Degradation of [^{14}C]Benzisothiazolone and formation of major metabolites in extracts of bioactive soil samples (Soil III; RefeSol 02-A) incubated at 20°C

Sampling times (days)	Benzisothiazolone	MET2	M5	M6 (incl. M6b)	M8	M9
	[% applied radioactivity]					
0.00	47.2	15.4	nd	2.4	16.9	nd
0.04	13.7	21.0	nd	22.1	2.0	nd
0.08	5.6	21.5	nd	28.5	2.1	nd
0.17	2.0	19.4	nd	31.6	2.2	nd
0.33	2.5	15.6	1.9	32.5	1.7	nd
1.0	1.2	7.0	4.3	35.0	2.4	nd
2.1	1.0	1.8	6.3	35.4	nd	nd
4	0.4	nd	7.9	36.2	nd	nd
7	nd	nd	6.0	35.9	nd	nd
14	1.0	nd	2.7	31.5	nd	nd
28	0.8	nd	nd	26.4	nd	nd
56	0.5	nd	nd	11.0	nd	nd
91	0.4	0.4	nd	2.1	nd	nd

nd: not detected

Table A7.2.1/01- 14: Degradation of [^{14}C]Benzisothiazolone and formation of major metabolites in extracts of bioactive soil samples (Soil IV; RefeSol 04-A) incubated at 20°C

Sampling times (days)	Benzisothiazolone	MET2	M5	M6 (incl. M6b)	M8	M9
	[% applied radioactivity]					
0.00	51.1	29.4	nd	nd	9.8	nd
0.04	53.2	22.3	nd	5.3	nd	nd
0.08	43.6	21.5	nd	8.1	5.9	nd
0.17	31.7	21.3	nd	18.8	nd	nd
0.33	19.8	15.0	nd	25.4	1.8	nd
1.0	4.9	10.4	nd	37.5	3.2	nd
2.1	2.8	7.4	nd	45.0	2.1	nd
4	2.5	4.1	nd	43.5	0.5	nd
7	1.3	1.9	nd	41.7	2.7	nd
14	1.4	1.1	nd	39.5	nd	nd
28	1.0	0.4	nd	35.0	nd	nd
56	1.1	nd	nd	22.4	nd	nd
91	1.2	nd	nd	2.6	0.7	nd

nd: not detected

Table A7.2.1/01- 15: Degradation of [^{14}C]Benzisothiazolone and formation of major metabolites in extracts of sterile soil samples (Soil I; Speyer 2.4) incubated at 20°C

Sampling times (days)	Benzisothiazolone	MET2	M5	M6 (incl. M6b)	M8	M9	M19
	[% applied radioactivity]						
0.00	62.7	9.4	nd	1.9	nd	nd	nd
0.91	17.1	28.0	4.8	8.5	nd	1.0	2.7
13	1.4	nd	20.5	26.2	nd	1.4	7.2
28	0.4	nd	22.7	28.1	nd	0.7	6.4
91	nd	nd	20.8	29.4	nd	2.4	7.1

nd: not detected

Table A7.2.1/01- 16: Degradation of [^{14}C]Benzisothiazolone and formation of major metabolites in extracts of sterile soil samples (Soil II; Speyer 5M) incubated at 20°C

Sampling times (days)	Benzisothiazolone	MET2	M5	M6 (incl. M6b)	M8	M9	M19
	[% applied radioactivity]						
0.00	73.7	12.7	nd	nd	nd	1.6	nd
0.91	19.7	35.6	2.4	4.8	nd	1.0	2.0
13	1.8	1.6	7.3	36.9	nd	2.2	6.2
28	1.0	nd	8.7	39.7	nd	1.0	6.9
91	1.0	0.5	9.5	38.6	nd	2.4	6.8

nd: not detected

Table A7.2.1/01- 17: Degradation of Name [^{14}C]Benzisothiazolone and formation of major metabolites in extracts of sterile soil samples (Soil III; RefeSol 02-A) incubated at 20°C

Sampling times (days)	Benzisothiazolone	MET2	M5	M6 (incl. M6b)	M8	M9	M19
	[% applied radioactivity]						
0.00	77.4	12	nd	nd	nd	nd	nd
0.91	19.4	33.8	1.9	7.0	1.7	nd	3.1
13	1.8	2.0	11.4	30.8	1.3	nd	12.0
28	1.4	nd	12.3	33.5	nd	0.9	12.0
91	1.3	0.7	14.2	33.2	0.5	1.3	12.3

nd: not detected

Table A7.2.1/01- 18: Degradation of [^{14}C]Benzisothiazolone and formation of major metabolites in extracts of sterile soil samples (Soil IV; RefeSol 04-A) incubated at 20°C

Sampling times (days)	Benzisothiazolone	MET2	M5	M6 (incl. M6b)	M8	M9	M19
	[% applied radioactivity]						
0.00	77.1	14.8	nd	nd	nd	nd	nd
0.91	36.0	23.3	nd	5.1	1.1	nd	0.9
13	2.8	15.0	1.5	25.5	0.5	0.5	5.5
28	2.6	11.4	1.5	28.0	0.6	nd	6.6
91	2.4	3.3	2.2	32.4	1.1	nd	6.9

nd: not detected

Table A7.2.1/01- 19: DT_{50} and DT_{90} values of [^{14}C]Benzisothiazolone in soil

	Degradation Kinetics for Bioactive Soils					
	DT_{50} [days]	DT_{90} [days]	Parameter	χ^2 error %	r^2	Prob > t
	Soil Speyer 2.4					
Parent (SFO)	0.0151	0.05	k = 46.02	11.2	0.9955	7.91E-013

Parent (FOMC)	0.00763	0.0568	α = 1.199 β = 0.009743	8.35	0.9955	n/a
Parent (DFOP)	0.0139	0.0509	k1 = 52.76 k2 = 0.4687	2.67	0.997	1.82E-011 0.1136
	Soil Speyer 5M					
Parent (SFO)	0.0346	0.115	k = 20.06	9.92	0.9963	1.20E-015
Parent (FOMC)	0.0307	0.143	α = 2.582 β = 0.09963	7.11	0.9972	n/a
Parent (DFOP)	0.0328	0.128	k1 = 22.82 k2 = 0.4671	2.14	0.9988	4.88E-017 0.01204
	Soil RefeSol 02-A					
Parent (SFO)	0.0237	0.0787	k = 29.25	15.3	0.9941	4.95E-017
Parent (FOMC)	0.0176	0.107	α = 1.539 β = 0.03093	11.2	0.9949	n/a
Parent (DFOP)	nd	0.0867	k1 = 34 k2 = 0.4603	6.74	0.9965	1.16E-016 0.03544
	Soil RefeSol 04-A					
Parent (SFO)	0.24	0.797	k = 2.89	10.8	0.9803	1.24E-010
Parent (FOMC)	0.233	0.947	α = 4.252 β = 1.318	10.8	0.9796	n/a
Parent (DFOP)	nd	0.871	k1 = 3.15 k2 = 0.009803	9.35	0.9809	1.26E-009 0.3306

Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

Official use only

- **REFERENCE**

- **Reference**: [redacted] (2020b): 1,2-Benzisothiazol-3(2H)-one: Confirmation of Identification of Metabolites from Soil Degradation Study [redacted] GLP, non-published, 29 January 2020.

- **Data protection**: Yes

Data owner: [redacted]

Criteria for data protection: Data on existing a.s. submitted for first entry into the European list of approved biocidal active substance

- **GUIDELINES AND QUALITY ASSURANCE**

- **Guideline study**: Yes
(OECD Guideline 307 (Adopted 24th April 2002)
OPPTS 835.4100, US EPA, October 2008)

- **GLP**: Yes

- **Deviations**: No

- **MATERIALS AND METHODS**

- **Test material**

General information: 1,2-Benzisothiazol-3(2H)-one; CAS number: 2634-33-5; Molecular formula: C_7H_5NOS; Molecular weight: 151.19 g/mol

Labelled test material (Lot/Batch number; purity): 1,2-[ring-U-^{14}C]Benzisothiazol-3(2H)-one (thereafter referred to as [^{14}C]Benzisothiazolone); [redacted]

Unlabelled test material (Lot/Batch number; purity, description): 1,2-Benzisothiazol-3(2H)-one [redacted] white to yellow and faint beige to beige powder)

Reference items: 1,2-Benzisothiazol-3(2H)-one (R0)
MET1 (R1): Hydroxy-1,2-benzisothiazolin-3-one
MET2 (R2): 1,2-Benzisothiazolin-3-one-1-oxide
MET3 (R3): Dihydroxy-1,2-benzisothiazolin-3-one
MET4 (R4): o-Sulphobenzamide (sodium salt)

Section A7.2.1/01 Aerobic degradation in soil, initial study

Annex Point IIIA, VII.4, XII.1.1

	MET7 (R7): N-(4-amino-4-hydroxy-buta-1,3-dienyl)-benzamide Saccharin (R8): 1,2-Benzisothiazolin-3-one-1-dioxide 2-Sulphanylbenzamide (R9) 2-Sulphobenzoic acid hydrate (R11) 2-Sulphamoylbenzoic acid (R12)
Stability	Concentrated soil extracts, generated in [redacted] and treated with [14C]-1,2-Benzisothiazol-3(2H)-one were used for analysis. Extracts (stored at -20°C) were thawed, centrifuged, and measured by LSC to determine the radioactive residues content. Storage recovery was between 85.3 and 102.5 %.
• **Study conduct**	Concentrated soil extracts were measured by LSC to determine the radioactive residues content, and then analysed by HPLC to confirm the presence of the radioactive fractions to be confirmed. Afterwards, the samples were re-analysed as applicable by co-chromatography with the reference item(s) with HPLC-RAD and HPLC-UV, and/or TLC with phosphorimaging, and/or LC-MS. Nine soil extracts were used for HPLC co-chromatography with reference item R12 and two soil extracts were taken for TLC co-chromatography.
• **Analytical method**	Volumes of extracts were determined and dispensed aliquots were assayed for radioactivity in duplicate. The aliquots were added directly to a known volume of scintillant and assayed by liquid scintillation counting (LSC). The quantity of radioactivity was determined using a calibrated Packard liquid scintillation counter equipped with DPM and luminescence options. Reversed-phase HPLC (RP-HPLC) was used for chromatographic profiling of the soil extracts. For identification, radioactive components were compared with reference standards by co-chromatography. Aliquots from extracts were mixed with solutions of reference items and the mixtures injected to the HPLC system. Additionally, Normal-phase TLC (NP-TLC) was used to confirm the HPLC chromatographic profile of sample extracts. Radioactive components were compared with reference standards by co-chromatography for their identification. The radiolabelled test item and metabolites were detected using a phosphorimager, and unlabelled test item and the reference items were detected using a UV lamp (254 nm). Mass spectrometry (MS) was used to confirm the identity of reference standards.
	• **RESULTS**
• **Storage stability**	Concentrated soil extracts, generated in the [redacted] and treated with [^{14}C]-1,2-Benzisothiazol-3(2H)-one were analysed after a storage period of approximately 1 year. HPLC profiles were compared to corresponding profiles in the [redacted] or the study raw data. Sufficient stability during storage and presence of metabolite M6 could be confirmed.
• **Analytical results**	The reference standard of 2-sulphamoylbenzoic acid (R12) was analysed by HPLC in water/MeCN (95/5) and in DMSO with three

Section A7.2.1/01 **Aerobic degradation in soil, initial study**

Annex Point IIIA, VII.4, XII.1.1

HPLC methods as well as two LC-MS methods. All HPLC and LC-MS analyses of both batches of the reference item R12 showed multiple peaks, irrespective of the solvent used for the solution, and of the HPLC method (mobile and stationary phase) used. Two peaks detected by LC-MS corresponded to the m/z value expected for 2-sulphamoylbenzoic acid, and one of them matched the retention time of M6 as well, however, this peak was found only in one of the reference standards R12 and showed the lowest intensity. The other two peaks, not matching m/z of R12, correspond to 2-sulphobenzoic acid and saccharin, the latter at ~70% ROI, both of which are possible products of hydrolysis of 2-sulphamoylbenzoic acid. Results suggest either instability during chromatographic analysis or instability during storage. Additionally, the R12 reference solutions when directly introduced into the ion source without chromatography showed the presence of the same components as observed with LC-MS. Nevertheless, selected soil samples were analysed with HPLC with co-chromatography with the reference standard R12. The results for all samples showed presence of M6 with the retention time observed analyses in the [redacted] To corroborate the presence of metabolite M6 in the soil samples, a selected extract was subjected to TLC co-chromatography with the reference standards, including 2-sulphamoylbenzoic acid.
The TLC analysis confirmed presence of an abundant, corresponding to the abundance of M6 that did not co-chromatograph with any of the available reference standards.

- **APPLICANT'S SUMMARY AND CONCLUSION**

- **Materials and methods**

Concentrated soil extracts, generated in the [redacted] were used for further analytical work. Sufficient stability was verified by comparison of HPLC profiles obtained in study [redacted] with new profiles. Soil extract samples were re-analysed as applicable by co-chromatography with the reference item(s) with HPLC-RAD and HPLC-UV, and/or TLC with phosphorimaging, and/or LC-MS.

- **Results and discussion**

All HPLC and LC-MS analyses of both batches of the reference item R12 showed multiple peaks, irrespective of the solvent used for the solution, and of the HPLC method used. Two peaks correspond to the m/z value expected for 2-sulphamoylbenzoic acid and one matched the retention time of M6 but was only found in at a very low intensity and only in one of the references for R12. Other peaks correspond to 2-sulphobenzoic acid and saccharin. This would suggest instability of the substance either during chromatographic analysis, or during storage. Nevertheless, soil extract samples were analysed with HPLC with co-chromatography with the reference standard R12. To corroborate the presence of metabolite M6 in the soil samples, a selected soil extract was subjected to TLC co-chromatography with the reference standards, including 2-sulphamoylbenzoic acid. The TLC analysis confirmed presence of an abundant metabolite, corresponding to the abundance of M6 that did not co-chromatograph with any of the available reference

Section A7.2.1/01 Aerobic degradation in soil, initial study

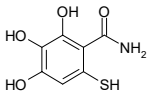
Annex Point IIIA, VII.4, XII.1.1

standards. In conclusion, following HPLC, TLC and LC-MS co-chromatography it could not be confirmed that metabolite M6 was 2-sulphamoylbenzoic acid.

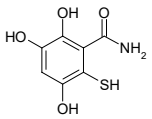
- **Conclusion**

Results of HPLC, TLC and LC-MS co-chromatography of selected soil samples with reference standards including R12 (2-sulphamoylbenzoic acid) and additional MS experiments showed, that metabolite M6 could not be confirmed to be 2-sulphamoylbenzoic acid.

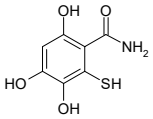
Within the original study [redacted] the molecular weights and molecular formulae of M6 and M9 (probably transient metabolite of M6) were reported although the positions of oxidations could not be determined. However, based on the reported results, the likely structures of M6 are:



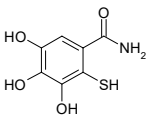
M6.1



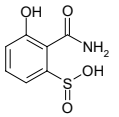
M6.2



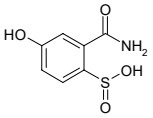
M6.3



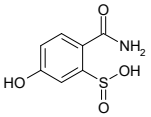
M6.4



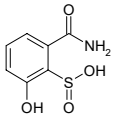
M6.5



M6.6

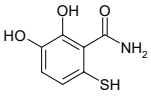


M6.7

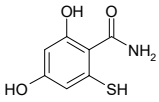


M6.8

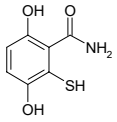
Similarly, based on the total information available of M6 likely structures, the likely structures of M9 are:



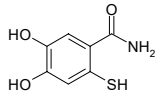
M9.1



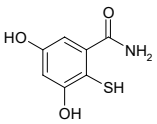
M9.2



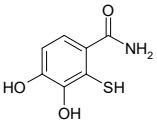
M9.3



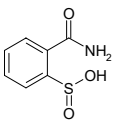
M9.4



M9.5



M9.6



M9.7

Reliability 1

Deficiencies No

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*17/02/20*
Materials and Methods	*Adopt applicant's version*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	*1*
Acceptability	*acceptable*

Section A7 **Subsection A7.2.2.1** **Annex Point IIIA 12.1.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AEROBIC DEGRADATION IN SOIL, FURTHER STUDIES** **THE RATE AND ROUTE OF DEGRADATION INCLUDING THE IDENTIFICATION OF THE PROCESSES INVOLVED AND IDENTIFICATION OF ANY METABOLITES AND DEGRADATION PRODUCTS IN AT LEAST THREE SOIL TYPES UNDER APPROPRIATE CONDITIONS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [...]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure […]	**Other justification […]**	
Detailed justification:	The data from aqueous photolysis (7.1.1.1.2) and ready biodegradation (7.1.1.2.1) of the active substance BIT are sufficient to drive the risk assessment and as a result studies on degradability in soil are not critical to evaluating the risk, and is not therefore required. 7.2.1: Aerobic degradation in soil, initial study Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment. 7.2.2.1: Aerobic degradation in soil, further studies Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment. 7.2.2.2: Field soil dissipation and accumulation Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment. 7.2.2.3: Extent and nature of bound residues. Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment. 7.2.2.4: Other soil degradation studies Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment.	
Undertaking of intended data submission [ ]	A soil transformation has been recently initiated for support of other product types. It will satisfy requirements 7.2.1 and 7.2.2.3.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE	

Section A7 **Subsection A7.2.2.1** **Annex Point IIIA 12.1.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AEROBIC DEGRADATION IN SOIL, FURTHER STUDIES** **THE RATE AND ROUTE OF DEGRADATION INCLUDING THE IDENTIFICATION OF THE PROCESSES INVOLVED AND IDENTIFICATION OF ANY METABOLITES AND DEGRADATION PRODUCTS IN AT LEAST THREE SOIL TYPES UNDER APPROPRIATE CONDITIONS**	
Date	*January 2011*	
Evaluation of applicant's justification	*Adopt applicant's version*	
Conclusion	*Adopt applicant's version*	
Remarks		

Section A7.2.2.3/01 Aerobic degradation in soil, further studies: Extent and nature of bound residues

Annex Point IIIA, XII.1.4

- **REFERENCE**

- **Reference**: [redacted] non-published, 20 December 2018, Amended 28 January 2020.
- **Data protection**: Yes

Data owner: [redacted]

Criteria for data protection: Data on existing a.s. submitted for first entry into the European list of approved biocidal active substance

- **GUIDELINES AND QUALITY ASSURANCE**

- **Guideline study**: Yes
  (OECD Guideline 307 (Adopted 24th April 2002)
  OPPTS 835.4100, US EPA, October 2008)
- **GLP**: Yes
- **Deviations**: No

- **MATERIALS AND METHODS**

- **Test material**: Test substance details are summarised below

General information: 1,2-Benzisothiazol-3(2H)-one; CAS number: 2634-33-5; Molecular formula: C_7H_5NOS; Molecular weight: 151.19 g/mol

Labelled test material (Lot/Batch number; purity): 1,2-[ring-U-^{14}C]Benzisothiazol-3(2H)-one (thereafter referred to as [^{14}C]Benzisothiazolone); [redacted]

Unlabelled test material (Lot/Batch number; purity, description): 1,2-Benzisothiazol-3(2H)-one; [redacted] white to yellow and faint beige to beige powder)

Reference items:
MET1 (R1): Hydroxy-1,2-benzisothiazolin-3-one
MET2 (R2): 1,2-Benzisothiazolin-3-one-1-oxide
MET3 (R3): Dihydroxy-1,2-benzisothiazolin-3-one
MET4 (R4): o-Sulphobenzamide (sodium salt)
MET7 (R7): N-(4-amino-4-hydroxy-buta-1,3-dienyl)-benzamide
Saccharin (R8): 1,2-Benzisothiazolin-3-one-1-dioxide
2-Sulphanylbenzamide (R9)
2-Sulphobenzoic acid hydrate (R11)

Section A7.2.2.3/01 Aerobic degradation in soil, further studies: Extent and nature of bound residues

Annex Point IIIA, XII.1.4

Stability	Stability was determined before and after application. Test substance was stable during the application procedure.
• **Test system**	Laboratory test
Soil type	Four standard representative fresh field soils with a wide range of soil properties were used: Soil I: Speyer 2.4 (loam), Soil II: Speyer 5M (sandy loam), Soil III: RefeSol 02-A (silt loam) and Soil IV: RefeSol 04-A (loamy sand).
• **Treatment and sampling**	Soil samples of 100 g (equivalent dry weight) were treated initial concentration of 0.5 mg per kg dry soil equivalent. Samples were incubated under aerobic conditions in the dark in an air-conditioned room at a temperature of 20.8 ± 0.2°C and 20.9 ± 0.2°C and a soil moisture content of pF 2.
• **Extraction and analytics**	After extraction of soil samples with acetonitrile, acetonitrile/water (4:1, v:v), acetonitrile/water (1:1, v:v) and acetonitrile/0.1 hydrochloric acid (1:1, v:v), Soxhlet extraction using acetonitrile/water/32% hydrochloric acid (80:20:0.1, v:v:v) was performed. If non-extractable radioactivity is > 10% AR after Soxhlet extraction, additional harsh extraction with 0.1 M hydrochloric acid under reflux conditions followed by organic matter fractionation according to Stevenson (1982) was performed, to determine the amount of radioactivity in humin fractions and fulvic and humic acids. Extracts from harsh extractions were concentrated under reduced pressure in a rotary evaporator at about 30 °C. The concentrated extracts were measured by LSC for recovery and submitted for HPLC analysis.

• **RESULTS**

• **Analytical results**	Non-extractable residues increased from 27.6, 10.3, 13.0 and 6.9% AR on 0.00 DAT to maximum levels of 52.0, 42.9, 44.6 and 45.6% AR on 56 DAT respectively for bioactive soils tested, and from 20.3, 5.8, 8.1 and 4.8% AR on 0.00 DAT to maximum levels of 40.7, 37.7, 36.4 and 47.0% AR on 13-28 DAT, respectively, for the four sterile soils tested. At the end of incubation, amounts were 48.6, 39.9, 43.2 and 41.9% AR respectively for the four bioactive soils and 36.2, 33.1, 31.3 and 41.8% AR, respectively, for the four sterile soils tested. Harsh extraction of bioactive soil samples from 56 DAT under reflux conditions further released 5.7, 3.7, 7.3 and 5.7 % AR from the soil matrix, proving that only small amounts might become bioavailable in addition. The HPLC analysis of the resulting extracts showed that they comprised of several discrete radio components, including parent and MET2. Benzisothiazolone was found at levels of ≤0.6% AR for all soils. The maximum level of any single degradate was ≤2.7% AR in all soils. Subsequent allocation of the non-extractable radioactivity to the organic matter fractions revealed that 8.0-12.7%, 2.1-14.8% and 4.7-32.3% AR were associated with the fulvic acid, humic acid and humin fractions, respectively.

• **APPLICANT'S SUMMARY AND CONCLUSION**

• **Materials and methods**	After incubation of treated soils samples, the soil samples were extracted four times at room temperature followed by Soxhlet extraction. If non-extractable radioactivity is > 10% AR after Soxhlet extraction, additional harsh extraction with

Section A7.2.2.3/01 Aerobic degradation in soil, further studies: Extent and nature of bound residues

Annex Point IIIA, XII.1.4

	0.1 M hydrochloric acid under reflux conditions followed by organic matter fractionation according to Stevenson (1982) was performed. Extracts were measured by LSC for recovery and submitted for HPLC analysis.
• **Results and discussion**	Non-extractable residues remaining >10 % AR after Soxhlet extraction were further characterised. Harsh extraction under reflux conditions further released 5.7, 3.7, 7.3 and 5.7 % AR from the soil matrix. The HPLC analysis of the resulting extracts showed that they comprised of several discrete radio components, including parent (≤0.6% AR) and MET2. Subsequent allocation of the non-extractable radioactivity to the organic matter fractions revealed that 8.0-12.7%, 2.1-14.8% and 4.7-32.3% AR were associated with the fulvic acid, humic acid and humin fractions, respectively.
• **Conclusion**	A fast degradation of [^{14}C]Benzisothiazolone in soil was observed. Bound residues were formed to maximum levels of 52.0, 42.9, 44.6 and 45.6% AR on 56 DAT respectively for bioactive soils tested, and to maximum levels of 40.7, 37.7, 36.4 and 47.0% AR on 13-28 DAT, respectively, for the four sterile soils tested. Harsh extraction further released 5.7, 3.7, 7.3 and 5.7 % AR from the soil matrix. Organic matter fractions revealed that 8.0-12.7%, 2.1-14.8% and 4.7-32.3% AR were associated with the fulvic acid, humic acid and humin fractions, respectively.
Reliability	1
Deficiencies	No

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*18/2/20*
Materials and Methods	*Adopt applicant's version*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's: A fast degradation of [14C]Benzisothiazolone in soil was observed. Bound residues were formed to maximum levels of 52.0, 42.9, 44.6 and 45.6% AR on 56 DAT respectively for bioactive soils tested, and to maximum levels of 40.7, 37.7, 36.4 and 47.0% AR on 13-28 DAT, respectively, for the four sterile soils tested. Harsh extraction further released 5.7, 3.7, 7.3 and 5.7 % AR from the soil matrix. Organic matter fractions revealed that 8.0 12.7%, 2.1-14.8% and 4.7-32.3% AR were associated with the fulvic acid, humic acid and humin fractions, respectively.*
Reliability	*1*
Acceptability	*acceptable*
Remarks	
	COMMENTS FROM
Date	*Give date of the comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*

Section A7 **Subsection A7.2.2.3** **Annex Point IIIA 12.1.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AEROBIC DEGRADATION IN SOIL, FURTHER STUDIES** **EXTENT AND NATURE OF BOUND RESIDUES**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [...]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure […]	**Other justification […]**	
Detailed justification:	The data from aqueous photolysis (7.1.1.1.2) and ready biodegradation (7.1.1.2.1) of the active substance BIT are sufficient to drive the risk assessment and as a result studies on degradability in soil are not critical to evaluating the risk, and is not therefore required. 7.2.1: Aerobic degradation in soil, initial study Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment. 7.2.2.1: Aerobic degradation in soil, further studies Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment. 7.2.2.2: Field soil dissipation and accumulation Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment. 7.2.2.3: Extent and nature of bound residues. Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment. 7.2.2.4: Other soil degradation studies Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment.	
Undertaking of intended data submission [ ]	A soil transformation has been recently initiated for support of other product types. It will satisfy requirements 7.2.1 and 7.2.2.3.	

Section A7 **Subsection A7.2.2.3** **Annex Point IIIA 12.1.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AEROBIC DEGRADATION IN SOIL, FURTHER STUDIES** **EXTENT AND NATURE OF BOUND RESIDUES**	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	*January 2011*	
Evaluation of applicant's justification	*Adopt applicant's version*	
Conclusion	*Adopt applicant's version*	
Remarks		

Section A7 **Subsection A7.2.3** **Annex Point IIIA XII 1.3**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ADSORPTION AND MOBILITY IN SOIL, FURTHER STUDIES**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [...]	**Other justification [ ]**	
Detailed justification:	7.2.3.1: Adsorption of Metabolites A waiver is requested for the performing mobility studies on the metabolites of BIT. Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment. 7.2.3.2: Mobility A waiver is requested from performing field mobility studies with BIT. Based on the use pattern there is limited exposure to the soil. Thus this study will have a negligible impact on the environmental risk assessment.	
Undertaking of intended data submission [ ]	No studies are planned.	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	*January 2011*	
Evaluation of applicant's justification	*Adopt applicant's version*	
Conclusion	*Adopt applicant's version*	
Remarks		

Section A7 **Subsection A7.3.1** **Annex Point: IIIA 12.3**		**Ecotoxicological Profile Including Environmental Fate and Behaviour** **PHOTOTRANSFORMATION IN AIR (ESTIMATION METHOD) (01)**	
		1 REFERENCE	**Officialuse only**
	1.1 Reference	**A7.3.1/01** [redacted] (2007) Calculation of Tropospheric Phototransformation of 1,2-Benzisothiazolin-3-one, [redacted] (April 19, 2007), Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes-Technical Guidance Document, Chapter 3, Section 7.3.1	
	2.2 GLP	Not Applicable (This is a calculation method and not a laboratory experiment)	
	2.3 Deviations	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	BIT (1,2-Benzisothiazolin-3-one)	
3.1.1	Lot/Batch number	Not applicable	
3.1.2	Specification	Not applicable	

Section A7 Subsection A7.3.1 Annex Point: IIIA 12.3		Ecotoxicological Profile Including Environmental Fate and Behaviour PHOTOTRANSFORMATION IN AIR (ESTIMATION METHOD) (01)	
3.1.3	Purity	Not applicable	
3.1.4	Radiolabeling	Not applicable	
3.1.5	UV/VIS absorption spectra and value	Not applicable	
3.1.6	Further relevant properties	Vapor Pressure at 25°C: 2.3 x 10^{-4} Pa Octanol:Water Partition Coefficient: 15.4 (pH = 7) Solubility in Water: 1.15 g/L at pH 7 and 20°C Aqueous Photolytic half-life: <9 hours	
3.2	**Reference**	Environment Monograph. Application of Structure-Activity Relationships to the Estimation of Properties Important in Exposure Assessment. No 67. Environment Directorate, Paris, 1993.	
3.3	**Test solution**	Not applicable	
3.4	**Testing procedure**	As described in the Technical Guidance Document, Chapter 3, section 7.3.1, a first approach to the phototransformation of a biocide in air is to determine the first order degradation rate constant by Structure-Activity Relationship (SAR) methods. SAR recognizes that organic compounds emitted into the troposphere are mainly removed by reactions with OH radicals during the daylight hours and NO_3 radicals during night. SAR utilizes the fact that a number of separate OH radical reactions occur and that they can be dealt with individually in terms of the rate constant, k_{OH}, including: a) hydrogen atom abstraction from C-H bonds in alkanes, carbonyls, and other saturated organics; b) addition to >C=C< and -C≡C- unsaturated bonds; c) addition to aromatic rings; and d) interaction with $–NH_2$, >NH, >N-, -SH, and –S- groups) *i.e.*: k_{OH} = k(hydrogen atom abstraction from C-H bonds) + k(radical addition to >C=C< and -C≡C- bonds) + k(radical addition to aromatic rings)	

Section A7 Subsection A7.3.1 Annex Point: IIIA 12.3		Ecotoxicological Profile Including Environmental Fate and Behaviour PHOTOTRANSFORMATION IN AIR (ESTIMATION METHOD) (01)	
		+ k(radical interaction with $–NH_2$, >NH, >N-, -SH, -S-) Since little is known about the reaction mechanism of NO_3 radicals with organic compounds and no database for NO_3 radical reactions is available, the rate constant k_{NO3} is estimated by correlations between k_{NO3} and k_{OH}, *i.e.*: $-\log k_{NO3} = -18.86 + 3.05 \times (-\log k_{OH})$ SAR calculates phototransformation half-life of a specific organic compound ($t_{1/2}$) based on its phototransformation rate constant k and the concentration of OH and NO_3 radicals in the troposphere, i.e.: $t_{½} = \ln 2/(k\ [C])$ Where k is the phototransformation rate constant and [C] is the concentration of the radicals in the troposphere such as OH and NO_3.	
3.4.1	Test system	Not applicable	
3.4.2	Properties of light source	Not applicable	
3.4.3	Determination of irradiance	Not applicable	
3.4.4	Temperature	Not applicable	
3.4.5	pH	Not applicable	
3.4.6	Duration of test	Not applicable	
3.4.7	Number of replicate	Not applicable	

Section A7 Subsection A7.3.1 Annex Point: IIIA 12.3		Ecotoxicological Profile Including Environmental Fate and Behaviour PHOTOTRANSFORMATION IN AIR (ESTIMATION METHOD) (01)	
3.4.8	Sampling	Not applicable	
3.4.9	Analytical method	Not applicable	
3.5	**Transformation products**	Potential phototransformation products are hypothesized based on previously conducted environmental fate studies, *i.e.* aqueous photolysis, hydrolysis, and water/soil metabolism: $H_2NC(O)PhSH$ $(H_2NC(O)PhS)_2$ HSPhCOOH $H_2NC(O)PhSO_3H$ $H_2NSO_2PhCOOH$ $HSO_3PhCOOH$ $HSO_3Ph(OH)OH$ HOPh(OH)COOH HOPhOH $HOOCCH_2CHCHC(O)COOH$ where Ph = phenyl ring	
3.5.1	Method of analysis for transformation procedure	Same as that of the parent (see section 3.4).	
		4 RESULTS	
4.1	**CMIT**		
4.1.1	K_{OH}	The first order degradation rate constant (k_{OH}) from OH^- radicals is calculated as the sum of bond k_{OH}'s. This is presented in Table A7.3.1-1. The k_{OH} for BIT is 287.47 x 10^{-13} cm^3. $molecule^{-1}$. sec^{-1}.	

Section A7 **Subsection A7.3.1** **Annex Point: IIIA 12.3**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **PHOTOTRANSFORMATION IN AIR (ESTIMATION METHOD) (01)**	
4.1.2 Half-life ($OH^•$)	The half-life due to the hydroxyl radical is determined as follows: $t_{½}$ = ln2/(k_{OH}) x [OH] = 0.693/(287.47 x 10^{-13} cm^3-molec.$^{-1}$-sec^{-1} x 6.5 x 10^5 molecule.cm^{-3}) = 3.71 x 10^4 sec = 10.3 hours	
4.1.3 K_{NO3}	The first order degradation rate constant (k_{NO3}) from $NO_3^•$ radicals is determined as follows: -logk_{NO3} = -18.86 + 3.05 x (-log k_{OH}) = -18.86 + 3.05 x (-log 287.47 x 10^{-13}) = -18,86 + 3.05 x (10.541) = -13.291 k_{NO3} = antilog (-13.291) = 0.512 x 10^{-13} cm^3.molecule^{-1}.sec^{-1}	
4.1.4 Half-life ($NO_3^•$)	The half-life due to the nitrate radical is calculated similarly to the hydroxyl (described above) and is 15.7 hours.	
4.2 Transformations products		
4.2.1 K_{OH}	The first order degradation rate constant (k_{OH}) from $OH^•$ radicals for the potential transformation products is presented in Table A7.3.1-2	
4.2.2 Half-life ($OH^•$)	The half-life of the potential transformation products due to the hydroxyl radical is presented in Table A7.3.1-2. The half-lives range from 5.2-237.1 hours.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The guideline followed is that described in the Technical Guidance Document, Chapter 3, Section 7.3.1 The phototransformation rate constant of BIT is calculated using SAR method. Global average OH and NO_3 radical concentrations in daylight and	

Section A7 Subsection A7.3.1 Annex Point: IIIA 12.3	Ecotoxicological Profile Including Environmental Fate and Behaviour PHOTOTRANSFORMATION IN AIR (ESTIMATION METHOD) (01)	
	night hours are used. Potential phototransformation products of BIT are hypothesized based on available information. The estimation is demonstrated to be accurate by comparing the rate constant of BITwith that of six compounds which have similar bond types.	
5.2 Results and discussion	Due to relative low vapor pressure and high water solubility, the concentration of BIT in the troposphere is expected to be low. This ensures that the photodegradation of the radicals with BIT follows a pseudo first-order kinetics required by SAR calculation method. Due to the presence of nitrogen and sulfur bonds, BIT has a large phototransformation rate constant. The parent compound quickly photodegrades during the daylight with half-life of 12.6 hours. All potential photodegradation products are expected to be very reactive to photodegradation with half-lives ranging from 5.4-237.1 hours.	
5.3 Conclusion	Daylight photolysis is the dominant phototransformation procedure for BIT and its potential metabolites. BIT photodegrades quickly with half-life of 10.3 hours and the half-lives of its metabolites range from5.4 – 237.1 hours. Due to very low production and usage volume, the effect from BIT and its potential photodegradation products towards global warming is minimal. Therefore, BIT and its photodegradation metabolites impose no effect to global warming.	
5.3.1 Reliability	1-valid without restrictions	
5.3.2 Deficiencies	There are no deficiencies.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2010*
Materials and Methods	*Applicant's version is accepted. Test method considers the photodegradation of BIT due to reactions with OH radicals and with NO_3 radicals.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.3.1 PHOTOTRANSFORMATION IN AIR (ESTIMATION METHOD) (01)

Annex Point: IIIA 12.3

Results and discussion	*Applicant's version is accepted, but with the following comments:* *3.5. Transformation products. It is recommended to carry out further studies focused on the environmental behaviour of the compound identified as metabolite 10. However, due to the low vapour pressure of BIT, its concentration ontroposphere is expected to be low.* *4.1 CMIT should read BIT*
Conclusion	*Daylight photolysis is the dominant phototransformation procedure for BIT and itspotential metabolites.* *BIT photodegrades quickly with half-life of 10.3 hours and the half-lives of its metabolites range from 5.4 – 237.1 hours.* *Due to very low production and usage volume, the effect from BIT and its potential photodegradation products towards global warming is minimal.* *Therefore, BIT and its photodegradation metabolites impose no effect to global warming.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A7.3.1-1: Hydroxyl Rate Constants of Different Types of Reactions for BIT

Bond Type	k_{OH} (10^{-13} $cm^3.molecule^{-1}.sec^{-1}$)	Number of Bonds	Total (10^{-13} $cm^3.molecule^{-1}.sec^{-1}$)
C-H	0.14	4	0.56
>C=C<	11.0	6	66.0
>C=O	0.31	1	0.31
>N-	60.2	3	180.6
-S-	20.0	2	40.0
			287.47

Table A7.3.1-2: Reaction Rate Constant k_{OH} and Half-Life of Transformation Products

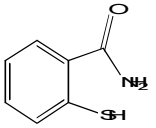
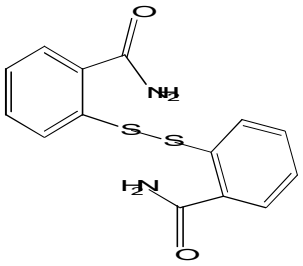
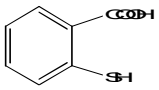
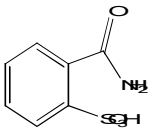
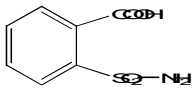
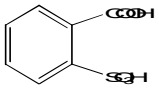
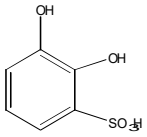
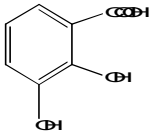
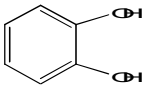
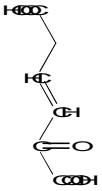
Compound	k_{OH} (10^{-13} $cm^3.molecule^{-1}.sec^{-1}$)	$t_{½}$ (hours)
	287.47	10.3
	574.94	5.2
	106.87	27.7
	287.47	10.3
	287.47	10.3
	106.87	27.7
	106.87	27.8
	66.56	44.4

Table A7.3.1-2 (continued): Reaction Rate Constant k_{OH} and Half-Life of Transformation Products

Compound	k_{OH} (10^{-13} $cm^3.molecule^{-1}.sec^{-1}$)	$t_{½}$ (hours)
	66.56	44.5
	12.49	237.1

Section A7 **Subsection A7.3.2** **Annex Point IIIA 12.3**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **FATE AND BEHAVIOUR IN AIR, FURTHER STUDIES**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Due to the rapid decline of parent and metabolites calculated in Section 7.3.1, BIT does not trigger the need for additional fate and behaviour in air studies.	
Undertaking of intended data submission [ ]	No studies are planned.	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	*January 2011*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version*	
Remarks		

Section A7
Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.1a
Annex Point IIA VII.7.1
ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, RAINBOW TROUT

		1 REFERENCE	Officialuse only
	1.1 Reference	**A7.4.1.1.a/01** [redacted] (2006a) 1,2-Benzisothiazolin-3-one: A 96-hour flow-through acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*); [redacted] Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD 203, US EPA OPPTS 850.1075	
	2.2 GLP	Yes	
	2.3 Deviations	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	not applicable	
3.1.5	Further relevant properties	not applicable	

Section A7 Subsection A7.4.1.1a Annex Point IIA VII.7.1		Ecotoxicological Profile Including Environmental Fate and Behaviour ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, RAINBOW TROUT	
3.1.6	Method of analysis	High performance liquid chromatography (HPLC) with UV detector	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see Table A7.4.1.1.a/01-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	not tested	
3.4	**Testing procedure**		
3.4.1	Dilution water	see Table A7.4.1.1.a/01-2	
3.4.2	Test organisms	see Table A7.4.1.1.a/01-3	
3.4.3	Test system	see Table A7.4.1.1.a/01-4	
3.4.4	Test conditions	see Table A7.4.1.1.a/01-5	
3.4.5	Duration of the test	96 h	
3.4.6	Test parameter	mortality: see Table A7.4.1.1.a/01-6	
3.4.7	Sampling	Water samples were collected from one test chamber of each treatment and control group three days prior to the start of the test after conditioning the diluter for three days. The samples were collected from mid-depth in each test chamber, placed in glass vials and processed immediately for analysis.	
3.4.8	Monitoring of TS concentration	Yes, 0, 48 and 96 hours of the study	
3.4.9	Statistics	Mortality data were canalyzed using the omputer program of C.E. Stephan (Methods for calculating an LC_{50}, *Aquatic Toxicology and Hazard Evaluations*. American Society for Testing and Materials. Publication Number STP 634, pages 65-84). Binomial probability was used to calculate the 24 and 48-hour LC_{50} values and the probit method was used to calculate the 72 and 96-hour LC_{50} values. The no-mortality and the NOEC were determined by visual interpretation of the mortality and observation data.	

Section A7
Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.1a
Annex Point IIA VII.7.1
ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, RAINBOW TROUT

4 RESULTS

4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal (mg BIT/L) 0.31, 0.63, 1.3, 2.5, 5.0
4.2.2	Actual concentrations of test substance	Measured concentrations (mg BIT/L) in test samples

Nominal	0 h	48 h	96 h	Mean
0.31	0.281	0.270	0.268	0.27
0.63	0.594	0.581	0.580	0.59
1.3	1.24	1.20	1.22	1.2
2.5	2.37	2.37	2.29	2.3
5.0	5.13	5.14	100% mortal-ity, no sample	5.1

4.2.3	Effect data (Mortality)	see Table A7.4.1.1.a/01-6; see Table A7.4.1.1.a/01-7
4.2.4	Concentration / response curve	See Figure A7.41.1.a/01-1.
4.2.5	Other effects	One lethargic fish in the 1.2 mg BIT/L group and one fish lying on the bottom of the tank in the 2.3 mg BIT/L group. All other surviving fish appeared normal at test termination. All test solutions appeared clear and colorless in the diluter mixing chambers and in the test chambers at test intiation and termination.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	no adverse effects
4.3.2	Nature of adverse effects	not applicable

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.4.1.1a	**ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, RAINBOW TROUT**
Annex Point IIA VII.7.1	

4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 203, US EPA OPPTS 850.1075, Acute flow-through 96h fish study with analytical confirmation of test solution concentrations.	
5.2	**Results and discussion**	96 h NOEC = 0.27 mg BIT/L	
	96 h-LC_0	96 h = 0.27 mg BIT/L	
	96 h-LC_{50}	96 h = 1.9 mg BIT/L	
	96 h-LC_{100}	96 h = 5.1 mg BIT/L	
5.3	**Conclusion**	see validity criteria summarized in table A7.4.1.1.a/01-8	
5.3.1	Other Conclusions	None	
5.3.2	Reliability	(1), valid without restriction	
5.3.3	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*April 2013*
Materials and Methods	*Accept the applicant's version*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A7.4.1.1.a/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes, sonic bath and mixed by inversion
Vehicle	Yes, Dimethyl formamide (DMF)
Concentration of vehicle	The concentration of DMF in the solvent control and all treatment groups was 0.1 mL/L
Vehicle control performed	Yes, DMF
Other procedures	not applicable

Table A7.4.1.1.a/01-2: Dilution water

Criteria	Details
Source	Filtered, UV-sterilized, well water, 40 meters deep located at the [redacted]
Alkalinity	182 mg/L (as $CaCO_3$)
Hardness	136 mg/L (as $CaCO_3$)
pH	8.2
Oxygen content	≥ 8.2 mg/L (76% of saturation)
Conductance	313 µMhos/cm
Holding water different from dilution water	No

Table A7.4.1.1.a/01-3: Test organisms

Criteria	Details
Species/strain	rainbow trout, *Oncorhynchus mykiss*
Source	[redacted]
Wild caught	No
Age/size	Juveniles, the length of the longest fish measured was no more than twice the length of the shortest fish.
Kind of food	Holding period: trout were fed a commercially prepared diet supplied by [redacted]
Amount of food	*ad libitum*
Feeding frequency	Daily during 2-week holding period
Pretreatment	The fish were not fed for at least two days prior to testing.
Feeding of animals during test	No

Table A7.4.1.1.a/01-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	A continuous flow diluter was used to deliver each concentration of TS, solvent control (DMF) and a negative control (dilution water). A calibrated syringe pump was used to deliver the TS and controls into mixing chambers. The diluter was adjusted so that each test chamber received approximately 10 volume additions of test water every 24 hours. The five stock solutions were injected into the diluting mixing chambers (at a rate of 20 µL/minute) where they were mixed with well water (at a rate of 200 mL/minute) to achieve the desired test concentrations.
Volume of test vessels	25 L stainless steel chambers filled with approximately 15 L of test water to achieve a depth of 18.7 cm.
Volume/animal	1.5 L
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of	No

TS	

Table A7.4.1.1.a/01-5: Test conditions

Criteria	Details
Test temperature (degree C)	11.3 – 12.6 ºC
Dissolved oxygen (mg/L)	≥ 8.2 mg/L (76% of saturation)
pH	8.0 – 8.1
Adjustment of pH	Not described
Aeration of dilution water	Yes, flow-through
Intensity of irradiation	Fluorescent light bulbs that emit wavelengths similar to natural sunlight
Photoperiod	16 h daylight, 8 h darkness

Table A7.4.1.1.a/01-6: Mortality data

Test-Substance Concentration (mean measured) [mg BIT/L]	Mortality									
	Number					Percentage				
	2.5 h	24 h	48 h	72 h	96 h	2.5 h	24 h	48 h	72 h	96 h
Negative control	0/20	0/20	0/20	0/20	0/20	0	0	0	0	0
DMF solvent control	0/20	0/20	0/20	0/20	0/20	0	0	0	0	0
0.27	0/20	0/20	0/20	0/20	0/20	0	0	0	0	0
0.59	0/20	0/20	0/20	0/20	1/20	0	0	0	0	5
1.2	0/20	2/20	3/20	3/20	3/20	0	10	15	15	15
2.3	0/20	1/20	4/20	6/20	12/20	0	5	20	30	60
5.1	0/20	19/20	20/20	20/20	20/20	0	95	100	100	100
Temperature [°C]	11.8-12.6	--	--	--	11.3-12.1					
pH	8.0-8.1	8.0-8.1	8.0	8.0-8.1	8.0					
Oxygen [mg/L]	8.7-9.0	8.2-8.6	8.2-8.5	8.2-8.4	8.4-8.7					

Table A7.4.1.1.a/01-7: Effect data

	24 h [mg BIT/L][1]	95 % C.I.	48 h [mg BIT/L][1]	95 % C.I.	72 h [mg BIT/L][1]	95 % C.I.	96 h [mg BIT/L][1]	95 % C.I.
LC_{50}	3.4	2.3 – 5.1	2.9 (m)	2.3 – 5.1	2.4	2.0 – 3.0	1.9 (m)	1.5 – 2.4

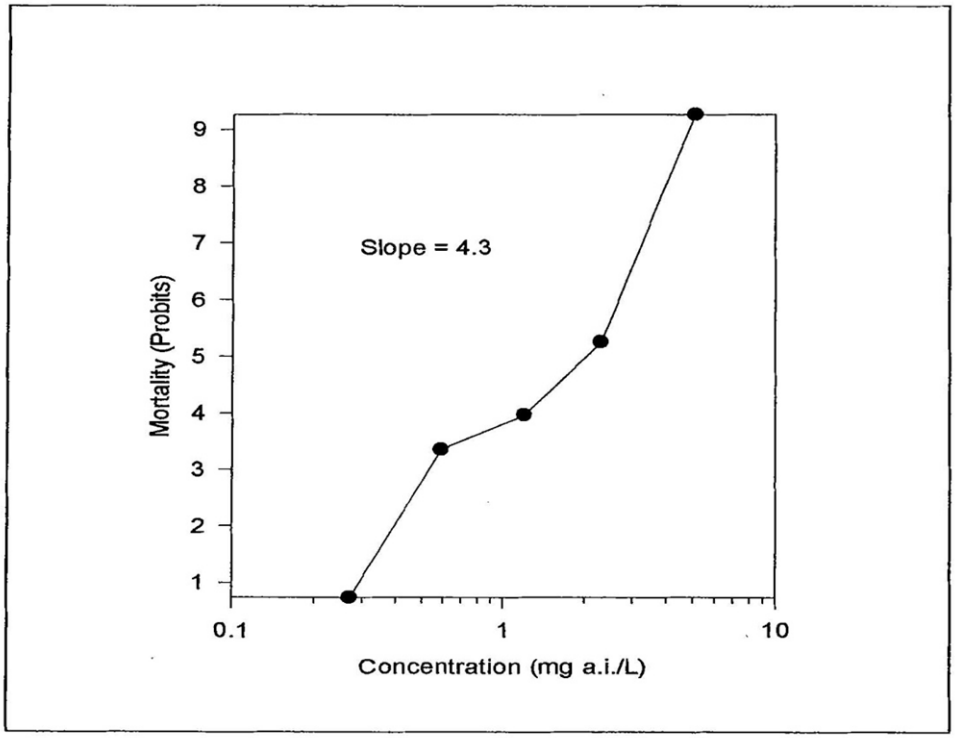
[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.4.1.1.a/01-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	yes	
Concentration of dissolved oxygen in all test vessels > 60% saturation	yes	
Concentration of test substance ≥80% of initial concentration during test	yes	

Figure A7.4.1.1.a/01-1: 96-hour dose-response line for Rainbow trout (*Oncorhynchus mykiss*) exposed to BIT

Concentration-Response Curve (96-Hour Mortality Data)



Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.4.1.2a(01)	**ACUTE TOXICITY TO INVERTEBRATES**
Annex Point IIA VII.7.2	*Daphnia magna*

			Officialuse only
		1 REFERENCE	
	1.1 Reference	**A7.4.1.2.a/01** [redacted] (September 28, 2006), GLP, Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD 202, US EPA 850.1010	
	2.2 GLP	Yes	**X**
	2.3 Deviations	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	not applicable	

Section A7		**Ecotoxicological Profile Including Environmental Fate and Behaviour**	
Subsection A7.4.1.2a(01)		**ACUTE TOXICITY TO INVERTEBRATES**	
Annex Point IIA VII.7.2		*Daphnia magna*	
3.1.5	Further relevant properties	not applicable	
3.1.6	Method of analysis	Reverse phase high performance liquid chromatography (HPLC)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see Table A7.4.1.2.a/01-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not tested	
3.4	**Testing procedure**		
3.4.1	Dilution water	see Table A7.4.1.2.a/01-2	
3.4.2	Test organisms	see Table A7.4.1.2.a/01-3	
3.4.3	Test system	see Table A7.4.1.2.a/01-4	**X**
3.4.4	Test conditions	see Table A7.4.1.2.a/01-5	
3.4.5	Duration of the test	48 h	
3.4.6	Test parameter	immobilization: see Table A7.4.1.2.a/01-6	
3.4.7	Sampling	The samples were collected from mid-depth in each test chamber, placed in glass vials and processed immediately for BIT concentration.	
3.4.8	Monitoring of TS concentration	Yes, 0 and 48 hours of the study	
3.4.9	Statistics	The 24 and 48 hour mortality and immobility data were analyzed using the computer program of C.E. Stephan ([redacted]. The binomial probability was used to calculate the 24 hour EC_{50} value and the probit method was used to calculate the 48 hour EC_{50} value.	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.4.1.2a(01) **ACUTE TOXICITY TO INVERTEBRATES**

Annex Point IIA VII.7.2 *Daphnia magna*

		4 RESULTS
4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal (mg BIT/L) 0, 1.3, 2.5, 5.0, 10, and 20
4.2.2	Actual concentrations of test substance	measured concentrations (mg BIT/L)

Nominal	**0 h**	**48 h**	**mean**
1.3	1.08	1.19	1.1
2.5	2.88	2.92	2.9
5.0	5.13	4.98	5.1
10	10.3	9.62	10
20	21.5	20.6	21

4.2.3	Effect data (Immobilisation)	see Table A7.4.1.2.a/01-6; see Table A7.4.1.2.a/01-7
4.2.4	Concentration / response curve	48 hour mortality/immobility data, see Figure A7.4.1.2.a/01-1.
4.2.5	Other effects	Mortality, lethargy
4.3	**Results of controls**	normal in appearance and behavior
4.4	**Test with reference substance**	Not performed
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	US EPA Guideline 72-2, Acute flow-through 48h *Daphnia magna* study with analytical confirmation of test solution concentrations. There were no guideline deviations.
5.2	**Results and**	*Daphnia magna* were exposed to five concentrations of BIT, a dilution water control (negative control) and a solvent control

Section A7 **Subsection A7.4.1.2a(01)** **Annex Point IIA VII.7.2**		**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ACUTE TOXICITY TO INVERTEBRATES** *Daphnia magna*	
	discussion	(dimethyl formamide) under flow-through conditions for 48 hours. The test solutions appeared clear and colorless in all test chambers at test initiation and termination. Analytical recoveries ranged from 83 to 115% of nominal concentrations on Day 0 and from 92 to 117% of nominal concentrations on Day 2. At test termination, all daphnids in the negative control and the solvent control appeared normal with no mortalities or immobile daphnids noted. Percent mortality/immobility at test termination in the 1.1, 2.9, 5.1, 10 and 21 mg BIT/L treatment groups was 5, 35, 60, 100 and 100%, respectively.	
5.2.1	EC_0	Not applicable	
5.2.2	EC_{50}	3.7 mg BIT/L (95% confidence interval of 2.9 to 6.4 mg BIT/L)	
5.2.3	EC_{100}	10 mg BIT/L	
5.3	**Conclusion**	see Table A7.4.1.2.a/01-8	
5.3.1	Reliability	(1), reliable without restriction	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*December 2010*
Materials and Methods	*Applicant's version is accepted with the following remark:* *3.4.3: The OECD Guideline 202 recommends four groups of 5 animals each instead of two groups of 10 animals.*
Results and discussion	*Adopt applicant's version*
Conclusion	*Applicant's version adopted.*
Reliability	*2*
Acceptability	*Acceptable*

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.4.1.2a(01) **ACUTE TOXICITY TO INVERTEBRATES**

Annex Point IIA VII.7.2 *Daphnia magna*

Remarks	

Table A7.4.1.2.a/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Mixed by inversion
Vehicle	DMF (dimethyl formamide)
Concentration of vehicle	0.1 mL/L in solvent control and in all BIT treatment groups
Vehicle control performed	yes
Other procedures	Not applicable

Table A7.4.1.2.a/01-2: Dilution water

Criteria	Details
Source	Well water, approximately 40 meters deep, located at [redacted]
Alkalinity	180 to 182 mg/L as $CaCO_3$
Hardness	Moderately hard, 132 to 136 mg/L as $CaCO_3$
pH	8.0 to 8.2
Ca / Mg ratio	Not described
Na / K ratio	Not described
Oxygen content	≥ 7.9 mg/L (88% of saturation)
Conductance	305 to 320 µmhos/cm
Holding water different from dilution water	Well water was sand filtered, pumped into a storage tank and aerated. Prior to use, the water was filtered to 0.45 µm and passed through an ultraviolet

	sterilizer.

Table A7.4.1.2.a/01-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	In-house daphnid culture
Age	first instar daphnids (<24 h old)
Breeding method	not described
Kind of food	Mixture of yeast, cereal grass media and trout chow and a suspension of freshwater green alga, Selenastrum capricornutum
Amount of food	*ad libitum*
Feeding frequency	Daily prior to test initiation
Pretreatment	None
Feeding of animals during test	No

Table A7.4.1.2.a/01-4: Test system

Criteria	Details
Renewal of test solution	Flow-through using a calibrated syringe pump to deliver the desired test concentration. Diluter was adjusted so that each test chamber received approximately 5 volume additions of test water every 24 hours.
Volume of test vessels	25 liter stainless steel aquarium containing 22 liters of test water
Volume/animal	2.2 liters
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2.a/01-5: Test conditions

Criteria	Details
Test temperature	19.9 to 20.1 °C
Dissolved oxygen	≥ 7.9 mg/L (88% of saturation)
pH	8.0 to 8.1
Adjustment of pH	not described
Aeration of dilution water	Yes
Quality/Intensity of irradiation	183 lux
Photoperiod	16 h daylight, 8 hours darkness

Table A7.4.1.2.a/01-6: Immobilisation data

Test-Substance Concentration (mean measured)[1] [mg BIT/L]	Mortality/Immobility *Daphnia*				Oxygen [mg/L] 48 h	pH 48 h	Temperature [°C] 48 h
	Number		Percentage (%)				
	24 h	48 h	24 h	48 h			
Negative control	0/10	0/10	0	0	8.4	8.0	20.1
DMF solvent control	0/10	0/10	0	0	8.4	8.0	20.1
1.1	0/10	1/10	0	5	8.5	8.1	20.0
2.9	0/10	3.5/10	0	35	8.2	8.1	20.0
5.1	0/10	6/10	0	60	8.3	8.0	20.0
10	0/10	10/10	0	100	8.2	8.0	19.9
21	10/10	10/10	100	100	8.3	8.0	19.9

[1] specify, if TS concentrations were nominal or measured

Table A7.4.1.2.a/01-7: Effect data

	EC_{50}[1]	95 % C.I	EC_0[1]	EC_{100}[1]
24 h [mg BT/L]			Not applicable	
48 h [mg BIT/L]	3.7	2.9 to 4.6	Not applicable	

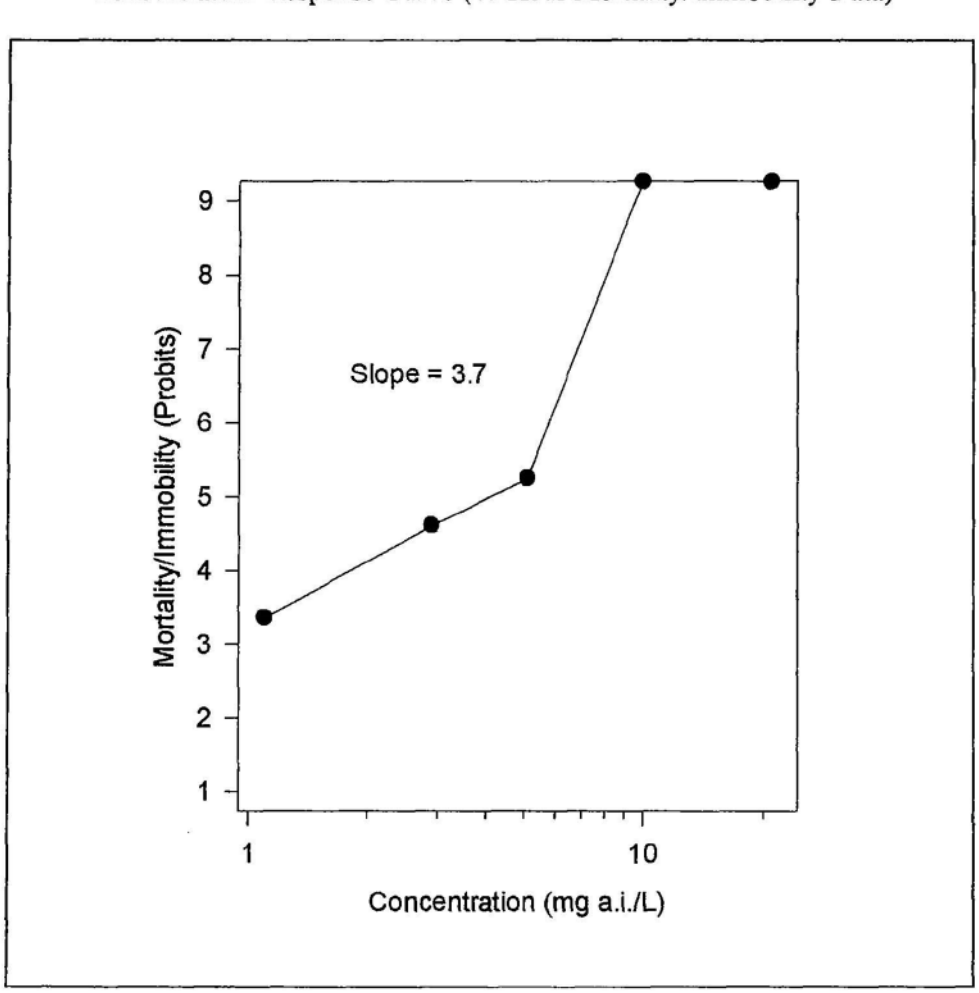
[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.4.1.2.a/01-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	Not fullfilled
Immobilisation of control animals <10%	yes	
Control animals not staying at the surface	yes	
Concentration of dissolved oxygen in all test vessels >3 mg/L	yes	
Concentration of test substance ≥80% of initial concentration during test	yes	

Criteria for poorly soluble test substances		

Figure A7.4.1.2.a/01-1: 48-hour dose-concentration response curve for *Daphnia magna* exposed to BIT



Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.4.1.3a(01) **GROWTH INHIBITION TEST ON ALGAE**

Annex Point IIA VII.7.3 *Pseudokirchnerielle subcapitata*

		1 REFERENCE	**Officialuse only**
	1.1 Reference	**A7.4.1.3.a/01** [redacted] (2006b) 1,2--Benzisothiazolin-3-one: A 96-hour toxicity test with the freshwater alga *(Pseudokirchneriella subcapitata)*, [redacted] (September 20, 2006), Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD Guideline 201, EEC Method C.3, US EPA OPPTS 850.5400	
	2.2 GLP	Yes	
	2.3 Deviations	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2--Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant	Due to the decline in BIT concentrations over the duration of the study, the biological endpoints were based on Day 0 measured	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.4.1.3a(01) **GROWTH INHIBITION TEST ON ALGAE**

Annex Point IIA VII.7.3 *Pseudokirchnerielle subcapitata*

		1 REFERENCE	Officialuse only
	properties	concentrations.	
3.1.6	Method of analysis	High performance liquid chromatography (HPLC) with UV detector	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see Table A7.4.1.3.a/01-1	
3.3	**Reference substance**	No	
3.4	**Testing procedure**		
3.4.1	Culture medium	Stock nutrient solutions were prepared by adding reagent-grade chemicals to purified well water. The pH of the medium was adjusted to pH 8.0 with 10% HCl and the medium was sterilized by filtration (0.22 µm) prior to use.	
3.4.2	Test organisms	see Table A7.4.1.3.a/01-2	
3.4.3	Test system	see Table A7.4.1.3.a/01-3	
3.4.4	Test conditions	see Table A7.4.1.3.a/01-4	**X**
3.4.5	Duration of the test	96 h	
3.4.6	Test parameter	cell multiplication inhibition	
3.4.7	Sampling	0 h: aliquots were collected from the individual batches of test solution prepared for each treatment and control group prior to addition of the algae. At 96 h: samples were from pooled replicates from each treatment and control group. All samples were collected in glass vials and were processed on the day of collection and analyzed as soon as possible.	
3.4.8	Monitoring of TS concentration	Yes, 0 and 96 h	
3.4.9	Statistics	The calculation of cell densities, area under the growth curve, growth rates and percent inhibition values, as well as all statistical analyses, were conducted using “SAS System for Windows”, Version 8.02 (SAS Institute, Inc., 1999, Cary, North Carolina, USA). The data were evaluated for normality and homogeneity of	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.4.1.3a(01) **GROWTH INHIBITION TEST ON ALGAE**

Annex Point IIA VII.7.3 *Pseudokirchnerielle subcapitata*

		1 REFERENCE	Officialuse only
		variance (p = 0.05) using the Shapiro-Wilk's and Levene's tests, respectively.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal: 0 (negative control), 0.018, 0.041, 0.091, 0.20, 0.45 and 1.0 mg BIT/L	
4.2.2	Actual concentrations of test substance	Day 0 Measured: negative control < LOQ (limit of quantitation), 0.019, 0.043, 0.095, 0.21, 0.47 and 1.1 mg BIT/L Day 4 (96 hours) all BIT concentrations were < LOQ.	
4.2.3	Growth curves	see attached Figure A7.4.1.3.a/01-1 for growth of *Pseudokirchneriella subcapitata* in the negative control	
4.2.4	Concentration / response curve	see attached Figure A7.4.1.3.a/01-2	
4.2.5	Cell concentration data	Not described in report	**X**
4.2.6	Effect data (cell multiplication inhibition)	72 h EC_{50} = 0.32 mg BIT/L 72 h E_rC_{50} = 0.80 mg BIT/L 72 h E_bC_{50} = 0.32 mg BIT/L	**X**
4.2.7	Other observed effects	Not applicable	
4.3	**Results of controls**	control results performed as expected	
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and**	US EPA OPPTS 850.5400, OECD Guideline 201, EEC Method	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.4.1.3a(01) **GROWTH INHIBITION TEST ON ALGAE**

Annex Point IIA VII.7.3 *Pseudokirchnerielle subcapitata*

		1 REFERENCE	Officialuse only
	methods	C.3, Acute static 96 h algal study with analytical confirmation of test solution concentrations.	
5.2	**Results and discussion**	The 96 hour EC_{50} is equal to 0.38 mg BIT/L. The freshwater alga was exposed to a geometric series of six test concentrations and a negative control under static conditions for 96 hours. All stock solutions and test solutions appeared clear and colourless at preparation and no precipitates were observed in the test solutions during the test. Samples of test medium collected and analyzed for BIT concentrations resulted in recoveries that ranged from 105 to 106% of nominal concentrations on Day 0 and all <LOQ on Day 4.	
5.2.1	NOE_rC	96 h = 0.47 mg BIT/L	
5.2.2	E_rC_{50}	96 h = 0.98 mg BIT/L	
5.2.3	E_bC_{50}	96 h = 0.36 mg BIT/L	
5.3	**Conclusion**	see validity criteria in Table A7.4.1.3.a/01-6	
5.3.1	Reliability	(1), reliable without restriction	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*April 2015*
Materials and Methods	*A 96-hour toxicity test with the marine diatom (P. subcapitata) of BIT [redacted] was conducted following OECD TG 201, EC Method C.3, and US EPA OPPTS 850.5400.* *The initial cell density was ca. 10000 cells/ml for each test flask.*
Results and discussion	*The test fulfills the Validity criteria in OECD 201:* • *It fulfills exponential growth criteria.* • *Mean coefficient of variation section by section at 96h = 0.169 and at 72h = 0.2. Meets the criteria and does not exceeds 35%.*

Section A7
Subsection A7.4.1.3a(01)
Annex Point IIA VII.7.3

Ecotoxicological Profile Including Environmental Fate and Behaviour
GROWTH INHIBITION TEST ON ALGAE
Pseudokirchnerielle subcapitata

	1 REFERENCE	**Officialuse only**

- *Coef. of variation of average specific growth rates for 72h = 0.0056 and for 96h = 0.022 meets the criteria and does not exceeds 7%.*
- *Initial cell density is 10000 cells/ml fulfilling criteria.*

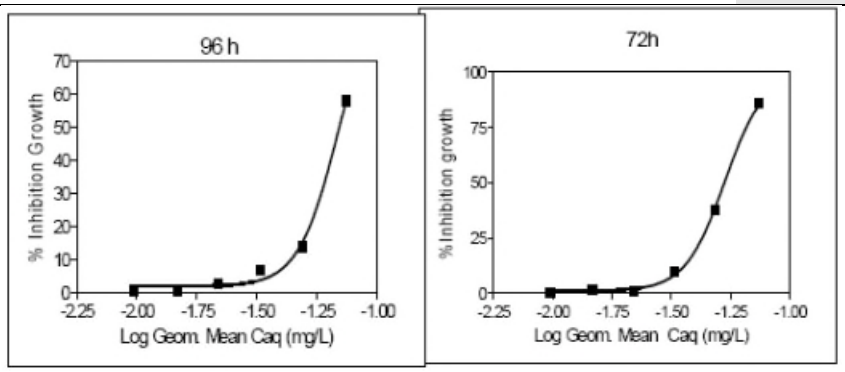
Cell Density By Replicate Over the 96-Hour Exposure Period

Day 0 Measured Test Concentration (mg a.i./L)	Replicate	Cell Density (cells/mL) 24 Hours[1]	48 Hours	72 Hours	96 Hours
Negative Control	A	34,334	190,493	1,233,029	6,572,691
	B	36,993	183,054	1,185,600	6,117,897
	C	32,655	174,583	1,170,767	5,008,024
0.019	A	31,684	189,538	1,212,067	5,962,431
	B	33,299	184,889	1,129,678	5,645,357
	C	30,520	178,450	1,211,324	5,095,540
0.043	A	27,793	170,002	1,261,301	5,864,374
	B	27,843	168,182	1,103,894	5,435,972
	C	29,883	184,396	1,303,178	5,673,872
0.095	A	24,085	155,191	1,042,784	5,162,959
	B	27,532	161,607	876,990	4,108,751
	C	27,885	175,917	1,133,576	5,539,338
0.21	A	23,365	153,781	827,483	4,029,426
	B	20,297	111,828	815,122	3,496,899
	C	23,118	136,224	593,587	3,930,307
0.47	A	18,014	100,331	680,436	3,548,696
	B	14,693	58,432	272,661	1,486,388
	C	13,987	66,990	449,224	2,661,253
1.1	A	11,257	42,862	121,509	661,433
	B	14,545	22,565	30,012	99,220
	C	10,829	21,495	21,802	46,757

[1] The initial cell density of the stock culture was determined and an inoculum volume was administered to each test chamber to yield a cell density of approximately 10,000 cells/mL at test initiation (0 hours).

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.4.1.3a(01)	**GROWTH INHIBITION TEST ON ALGAE**
Annex Point IIA VII.7.3	*Pseudokirchnerielle subcapitata*

1 REFERENCE	**Officialuse only**

	
Conclusion	*The endpoints were recalculated.* *Initial measured concentrations were used for endpoints calculation since 24h represents the most sensitive endpoint. An E_rC50 = 0.33 mg BIT/l and a ErC10 = 0.032 mg BIT/l was calculated.*
Reliability	*2*
Acceptability	*Acceptable*

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.4.1.3a(01) **GROWTH INHIBITION TEST ON ALGAE**

Annex Point IIA VII.7.3 *Pseudokirchnerielle subcapitata*

	1 REFERENCE	**Officialuse only**

Remarks

Calculation of endpoints:

The endpoints evaluated were the 50% effect concentration for growth rate (ErC50), 10% effect concentration (ErC10) and were derived by generating a logistic sigmoid curve from 0% to 100%, applying a logistic model using a nonlinear (weighted) regression.

Period	eCA		
	ErC50	ErC10	NOEC
0-24	0.33 (0.26-0.4)	0.032 (0.01 -0.05)	0.04
0-48	0.8(0.59-1.02)	0.19 (0.14 -0.25)	0.21
0-72	0.99 (0.74 - 1.24)	0.24 (0.16 - 0.32)	0.47
0-96	1.31 (0.88 - 1.74)	0.34 (0.25 – 0.45)	0.47

Table A7.4.1.3.a/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	Yes, purified well water
Concentration of vehicle	Not applicable
Vehicle control performed	Yes dilution water control
Other procedures	Not applicable

Table A7.4.1.3.a/01-2: Test organisms

Criteria	Details
Species	Pseudokirchneriella subcapitata
Strain	Not applicable
Source	[redacted]
Laboratory culture	Yes
Method of cultivation	sterile algal medium identical to medium used in the toxicity test
Pretreatment	Actively growing in culture medium for at least two weeks prior to test initiation
Initial cell concentration	1.0 x 106 cells/mL; each test vessel was inoculated with 1.0 mL to yield 10,000 cells/mL at test initiation

Table A7.4.1.3.a/01-3: Test system

Criteria	Details
Volume of culture flasks	250 mL containing 100 mL test solution
Culturing apparatus	haemocytometer and a microscope
Light quality	cool-white fluorescent lights
Procedure for suspending algae	rotary shaker adjusted to 100 rpm
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	Erlenmeyer flasks were plugged with foam stoppers

Table A7.4.1.3.a/01-4: Test conditions

Criteria	Details
Test temperature	24 ± 2 °C
pH	7.9 to 8.0 on Day 0 and 8.1 to 8.4 on Day 4
Aeration of dilution water	Not described
Light intensity	4300 ± 10% lux
Photoperiod	24 h photoperiod daily

Table A7.4.1.3.a/01-5: Cell concentration data

Test-Substance Concentration (nominal)[1] [mg BIT/L]	Cell density (mean values) [cells x 103/mL]							
	Mean cell density				Percent inhibition			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0 (control)	35	183	1196	5899	--	--	--	--
0.019	32	184	1184	5568	8.2	-0.87	1.0	5.6
0.0.43	29	174	1223	5658	18	4.6	-2.2	4.1
0.095	27	164	1018	4937	24	10	15	16
0.21	22	134	745	3819	36	27	38	35
0.47	16	75	467	2565	55	59	61	57
1.1	12	29	58	269	65	84	95	95
Temperature [°C]	24.5	24.6	24.5	24.0				
pH	7.9 to 8.0 on Day 0; 8.1 to 8.4 on Day 4							

[1] specify, if TS concentrations were nominal or measured

Table A7.4.1.3.a/01-6: Validity criteria for algal growth inhibition test

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	yes	
Concentration of test substance ≥80% of initial concentration during test		yes

Criteria for poorly soluble test substances		

Figure A7.4.1.3.a/01-1: Growth of the freshwater alga, *Pseudokirchneriella subcapitata*, in the negative control during the toxicity test with BIT

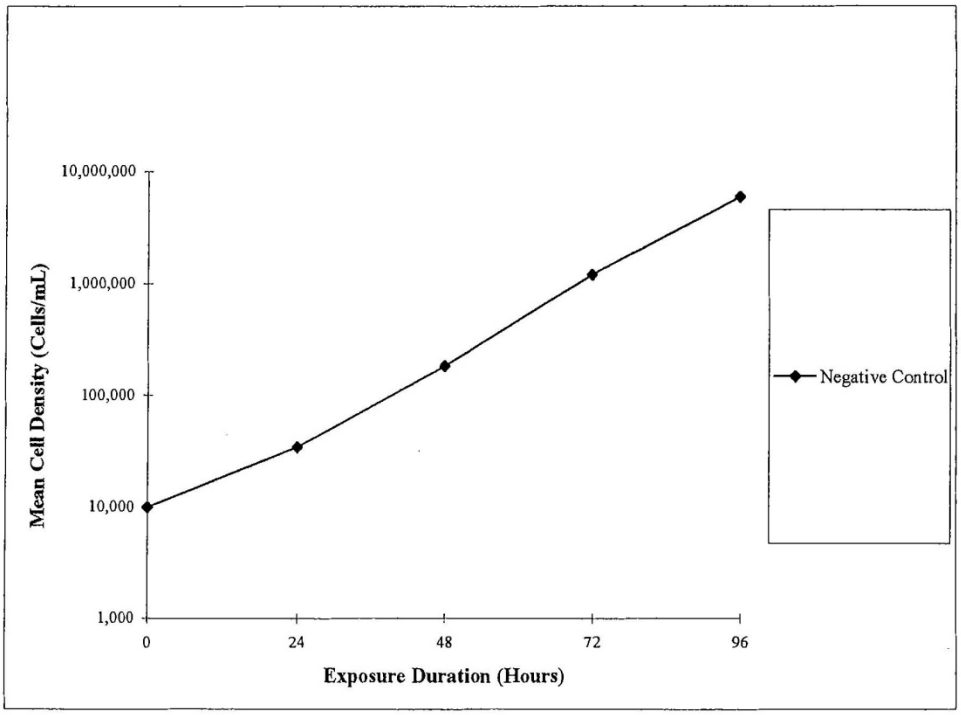
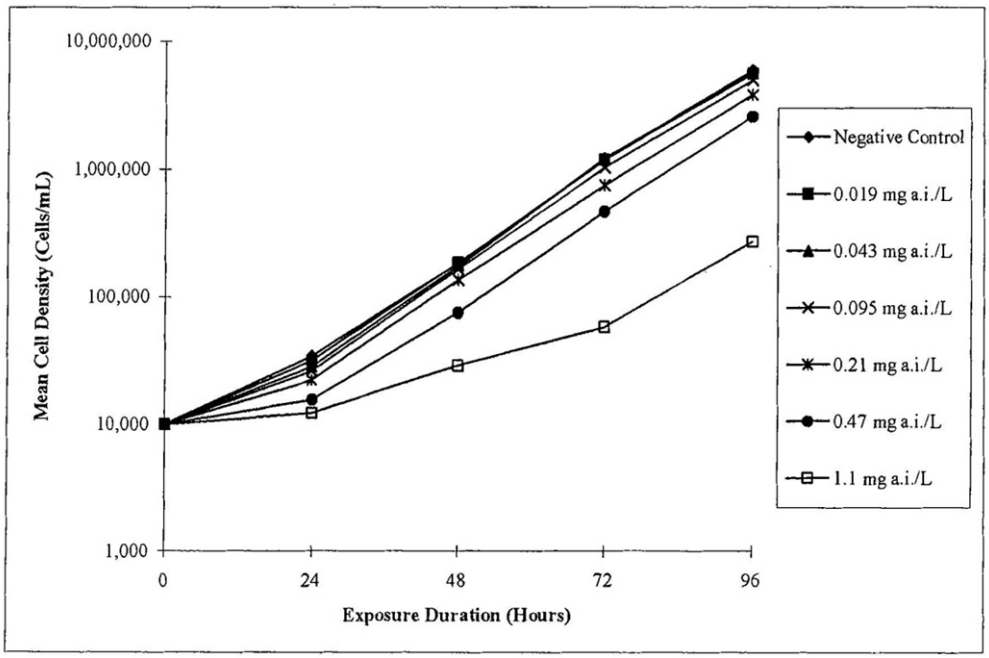


Figure A7.4.1.3.a/01-2: Concentration response curve for *Pseudokirchneriella subcapitata*, exposed to BIT for 96 hours, expressed as cell density



Section A7

Subsection A7.4.1.4(01)

Annex Point IIA VII.7.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

			Officialuse only
		1 REFERENCE	
	1.1 Reference	**A7.4.1.4/01** [redacted] (August 14, 2006), Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD 209	
	2.2 GLP	Yes	
	2.3 Deviations	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2.	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	

Section A7
Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4(01)
INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

3.1.6	Method of analysis	High performance liquid chromatography (HPLC)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see Table A7.4.1.4/01-1	
3.3	**Reference substance**	3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance	Not measured in this assay.	
3.4	**Testing procedure**		
3.4.1	Culture medium	Activated sludge collected from the [redacted] was utilized as the inoculum for the test. The [redacted] facility receives wastes from predominately domestic sources. The sludge was sieved using a 2 mm screen and allowed to settle for approximately 30 minutes. The supernatant above the settled solids was removed and the total suspended solids (TSS) concentration of the settled sludge was determined. Total suspended solids in the settled sludge were adjusted to a nominal concentration of approximately 4000 mg/L by dilution with municipal water. 50 mL of synthetic sludge was added to each liter of adjusted sludge. The sludge was maintained at a temperature of 20 ± 2 ºC and continuously aerated overnight. Before use, the pH and total suspended solids concentration of the activated sludge were determined.	
3.4.2	Inoculum / test organism	see Table A7.4.1.4/01-2	
3.4.3	Test system	see Table A7.4.1.4/01-3	
3.4.4	Test conditions	see Table A7.4.1.4/01-4	**X**
3.4.5	Duration of the test	3 h contact time for each concentration of the reference substance or the TS with the activated sludge	
3.4.6	Test parameter	respiration inhibition	
3.4.7	Analytical parameter	dissolved oxygen concentrations	
3.4.8	Sampling	respiration rate was measured at 10 second intervals over a 10 minute period or until dissolved oxygen concentrations fell below	

Section A7
Subsection A7.4.1.4(01)
Annex Point IIA VII.7.4

Ecotoxicological Profile Including Environmental Fate and Behaviour
INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

		1.0 mg/L using a dissolved oxygen meter.
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	The control contained 9.6 mL of synthetic sewage, 120 mL of inoculum and municipal water to bring up the total volume to 300 mL.
3.4.11	Statistics	A respiration rate was calculated for each test mixture and expressed in mg O_2/L/hour. The rate was calculated using dissolved oxygen (DO) values between 6.5 mg O_2/L and 2.5 mg O_2/L, or over a 10 minute period if the dissolved oxygen did not reach approximately 2.5 mg O_2/L. The respiration rate was calculated using the following calculation: Respiration rate=[(initial DO-final DO)/(final time-initial time)] x 3600 seconds/hour Percent inhibition was calculated using the following calculation: Percent Inhibition=[1 - (2Rs/RC1 + RC2)] x 100 Rs = oxygen consumption rate at a given concentration of the TS RC1 = oxygen consumption rate, control 1 RC2 = oxygen consumption rate, control 2 When the dose response pattern (percent inhibition versus TS concentration) allowed for the calculation on an EC_{50} value, the data were analyzed using the computer program of C.E. Stephan (1977. "Methods for Calculating an LC_{50}", *Aquatic Toxicology and Hazard Evaluations*. American Society for Testing and Materials. Publication Number STP 634, pages 65-84). The program was designed to calculate the EC_{50} value and the 95% confidence interval by probit analysis, the moving average, or binomial probability with nonlinear interpolation.
		4 RESULTS
4.1	**Preliminary test**	Not Performed
4.1.1	Concentration	Not applicable
4.1.2	Effect data	Not applicable
4.2	**Results test substance**	

Section A7

Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4(01)

INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

4.2.1	Initial concentration of test substance	Nominal: 1, 3, 10, 30, 100, 300 and 1000 mg a.i./L
4.2.2	Actual concentrations of test substance	Not applicable
4.2.3	Growth curves	Not applicable
4.2.4	Cell concentration data	Not applicable
4.2.5	Concentration/ response curve	See Figure A7.4.1.4/01-1
4.2.6	Effect data	Nominal concentration mg BIT per liter

Concentration	Respiration Rate (mg O_2/L/hour)	Percent Inhibition
1	52.4	-36.3
3	33.5	12.9
10	33.5	12.9
30	18.3	52.4
100	6.1	84.1
300	1.8	95.3
1000	0.6	98.4

4.2.7	Other observed effects	
4.3	**Results of controls**	respiration rate = 36.9 and 40.0 mg O_2/L/h
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	3,5-dichlorophenol: 3.0, 15, 50 mg/L
4.4.2	Results	EC_{50} = 15.86 mg/L (95% confidence limits: 3 and 50 mg/L)

Section A7

Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4(01)

INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 209, Activated sludge, respiration inhibition test	
5.2	**Results and discussion**	Nominal concentrations of BIT were used for all calculations. The respiration rates observed in the two controls were 36.9 and 40.0 mg O_2/L/h with a difference of approximately 7.8%. The EC_{50} value for the reference substance was 15.86 mg/L with 95% confidence limits of 3 and 50 and was within the 5 to 30% mg/L range considered acceptable for the test. The EC_{50} value for 1,2-Benzisothiazolin-3-one was 28.52 mg/L with 95% confidence limits of 10 and 100. The EC_{50} and 95% confidence limits were calculated using binomial probability with nonlinear interpolation. Inhibitory effects upon respiration by 1,2-Benzisothiazolin-3-one at the concentrations evaluated in this study exhibited a concentration dependent dose response pattern.	
5.2.1	NOEC	3 h = between 1 and 3 mg BIT/L	
5.2.2	EC_{50}	3 h = 28.52 mg BIT/L (95% C.I. 10 and 100 mg/L)	
5.2.3	EC_{80}	Not calculated. 84.1% inhibition was observed at 100 mg BIT/L.	
5.3	**Conclusion**		
5.3.1	Reliability	(1), reliable without restriction	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*December 2010*
Materials and Methods	*Adopt applicant's version with the following remark:* *3.4.4 It is neccesary to describe aireation of the dilution water and the air flow.*
Results and discussion	*Adopt applicant's version.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4(01) INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

Conclusion	*Adopt applicant's version.* *Additionally eCA calculated EC10 using linear regression and the Michaelis Menten model which results in a EC10 = 4.12 mg a.s. /l BIT.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.4.1.4/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	Not applicable

Table A7.4.1.4/01-2: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	Not applicable
Strain	Not applicable
Source	municipal wastewater treatment plant in [redacted] which treats predominantly domestic waste
Sampling site	Not described
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	The sludge was sieved using a 2 mm screen and allowed to settle for approximately 30 minutes. The supernatant above the settled solids was removed and the total suspended solids (TSS) concentration of the settled sludge was determined. The sludge was maintained at a temperature of 20 ± 2 ºC and continuously aerated overnight. Before use, the pH and total suspended solids concentration of the activated sludge were determined.
Pretreatment	Not described
Initial cell concentration	Total suspended solids in the settled sludge were adjusted to a nominal concentration of approximately 4000 mg/L by dilution with municipal water. 50 mL of synthetic sludge was added to each liter of adjusted sludge.

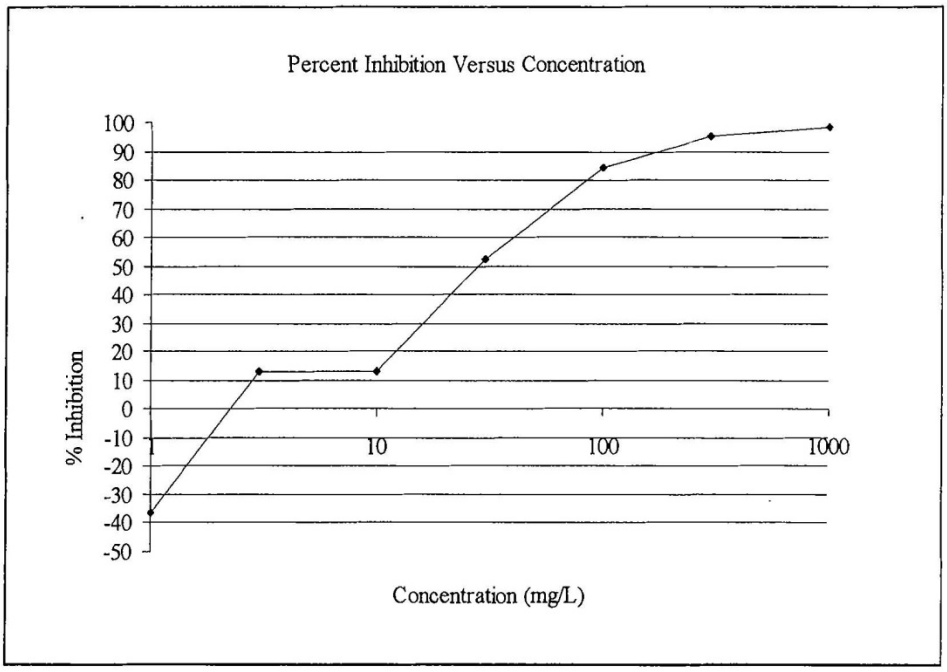
Table A7.4.1.4/01-3: Test system

Criteria	Details
Culturing apparatus	500 mL plastic Erlenmeyer flasks were used for the 3 hr incubation period then placed into BOD bottles
Number of culture flasks/concentration	2 controls and 1 for each reference substance and test substance concentration
Aeration device	vessels were aerated for 3 h using pressurized laboratory air
Measuring equipment	dissolved oxygen was measured with YSI Model 50B dissolved oxygen meter
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.4/01-4: Test conditions

Criteria	Details
Test temperature	20 + 2 °C
pH	7.8 at test initiation
Aeration of dilution water	Not described
Suspended solids concentration	4327 mg/L at test initiation

Figure A7.4.1.4/01-1: Percent Inhibition versus Concentration for 1,2-Benzisothiazolin-3-one



Section A7 **Subsection A7.4.2** **Annex Point IIA, VII.7.5**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ESTIMATION OF BIOCONCENTRATION**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X].**	
Detailed justification:	A waiver is requested for performing a fish bio-accumulation study for BIT. This request is based on the logP (log octanol:water partition coefficient) for this compound. • Log P < 1.5 This value indicates that the potential for BIT to bioaccumulate will be minimal. Many regulatory schemes have established that a logP of less than 3 precludes the need to perform a bio-accumulation study. Additionally using theUS EPA's EPIWIN software suite the calculated Bioconcentration Factor (BCF)is 1.646 (LogBCF = 0.216). Therefore, based on the logP values, bio-accumulation studies would not provide any necessary additional data but would result in the unnecessary sacrifice of animals.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	*January 2011.*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version*	
Remarks		

Section A7 **Subsection A7.4.3.1** **Annex Point IIIA 12.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **PROLONGED TOXICITY TO AN APPROPRIATE SPECIES OF FISH**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X].**	
Detailed justification:	As outlined in the "Technical guidance document in support of the directive 98/8/EC concerning the placing of biocidal products on the market", this test is not required as it does not add information as needed in the risk assessment. The existing guidelines are not sufficient. Other studies are available under section A7.4.3.2.	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	*January 2011*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version*	
Remarks		

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.2 EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH

Annex Point IIIA XIII 2.2

			Officialuse only
		1 REFERENCE	
	1.1 Reference	**A7.4.3.2.a/01** [redacted] (2007b). 1,2-Benzisothiazolin-3-one: An early life-stage toxicity test with the fathead minnow (*Pimephales promelas*), [redacted] (January 16, 2007), Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD 210 and US EPA OPPTS 850.1400	
	2.2 GLP	Yes	
	2.3 Deviations	No	
		3 METHOD	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	

Section A7 Subsection A7.4.3.2 Annex Point IIIA XIII 2.2		Ecotoxicological Profile Including Environmental Fate and Behaviour EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	High performance liquid chromatography with UV detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see Table A7.4.3.2.a/01-1	
3.3	**Reference substance**	No	
3.4	**Testing procedure**		
3.4.1	Dilution water	see Table A7.4.3.2.a/01-2	
3.4.2	Test organisms	see Table A7.4.3.2.a/01-3	
3.4.3	Handling of embryos and larvae (OECD 210/212)	Embryos were removed from 10 individual spawning substrates and examined under a dissecting microscope to select healthy, viable specimens at approximately the same stage of development. Embryos were added to incubation cups in the test chambers. After a 5-day embryo hatching period, the larvae were released into the test chambers where exposure to BIT continued during a 28-day post-hatch juvenile growth period.	
3.4.4	Test system	see Table A7.4.3.2.a/01-4	
3.4.5	Test conditions	see Table A7.4.3.2.a/01-5	**X**
3.4.6	Duration of the test	33 days (5 day hatch and 28 day post-hatch)	
3.4.7	Test parameter(s)	Time to hatch, hatching success, growth and survival	
3.4.8	Examination / Sampling	During the first day of exposure, embryos were examined twice for mortality and eggs with fungus. Observations of embryo mortality and the removal of dead embryos were performed once daily during the hatching period. During the 28-day post-hatch period, the larvae were observed daily for mortality, clinical signs of toxicity and abnormal behavior. Total length, wet weight and dry weight were measured on surviving fish.	

Section A7 Subsection A7.4.3.2 Annex Point IIIA XIII 2.2		Ecotoxicological Profile Including Environmental Fate and Behaviour EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH	
3.4.9	Monitoring of TS concentration	Samples were collected from each treatment group and control group on Days 0, 7, 14, 21, 28 and 33 (test termination) and processed immediately for analysis.	
3.4.10	Statistics	Post-hatch survival was calculated as the number of larvae surviving to test termination divided by the total number of embryos hatched successfully. Time to hatch data were evaluated by visual interpretation of the data. Hatching success and survival were analysed using Chi-square and Fisher's Exact tests.growth data were evaluated for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test ($p = 0.01$). Those treatments that were significantly different from the control means were identified using Dunnett's t-test ($p = 0.05$). All statistical test were performed with SAS software (The SAS System for Windows. 2001. Version 8.2. SAS Institute, Inc., Cary, North Carolina, USA)	
		4 RESULTS	
4.1	**Range finding test**		
4.1.1	Concentrations	Not described in report	
4.1.2	Number/ percentage of animals showing adverse effects	Not described in report	
4.1.3	Nature of adverse effects	Not described in report	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentrations (mg BIT/L) 0.31, 0.63, 1.3, 2.5 and 5.0	
4.2.2	Actual concentrations of test substance	Mean measured concentrations (mg BIT/L):	**X**

Nominal concentration	Mean measured
Negative control	<LOQ
Solvent control	<LOQ
0.31	0.28

Section A7
Subsection A7.4.3.2
Annex Point IIIA XIII 2.2

Ecotoxicological Profile Including Environmental Fate and Behaviour

EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH

	0.63	0.59
	1.3	1.2
	2.5	2.4
	5.0	4.8

4.2.3 Effect data

The majority of fish in the 0.28, 0.59 and 1.2 mg BIT/L treatment groups appeared normal throughout the test. Several fish in the 2.4 mg BIT/L group were surfacing between days 2 and 4 but the fish appeared normal from day 5 through test termination. Several fish in the 4.8 mg BIT/L group were weak, surfacing, swimming erractically or with morphological abnormalities such as crooked spines. Most of these 4.8 mg BIT/L weakened fish died prior to test termination.

4.2.4 Concentration / response curve

Not described in report

4.2.5 Other effects

BIT concentration	Fish total length (mm)	Fish wet weight (mg)	Fish dry weight (mg
Negative control	22.7	84.7	15.8
Solvent control	23.0	93.8	16.6
0.28 mg BIT/L	22.9	91.5	16.8
0.59 mg BIT/L	22.3 *	85.3 *	15.4
1.2 mg BIT/L	22.8	88.5	16.6
2.4 mg BIT/L	22.1	81.3	15.6
4.8 mg BIT/L	21.1	67.7	12.7

The 1.2, 2.4 and 4.8 mg BIT/L groups were excluded from analyses of growth due to significant effects on larval survival.

*statistically significantly different from the pooled control (total length and dry weight) or the solvent control (wet weight) using Dunnett's test ($p \leq 0.05$).

Day 28 post-hatch mortality:

Section A7

Subsection A7.4.3.2

Annex Point IIIA XIII 2.2

Ecotoxicological Profile Including Environmental Fate and Behaviour

EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH

BIT concentration	Number dead / Number hatched
Negative control	4 / 77
Solvent control	8 / 76
0.28 mg BIT/L	10 / 80
0.59 mg BIT/L	8 / 78
1.2 mg BIT/L	13 / 79
2.4 mg BIT/L	34 / 75
4.8 mg BIT/L	50 / 79

4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	Not applicable
4.3.2	Nature of adverse effects	Not applicable
4.4	**Test with reference substance**	Not performed

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.4.3.2	**EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH**
Annex Point IIIA XIII 2.2	

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	**OECD 210 and US EPA OPPTS 850.1400, Early life stage toxicity study to fish under flow-through conditions with analytical confirmation of TS concentrations.**	
5.2	**Results and discussion**	All environmental conditions were within acceptable limits during the test. Test solutions appeared clear and colorless in all test chambers with no precipitates noted during the test. There were no treatment-related effects on time to hatch or hatching success. All surviving fish appeared normal at 28 days post-hatch. The most sensitive end point was growth. The Maximum Acceptable Toxicant Concentration (MATC) = 0.41 mg BIT/L.	
5.2.1	NOEC	0.28 mg BIT/L, based on growth-related effects	
5.2.2	LOEC	0.59 mg BIT/L, based on growth-related effects	
5.3	**Conclusion**	see Table A7.4.3.2.A/01-6	
5.3.1	Other Conclusions	Not applicable	
5.3.2	Reliability	(1), reliable without restriction	
5.3.3	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*December 2010*
Materials and Methods	*Adopt applicant's version with the following comment:* *3.4.5. The water temperature differ more than ±1.5 ºC*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*

Section A7
Subsection A7.4.3.2
Annex Point IIIA XIII 2.2

Ecotoxicological Profile Including Environmental Fate and Behaviour

EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH

Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.4.3.2.a/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes, sonicated and mixed by inversion
Vehicle	Dimethyl formamide (DMF)
Concentration of vehicle	0.1 mL/L in solvent control and in all BIT treatment groups
Vehicle control performed	Yes
Other procedures	Not applicable

Table A7.4.3.2.a/01-2: Dilution water

Criteria	Details
Source	Filtered and sterilized freshwater obtained from a well approximately 40 meters deep located on the [redacted]
Salinity	Not applicable
Hardness	136 to 144 mg/L as $CaCO_3$
pH	8.1
Oxygen content	8.3 to 8.4 mg/L
Conductance	340 to 350 µmhos/cm
Alkalinity	180 to 185 mg/L as $CaCO_3$
Holding water different from dilution water	No

Table A7.4.3.2.a/01-3: Test organisms

Criteria	Details
Species/strain	Fathead minnow (*Pimephales promelas*)
Source	[redacted]
Wild caught	no
Age/size	Embryos < 24 h old
Kind of food	Live brine shrimp nauplii (Artemia species)
Amount of food	*Ad libitum*
Feeding frequency	3 times per day during first 7 days post-hatch. 3 times per day on weekdays and two times per day on weekends for the next 19 days. Fish were not fed for the 48 h prior to study termination to allow for clearance of the digestive tracts before weight measurements were made.
Post-hatch transfer time	5 days post-hatch
Time to first feeding	7 days post-hatch
Feeding of animals during test	yes
Treatment for disease within 2 weeks preceding test	No

Table A7.4.3.2.a/01-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	A continuous-flow diluter and syringe pump were used to deliver the controls and BIT solutions into mixing chambers where the controls and BIT solutions were diluted with water and delivered to the test chambers. The diluter flow rate was adjusted to provide 10 volume additions of test solutions in each test chamber per day.
Volume of test vessels	9 liter glass aquaria containing 7 liters of test solution
Volume/animal	0.35 liters
Number of animals/vessel	20
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	Not applicable

Table A7.4.3.2.a/01-5: Test conditions

Criteria	Details
Test temperature	24.0 – 25.7 °C
Dissolved oxygen	≥ 6.9 mg/L (84% of saturation)
pH	8.0 – 8.2
Adjustment of pH	Not described
Aeration of dilution water	Yes
Intensity of irradiation	Fluorescent light bulbs, 442 lux
Photoperiod	16 h daylight and 8 h darkness with a 30 minute transition period of low light intensity

Table A7.4.3.2.a/01-6: Validity criteria for fish tests according to OECD Guidelines 210

	fulfilled	Not fullfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	yes	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	yes	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	yes	
Test substance concentrations maintained within ± 20% of mean measured values	yes	
No effect on survival nor any other adverse effect found in solvent control	yes	
Further criteria for poorly soluble test substances	yes	

Section A7 **Subsection A7.4.3.3** **Annex Point IIIA XIII.2.3**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **BIO-ACCUMULATION IN AQUATIC ORGANISMS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X].**	
Detailed justification:	7.4.3.3.1 Bioaccumulation in fish A waiver is requested for performing a fish bio-accumulation study for BIT. This request is based on the logP (log octanol:water partition coefficient) for this compound. • LogP < 1.5 This value indicates that the potential for BIT to bioaccumulate will be minimal. Many regulatory schemes have established that a log P of less than 3 precludes the need to perform a bio-accumulation study. Additionally using theUS EPA's EPIWIN software suite the calculated Bioconcentration Factor (BCF) is 1.646 (LogBCF = 0.216). Therefore, based on the logP values, bio-accumulation studies would not provide any necessary additional data but would result in the unnecessary sacrifice of animals. 7.4.3.3.2 Bioaccumulation in invertebrates A waiver is requested for performing a fish bio-accumulation study for BIT This request is based on the logP (log octanol:water partition coefficient) for this compound. • LogP < 1.5 This value indicates that the potential for BIT to bioaccumulate will be minimal. Many regulatory schemes have established that a logP of less than 3 precludes the need to perform a bio-accumulation study. Additionally using theUS EPA's EPIWIN software suite the calculated Bioconcentration Factor (BCF) is 1.646 (LogBCF = 0.216). Therefore, based on the logP values, bio-accumulation studies would not provide any necessary additional data but would result in the unnecessary sacrifice of animals.	
Undertaking of intended data submission [ ]	No studies planned.	

Section A7 **Subsection A7.4.3.3** **Annex Point IIIA XIII.2.3**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **BIO-ACCUMULATION IN AQUATIC ORGANISMS**	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	*December 2010*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version*	
Remarks		

Section A7

Subsection A7.4.3.4a(01)

Annex Point IIIA XIII 2.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

EFFECTS ON REPRODUCTION AND GROWTH RATE WITH AN INVERTEBRATE SPECIES

			Officialuse only
		1 REFERENCE	
	1.1 Reference	**A7.4.3.4.a/01** [redacted] (2007c) 1,2-Benzisothiazolin-3-one: A flow-through life-cycle toxicity test with the cladoceran (*Daphnia magna*), [redacted] (January 17, 2007), GLP, Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD 211 and US EPA OPPTS 850.1300	
	2.2 GLP	Yes	
	2.3 Deviations	No	
		3 METHOD	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	

Section A7 Subsection A7.4.3.4a(01) Annex Point IIIA XIII 2.4	Ecotoxicological Profile Including Environmental Fate and Behaviour EFFECTS ON REPRODUCTION AND GROWTH RATE WITH AN INVERTEBRATE SPECIES	
3.1.5 Further relevant properties	Not applicable	
3.1.6 Method of analysis	High performance liquid chromatography	
3.2 Preparation of TS solution for poorly soluble or volatile test substances	see Table A7.4.3.4.a/01-1	
3.3 Reference substance	No	
3.4 Testing procedure		
3.4.1 Dilution water	see Table A7.4.3.4.a/01-2	**X**
3.4.2 Test organisms	see Table A7.4.3.4.a/01-3	
3.4.3 Handling of offspring	Following the onset of reproduction, the numbers of second-generation daphnids were counted three times per week and at test termination.	
3.4.4 Test system	see Table A7.4.3.4.a/01-4	
3.4.5 Test conditions	see Table A7.4.3.4.a/01-5	**X**
3.4.6 Duration of the test	21 days	
3.4.7 Test parameter	Mortality, immobility, sublethal signs of toxicity, onset of reproduction, mean lengths and dry weights in the first generation daphnids. First day of brood production and number of neonates indicated reproduction effects.	
3.4.8 Examination / Sampling	First-generation daphnids were observed daily. The numbers of second generation daphnids were counted three times per week and at test termination (day 21). Body lengths and dry weights of the surviving first generation daphnids were measured at the end of the exposure period.	
3.4.9 Monitoring of TS concentration	Yes, days –2, 0, 7, 14, 21. All samples were collected mid-depth, placed in glass scintillation vials and processed immediately for analysis.	
3.4.10 Statistics	Survival data were anlyzed using Chi-square and Fisher’s Exact	

Section A7 Subsection A7.4.3.4a(01) Annex Point IIIA XIII 2.4	Ecotoxicological Profile Including Environmental Fate and Behaviour EFFECTS ON REPRODUCTION AND GROWTH RATE WITH AN INVERTEBRATE SPECIES	

tests. Reproduction and growth data were evaluated for normality using Shapiro-Wilk's test and for homogeneity using Levenes or Bartlett's tests (p = 0.01). Analysis of Variance (ANOVA) was used to determine if statistically significant differences existed among the BIT treatment groups (p = 0.05). The BIT treatments that were significantly different from the pooled control means were identified using Bonferroni's t-test (p ≤ 0.05). All statisitical tests were performed using TOXSTAT (West, Inc. and D.D. Gulley. 1996. TOXSTAT® Version 3.5. Western EcoSystems Technology, Inc., Cheyenne, Wyoming, USA) or SAS (The SAS system for Windows. 1999-2001 Version 8.2, Cary, North Carolina, USA) software.

4 RESULTS

4.1 Range finding test — Not described

4.2 Results test substance

4.2.1 Initial concentrations of test substance — Nominal (mg BIT/L): 0.25, 0.50, 1.0, 2.0, and 4.0

4.2.2 Actual concentrations of test substance — mg BIT/L

Nominal concentration	Mean measured concentration	Percent of nominal
0.25	0.21	84
0.50	0.46	92
1.0	0.91	91
2.0	1.9	95
4.0	3.8	95

4.2.3 Effect data — See Table A7.4.3.4.a/01-6. One daphnid was lethargic and discoloured (pale) in the 3.8 mg BIT/L group **X**

4.2.4 Concentration / response curve — See figure A7.4.3.4.a/01-1

Section A7 Subsection A7.4.3.4a(01) Annex Point IIIA XIII 2.4	Ecotoxicological Profile Including Environmental Fate and Behaviour EFFECTS ON REPRODUCTION AND GROWTH RATE WITH AN INVERTEBRATE SPECIES	
4.2.5 Other effects	Not applicable	
4.3 Results of controls	After 21 days survival in the negative and solvent control groups was 95% and 100%, respectively. The first day of brood production in the negative and solvent control groups was Day 8 of the test.	
4.4 Test with reference substance	Not performed	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	OECD 211 and US EPA OPPTS 850.1300, Aquatic invertebrate life-cycle study with analytical confirmation of TS concentrations.	
5.2 Results and discussion	Since no significant differences between the control groups were found for any parameter tested ($p > 0.05$) the control data were pooled for comparison with the BIT treatment groups. After 21 days survival in the negative and solvent control groups was 95% and 100%, respectively. The control data was pooled for comparisons with the BIT treatment groups. The first day of brood production in the negative control, solvent control and the BIT treatment groups groups was Day 8 of the test indicating there was no apparent delay in the onset of production at any BIT concentration tested.	
5.2.1 NOEC (21 d)	0.91mg BIT/L	
5.2.2 LOEC (21 d)	1.9mg BIT/L	
5.2.3 EC_{50} (EC_x)	2.5mg BIT/L, 21-day mortality/immobility (95% C.I.: 1.9 to 3.8 mg BIT/L) >3.8 mg BIT/L, reproduction	
5.2.4 MATC	1.3 mg BIT/L	
5.3 Conclusion	see Table A7.4.3.4.a/01-7	
5.3.1 Reliability	(1), reliable without restrictions	
5.3.2 Deficiencies	No	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.4a(01) EFFECTS ON REPRODUCTION AND GROWTH RATE WITH AN INVERTEBRATE SPECIES

Annex Point IIIA XIII 2.4

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*December 2010*
Materials and Methods	*Applicant's version is accepted with the following remarks:* *3.4.1: It is recommended by the OECD Guideline#211 to estimate theTOC levels in the medium.* *3.4.5: The light intensity was lower than the recommended by the OECDGuideline (15-20 µE*m²/s).*
Results and discussion	*Applicant's version is accepted with the following remarks:* • *4.2.3: The following results are missing in the report:* *Coefficient of variation for control fecundity (based of total number ofliving offspring per parent animal alive).* *The plot of total number of living offspring per parent animal (for each replicate) alive at the end of the test vs concentration,*
Conclusion	*Applicant's version adopted.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.4.3.4.a/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes, sonicated and mixed by inversion
Vehicle	Yes, dimethyl formaide (DMF)
Concentration of vehicle	0.1 mL/L
Vehicle control performed	Yes
Other procedures	Not applicable

Table A7.4.3.4.a/01-2: Dilution water

Criteria	Details
Source	Fresh well water collected at the [redacted]
Alkalinity	178 to 182 mg/L as $CaCO_3$
Hardness	128 to 138 mg/L as $CaCO_3$
TOC	Not described
Holding water different from dilution water	No

Table A7.4.3.4.a/01-3: Test organisms

Criteria	Details
Strain / Clone	*Daphnia magna*
Source	in-house culture
Age	less than 24 h old at test initiation
Breeding method	Not described
Kind of food	A mixture of yeast, cereal grass media and trout chow (YCT) as well as a suspension of *Pseudokirchneriella subcapitata*
Amount of food	At each feeding, each test chamber initially was fed 0.75 ml of YCT and 1.5 mL of algae. The amounts were increased to 1.0 mL YCT and 2.0 mL of algae on Day 16 of the test after dilution water flow rates were increased.
Feeding frequency	3 times per day through Day 7 and 4 times per day until the last day of the test
Pretreatment	Adult daphnids were cultured in water from the same source and at approximately the same temperature as used during the test.
Feeding of animals during test	Yes

Table A7.4.3.4.a/01-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	The diluter flow rate was adjusted to provide approximately 5 volume additions of test water in each test chamber per day until Day 15 of the test. On Day 15, the flow rate was increased to aid in maintaining dissolved oxygen concentrations and provided approximately 8 volume additions of test water in each test chamber per day through test termination.
Volume of test vessels	Two 300 mL glass beakers suspended in 25 L stainless steel aquaria filled with approximately 22 L test solution
Volume/animal	27 mL
Number of animals/vessel	10/beaker
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.4.a/01-5: Test conditions

Criteria	Details
Test temperature	19.6 to 20.2 °C
Dissolved oxygen	≥ 6.2 mg/L (≥ 69% saturation)
pH	8.0 to 8.2
Adjustment of pH	Not described
Conductivity	300 to 320 µmhos/cm
Aeration of dilution water	Yes
Quality/Intensity of irradiation	219 lux
Photoperiod	16 h light, 8 h dark with 30 minute transition period of low light intensity

Table A7.4.3.4.a/01-6: Effect data

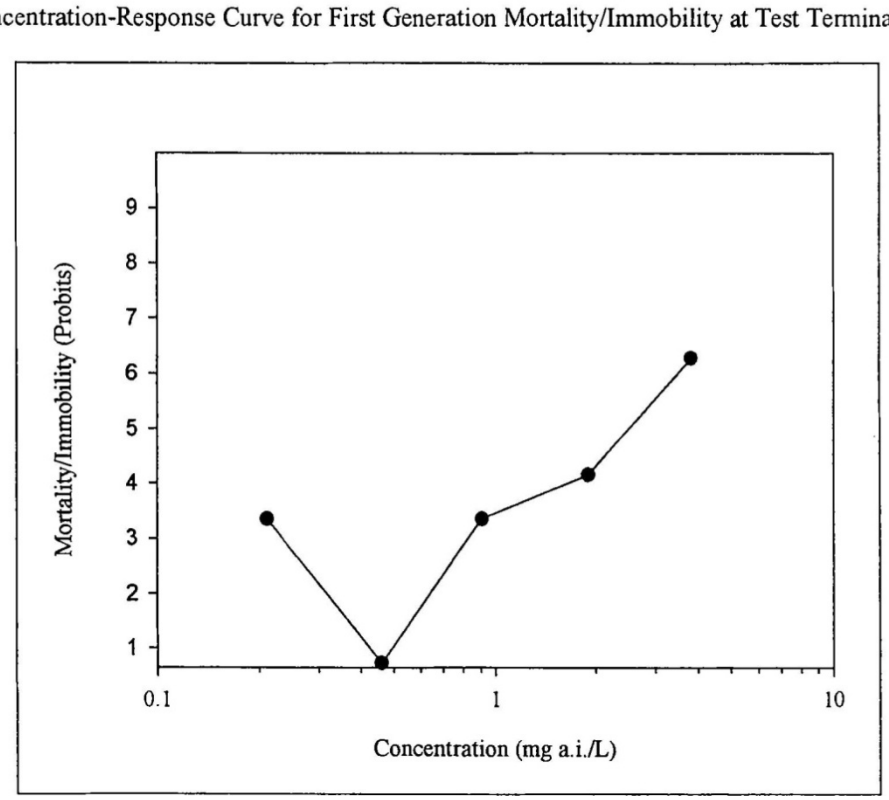
Mean measured concentration (µg DCOIT/L)	% survival at 21 days	Mean no. of young produced per reproductive day	Day of first brood	Treatment mean length (mm)	Treatment mean dry weight (mg)
Negative control	95	11.2	8	5.7	1.12
DMF solvent control	100	11.4	8	5.7	1.10
0.21	95	12.6	8	5.8	1.16
0.46	100	11.3	8	5.7	1.02
0.91	95	11.8	8	5.8	1.09
1.9	80*	10.5	8	5.5	0.99
3.8	10*	7.0	8	5.3	1.08

*Statistically significant decrease in survival in comparison to the pooled control (98%) using Fisher's Exact Test ($p \leq 0.05$)

Table A7.4.3.4.a/01-7: Validity criteria for invertebrate reproduction test according

	fulfilled	Not fullfilled
Mortality of parent animals < 20% at test termination	yes	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	yes	

Figure A7.4.3.4.a/01-1: Concentration-response curve for First Generation Mortality/Immobility at Test Termination



Section A7

Subsection A7.4.3.5.1a

Annex Point IIIA, XIII.3.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

EFFECTS ON SEDIMENT DWELLING ORGANISMS

Chironomus tentans

			Officialuse only
		1 REFERENCE	
	1.1 Reference	**A7.4.3.5.1a/01** [redacted] (2007) 1,2-Benzisothiazolin-3-one: A survival and growth sediment toxicity test with *Chironomus tentans* using spiked sediment, [redacted] (March 9, 2007), Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, US EPA OPPTS 850.1735	
	2.2 GLP	Yes	
	2.3 Deviations	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	

Section A7 **Subsection A7.4.3.5.1a** **Annex Point IIIA, XIII.3.4**		**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON SEDIMENT DWELLING ORGANISMS** *Chironomus tentans*	
3.1.6	Method of analysis	High performance liquid chromatography with UV detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see Table A7.4.3.5.1.a/01-1	
3.3	**Reference substance**	No	
3.4	**Testing procedure**		
3.4.1	Dilution water, Test sediment	see Table A7.4.3.5.1.a/01-2	
3.4.2	Test organisms	see Table A7.4.3.5.1.a/01-3	
3.4.3	Test system	see Table A7.4.3.5.1.a/01-4	
3.4.4	Test conditions	see Table A7.4.3.5.1.a/01-5	**X**
3.4.5	Duration of the test	10 days	
3.4.6	Test parameter	survival, growth parameters	
3.4.7	Sampling	TS concentration was measured in the overlying water, pore water and sediment samples at test initiation and termination	
3.4.8	Monitoring of TS concentration	Yes, test initiation and termination	
3.4.9	Statistics	The ash-free dry weight data were analyzed using the computer program TOXSTAT version 3.5 (West, Inc. and D.D. Gulley. TOXSTAT version 3.5. Copyright 1996. Western Ecosystems Technology, Inc., Cheyenne, Wyoming, USA). The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analyses of the survival and mean individual ash-free dry weight data. The ash-free dry weight (growth) data were evaluated for normality (Chi-Square) and homogeneity of variances (Levene's Test). The negative and solvent control growth data were compared using two-tailed t-test ($p = 0.05$). There were significant differences between the negative and solvent control groups, therefore treatment groups were compared to the solvent control.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	

Section A7

Subsection A7.4.3.5.1a

Annex Point IIIA, XIII.3.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

EFFECTS ON SEDIMENT DWELLING ORGANISMS

Chironomus tentans

4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	6.3, 13, 25, 50 and 100 mg BIT/kg (nominal)
4.2.2	Actual concentrations of test substance	Measured BIT concentrations in sediment samples:

Nominal	Measured Day 0	Measured Day 10
Negative control	<LOQ	<LOQ
Solvent control	<LOQ	<LOQ
6.3 mg BIT/kg	3.38	<LOQ
13 mg BIT/kg	6.13	2.85
25 mg BIT/kg	15.4	5.91
50 mg BIT/kg	32.8	13.0
100 mg BIT/kg	45.9	22.2

Measured BIT concentrations in overlying water samples:

Nominal	Measured Day 0	Measured Day 10
Negative control	<LOQ	<LOQ
Solvent control	<LOQ	<LOQ
6.3 mg BIT/kg	<LOQ	<LOQ
13 mg BIT/kg	<LOQ	<LOQ
25 mg BIT/kg	<LOQ	<LOQ
50 mg BIT/kg	<LOQ	<LOQ
100 mg BIT/kg	0.312	<LOQ

Measured BIT concentrations in pore water samples:

Nominal	Measured Day 0	Measured Day 10

Section A7

Subsection A7.4.3.5.1a

Annex Point IIIA, XIII.3.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

EFFECTS ON SEDIMENT DWELLING ORGANISMS

Chironomus tentans

Negative control	<LOQ	<LOQ
Solvent control	<LOQ	<LOQ
6.3 mg BIT/kg	8.41	1.26
13 mg BIT/kg	21.0	7.29
25 mg BIT/kg	33.8	14.8
50 mg BIT/kg	93.3	32.6
100 mg BIT/kg	173	66.5

LOQ, limit of quantitation = 0.100 mg BIT/L

4.2.3	Effect data	see Table A7.4.3.5.1.a/01-6 and see Table A7.4.3.5.1.a/01-7
4.2.4	Concentration / response curve	Not described in report
4.2.5	Other effects	The organisms generally appeared normal and healthy throughout the study. A few organisms were observed on the surface of the sediment or climbing the walls of the test compartments in all BIT treatment groups and controls.
4.3	**Results of controls**	see Table A7.4.3.5.1.a/01-6
4.4	**Test with reference substance**	Not performed
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	US EPA OPPTS 850.1735, Acute flow-through toxicity study in whole sediment to midge larvae with analytical confirmation of TS concentrations.BIT was added to the sediment. At test termination, midges were rinsed of excess sediment, placed into a pre-weighed crucible and dried for approximately 42 hours at 60 ºC. The midges were weighed then placed into a furnace for approximately 2 hours at 550 ºC to determine ash-free dry weights.
5.2	**Results and discussion**	The overlying water appeared clear and colorless in all test compartments at test initiation and at test termination. All water quality parameters were within acceptable limits during the test. The organisms generally appeared normal and healthy throughout the study. Percent mortality at test termination was 0% in all treatment groups. One small midge in the 6.3 mg BIT/kg group and

Section A7 **Subsection A7.4.3.5.1a** **Annex Point IIIA, XIII.3.4**		**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON SEDIMENT DWELLING ORGANISMS** *Chironomus tentans*	
		three small midges in the 25 mg BIT/kg group were noted. The NOEC was 50 mg BIT/kg and the LOEC was 100 mg BIT/kg, based on ash-free dry weights.	
	EC_0	50 mg BIT/kg	
	EC_{50}	>100 mg BIT/kg	
	EC_{100}	Not applicable	
5.3	**Conclusion**	see Table A7.4.3.5.1.a/01-8	
5.3.1	Reliability	(1), reliable without restriction	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*March 2013*
Materials and Methods	*Applicant's version is accepted with the following remark:* *3.4.4: The light intensity used in the study is lower than those recommended bythe OECD (219 lux at water surface vs. 500 to 1000 lux).*
Results and discussion	*Applicant's version is accepted with the following remark:* *ECx values should consider the measured concentration at the beginning of thetest (as recommended by the guidances) and not be based on nominals. Therefore:* *EC_0 = 32.8 mg/kg* *$EC_{50} \geq$ 45.9 mg/kg* *EC_{100}= Not applicable.*
Conclusion	*Applicant's version is adopted.*
Reliability	*2*
Acceptability	*Acceptable*

Section A7 **Subsection A7.4.3.5.1a** **Annex Point IIIA, XIII.3.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON SEDIMENT DWELLING ORGANISMS** *Chironomus tentans*
Remarks	

Table A7.4.3.5.1.a/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	Acetone
Concentration of vehicle	Not applicable
Vehicle control performed	Yes
Other procedures	A primary stock solution was prepared by dissolving BIT in acetone at a nominal concentration of 10.0 mg BIT/mL.

Table A7.4.3.5.1.a/01-2: Dilution water

Criteria	Details
Source	Well fresh water, 40 meters deep
Alkalinity	178-180 mg/L as $CaCO_3$
Hardness	136 mg/L as $CaCO_3$
pH	8.0 – 8.2
Ca / Mg ratio	Not described
Na / K ratio	Not described
Oxygen content	Aerated, not measured
Conductance	300-320 µmhos/cm
Holding water different from dilution water	No

Table A7.4.3.5.1.a/01-3: Test organisms

Criteria	Details
Strain	midge larvae (*Chironomus tentans*)
Source	[redacted]
Age	10 days
Breeding method	Not described
Kind of food	Flake food (TetraMin Flakes)
Amount of food	1.5 mL of a 4 g/L suspension of flake food
Feeding frequency	Days 0 through 9
Pretreatment	Midges were held for 3 days at approximately the same temperature of water used in the test
Feeding of animals during test	Yes, Days 0 through 9

Table A7.4.3.5.1.a/01-4: Test system

Criteria	Details
Renewal of test solution	Flow-through. The diluter was adjusted so that approximately 786 mL of water was delivered every minute for 4 minutes to each splitting chamber 2 times per day resulting in approximately two volume additions in each test compartment per day.
Volume of test vessels	300 mL glass beakers with 2 stainless steel mesh-covered holes on opposite to allow for the flow of water throught the test compartment. Each beaker contained approximately 100 mL of sediment and 150 mL of overlying water.
Volume/animal	10 mL sediment per midge and 15 mL water per midge
Number of animals/vessel	10 midges
Number of vessels/ concentration	8 replicates with midge, 2 replicates for analytical purposes
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.5.1.a/01-5: Test conditions

Criteria	Details
Test temperature	23 ±1 ºC
Dissolved oxygen	≥ 5.6 mg/L (66% of saturation)
pH	7.9 – 8.2
Adjustment of pH	Not described
Aeration of dilution water	Yes
Quality/Intensity of irradiation	Fluorescent tubes that emit wavelengths similar to natural sunlight
Photoperiod	16 hours daylight, 8 hours darkness with 30-minute transition period of low light intensity

Table A7.4.3.5.1.a/01-6: Effect and Mortality data

Test-Substance Concentration (effective)[1] [mg BIT/kg dry sediment]		
	Percent Survival	Mean Individual Ash-Free Dry Weight (mg)
Negative control	100	1.54
Acetone control	99	1.76
6.3	100	1.54
13	100	1.95
25	100	1.56
50	100	1.63
100	100	1.30[2]

[1] specify, if TS concentrations were nominal or measured
[2]There was a statistically significant difference ($p < 0.05$) from the solvent control using Dunnett's test.

Table A7.4.3.5.1.a/01-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
10 d [mg BIT/kg dry sediment]	> 100 (n)	Not applicable	50 (n)	Not applicable

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.4.3.5.1.a/01-8: Validity criteria

	fulfilled	Not fullfilled
Mortality of control animals <10%	yes	
Concentration of test substance ≥80% of initial concentration during test	yes	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.4.3.5.1a	**EFFECTS ON SEDIMENT DWELLING ORGANISMS**
Annex Point IIIA, XIII.3.4	*Chironomus riparius*

			Officialuse only
		1 REFERENCE	
	1.1 Reference	**A7.4.3.5.1a/02** [redacted] (2007) 1,2-Benzisothiazolin-3-one: A prolonged sediment toxicity test with *Chironomus riparius* using spiked sediment, [redacted] (March 6, 2007), Unpublished.	
	1.2 Data protection	Yes	
1.2.4	Data owner	[redacted]	
1.2.5	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.6	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD Guideline 218	
	2.2 GLP	Yes	
	2.3 Deviations	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant	Not applicable	

Section A7 Subsection A7.4.3.5.1a Annex Point IIIA, XIII.3.4	Ecotoxicological Profile Including Environmental Fate and Behaviour EFFECTS ON SEDIMENT DWELLING ORGANISMS *Chironomus riparius*	

	properties		
3.1.6	Method of analysis	High performance liquid chromatography with UV detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see Table A7.4.3.5.1.a/02-1	
3.3	**Reference substance**	No	
3.4	**Testing procedure**		
3.4.1	Dilution water, Test sediment	see Table A7.4.3.5.1.a/02-2	
3.4.2	Test organisms	see Table A7.4.3.5.1.a/02-3	
3.4.3	Test system	see Table A7.4.3.5.1.a/02-4	**X**
3.4.4	Test conditions	see Table A7.4.3.5.1.a/02-5	**X**
3.4.5	Duration of the test	28 days	
3.4.6	Test parameter	Development rates, development times, emergence rates and toal number of adults emerged	
3.4.7	Sampling	overlying pore water, pore water and sediment samples.	
3.4.8	Monitoring of TS concentration	test initiation, day 7, and test termination	
3.4.9	Statistics	The 28-day EC_{50} was calculated using TOXSTAT version 3.5 using the mortality data at the end of the study (West, Inc. and D.D. Gulley. TOXSTAT version 3.5. Copyright 1996. Western Ecosystems Technology, Inc., Cheyenne, Wyoming, USA)The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analyses of the mean development times, emergence rates and development rates.The emergence rate and development rate were calculated for each replicate of each control and treatment group using SAS System for Windows version 8.2 (The SAS System for Windows. 1999-2001. Release 8.2 (TS2M0). SAS Institute, Inc., Cary, North Carolina, USA). The data were analyzed using an	

Section A7 **Subsection A7.4.3.5.1a** **Annex Point IIIA, XIII.3.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON SEDIMENT DWELLING ORGANISMS** *Chironomus riparius*

appropriate t-test to determine any statistical differences between solvent and negative control groups. The percent survival data were analyzed using a Bonferroni's t-test to identify those treatment levels that were statistically different ($p<0.05$) from the pooled control group (D.J. Finney. 1971. Statistical Methods in Biological Assay. Second edition. Griffin Press, London)(W.R. Thompson. 1947. Bacteriological Reviews. Volume II, No. 2, pp. 115-145). A Chi-square test was performed to check normality and the homogeneity of variance was checked using the Levene's test.

4 RESULTS

4.1 Limit Test — Not performed

4.2 Results test substance

4.2.1 Initial concentrations of test substance — 6.3, 13, 25, 50, 100 mg BIT/kg

4.2.2 Actual concentrations of test substance

Measured BIT concentration in sediment samples:

Nominal	***Measured Day 0***	***Measured Day 7***	***Measured Day 28***
Negative control	< LOQ	< LOQ	< LOQ
Solvent control	< LOQ	< LOQ	< LOQ
6.3 mg BIT/kg	5.01	< LOQ	< LOQ
13 mg BIT/kg	5.00	< LOQ	< LOQ
25 mg BIT/kg	11.7	1.48	< LOQ
50 mg BIT/kg	24.5	5.42	< LOQ
100 mg BIT/kg	48.5	11.2	2.36

Measured BIT concentration in overlying pore water samples:

Nominal	**Measured**	**Measured**	**Measured**

Section A7

Subsection A7.4.3.5.1a

Annex Point IIIA, XIII.3.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

EFFECTS ON SEDIMENT DWELLING ORGANISMS

Chironomus riparius

	Day 0	Day 7	Day 28
Negative control	<LOQ	<LOQ	<LOQ
Solvent control	<LOQ	<LOQ	<LOQ
6.3 mg BIT/kg	0.565	<LOQ	<LOQ
13 mg BIT/kg	1.28	0.336	<LOQ
25 mg BIT/kg	2.32	0.413	<LOQ
50 mg BIT/kg	5.13	4.32	0.152
100 mg BIT/kg	9.88	6.59	3.74

Measured BIT concentration in pore water samples:

Nominal	Measured Day 0	Measured Day 7	Measured Day 28
Negative control	<LOQ	<LOQ	<LOQ
Solvent control	<LOQ	<LOQ	<LOQ
6.3 mg BIT/kg	8.59	0.248	<LOQ
13 mg BIT/kg	12.7	1.13	<LOQ
25 mg BIT/kg	40.5	4.80	0.251
50 mg BIT/kg	59.6	17.3	0.613
100 mg BIT/kg	111	28.1	4.56

LOQ, limit of quantitation = 0.100 mg BIT/L

4.2.3	Effect data	see Table A7.4.3.5.1.a/02-6 and see Table A7.4.3.5.1.a/02-7 Percent mortality at test termination was 10, 7.5, 6.3, 13, 15, 54 and 60 in the negative control, acetone control, 6.3, 13, 25, 50 and 100 mg BIT/kg treatment groups, respectively.
4.2.4	Concentration /	Not described in report

Section A7 **Subsection A7.4.3.5.1a** **Annex Point IIIA, XIII.3.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON SEDIMENT DWELLING ORGANISMS** *Chironomus riparius*	

	response curve		
4.2.5	Other effects	The organisms generally appeared normal and healthy throughout the study. During the study there were a few observations of organisms on the surface of the sediment in all treatment groups and controls. There were also a few observations of organisms swimming in the water column and climbing the walls of the test chamber. These observations were few in number and were not treatment related.	
4.3	**Results of controls**	see Table A7.4.3.5.1.a/02-6	
4.4	**Test with reference substance**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD Guideline 218, Chronic toxicity to sediment dwelling organisms with analytical confirmation of BIT concentrations. Midges were exposed to BIT concentrations for 28 days under static test conditions. Observations of mortality and abnormal behavior were made daily during the test. The total number of adults emerged at the end of the test period was recorded. Sediment samples were fortified with stock solution of BIT prepared in acetone.	
5.2	**Results and discussion**	The overlying water appeared slightly tan and had a cloudy appearance in all test compartments at test initation and termination. The organisms generally appeared normal and healthy throughout the study. Percent mortality at test termination was 10, 7.5, 6.3, 13, 15, 54 and 60 in the negative control, acetone control, 6.3, 13, 25, 50 and 100 mg BIT/kg treatment groups, respectively. There were treatment related effects observed on development times in the 100 mg BIT/kg group and on mean emergence rates and development rates in the 50 and 100 mg BIT/kg treatment groups. Mean development time was 19.3, 21.5, 19.6, 19.6, 20.8, 22.7 and 25.1 days in the negative control, acetone control, 6.3, 13, 25, 50 and 100 mg BIT/kg treatment groups, respectively. The NOEC and LOEC for development time were based in the 100 mg BIT/kg values.	
	LOEC	100 mg BIT/kg, development time 50 mg BIT/kg, emergence rate and development rate	
	NOEC	50 mg BIT/kg, development time 25 mg BIT/kg, emergence rate and development rate	
	EC_{50}	52 mg BIT/kg (95% confidence interval of 40 – 95 mg BIT/kg), based	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.4.3.5.1a	**EFFECTS ON SEDIMENT DWELLING ORGANISMS**
Annex Point IIIA, XIII.3.4	*Chironomus riparius*

		on percent survival	
5.3	**Conclusion**		
5.3.3	Reliability	(1), reliable without restriction	
5.3.4	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*December 2010*
Materials and Methods	*Applicant's version is adopted with the following remark:* • *3.4.4: The light intensity used in the study is lower than those recommended by the OECD (338 lux at water surface vs. 500 to 1000lux).* • *According to the OECD 218 guidance, effect concentrations should be based on measured sediment concentrations at the beginning of the test.*
Results and discussion	*Applicant's version is adopted.* *The final effect concentrations based on measurements are resulted as follows:* *LOEC based on development time = 48.5 mg/kg* *LOEC based on emergence rate and development time = 24.5 mg/kg* *NOEC based on development time = 24.5 mg/kg* *NOEC based on emergence rate and development time = 11.7 mg/kg* *EC_{50}= 32.79 mg/kg (19.39-55.46 mg/kg)*
Conclusion	*Applicant's version is adopted.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.4.3.5.1.a/02-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	yes
Vehicle	Acetone
Concentration of vehicle	Not applicable
Vehicle control performed	Yes
Other procedures	The BIT primary stock solution was prepared by dissolving BIT in acetone at a nominal concentration of 10.0 mg BIT/mL.

Table A7.4.3.5.1.a/02-2: Dilution water

Criteria	Details
Source	Well freshwater, 40 meters deep
Alkalinity	178 – 182 mg/L as $CaCO_3$
Hardness	136 mg/L as $CaCO_3$
pH	8.0 – 8.2
Ca / Mg ratio	Not described
Na / K ratio	Not described
Dissolved Oxygen content	Not described
Conductance	300-320 µmhos/cm
Holding water different from dilution water	No

Table A7.4.3.5.1.a/02-3: Test organisms

Criteria	Details
Strain	midge larvae (*Chironomus riparius*)
Source	[redacted]
Age	1-4 days
Breeding method	Not described
Kind of food	Hartz pet rabbit food
Amount of food	10-30 mg
Feeding frequency	Approximately 3 times per week during the test. Organisms were not fed on day 27 due to the presence of fungal growth in the controls.
Pretreatment	Egg masses were held for four days prior to the start of the test at approximately the same temperature and water source as used during the test.
Feeding of animals during test	

Table A7.4.3.5.1.a/02-4: Test system

Criteria	Details
Renewal of test solution	No, static toxicity study
Volume of test vessels	Quart jars containing 2 cm of sediment and 600 mL of overlying water
Volume/animal	30 mL/midge
Number of animals/vessel	20 midges
Number of vessels/ concentration	4 containing midges and 3 for analytical sampling
Sediment	< 1% humic acid and dolomite, 5% *alpha*-cellulose, 14% silt and clay (Kaolin clay) and 80% industrial quartz sand
Test performed in closed vessels due to significant volatility of TS	Loose plastic covers were placed over each test chamber during the test

Table A7.4.3.5.1.a/02-5: Test conditions

Criteria	Details
Test temperature	20.4 – 21.0 ºC
Dissolved oxygen	≥ 5.9 mg/L (66 % of saturation)
pH	8.0 – 8.4
Adjustment of pH	Not described
Total hardness	136 mg/L as $CaCO_3$
Ammonia	Not described
Aeration of dilution water	Aeration was applied to each test chamber through a glass pipette that extended no closer than 2 cm from the surface of the sediment
Quality/Intensity of irradiation	Fluorescent tubes that emitted wavelengths similar to natural sunlight. Light intensity = 338 lux at water surface
Photoperiod	16 hours light and 8 hours darkness with 30-minute transition perion of low light intensity

Table A7.4.3.5.1.a/02-6: Effect and Mortality data

Test-Substance Concentration (nominal)[1] [mg BIT/kg dry sediment]	Percent emergence	Percent mortality	Mean development time (days)	Mean emergence rate	Mean development rate
Negative control	93	10	19.3	0.93	0.0540
Acetone control	93	7.5	21.5	0.93	0.0486
6.3	96	6.3	19.6	0.96	0.0532
13	91	13	19.6	0.91	0.0531
25	85	15	20.8	0.85	0.0498
50	58	54	22.7	0.58*	0.0453*
100	61	60	25.1	0.61*	0.0409*

[1] specify, if TS concentrations were nominal or measured
* Statistically significant ($p<0.05$) differences from the pooled control using Dunnett's test

Table A7.4.3.5.1.a/02-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
28 d [mg BIT/kg dry sediment]	52 (n)	40 – 95 (n)	25 (n)	Not applicable

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.4.3.5.1.a/02-8: Validity criteria

	fulfilled	Not fullfilled
Mortality of control animals <10%	Yes	
Concentration of test substance ≥80% of initial concentration during test	yes	

Section A7 **Subsection A7.4.3.5.2** **Annex Point IIIA 13.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AQUATIC PLANT TOXICITY**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X].**	
Detailed justification:	Considering the environmental properties of BIT (not persistent, not accumulating, rapidly photolytically degradable, rapidly biodegraded), and the use pattern for BIT in the product type in question, which predicts low direct exposure to the aquatic and terrestrial environment, a long term exposure of the aquatic environment to high concentration of BIT is not expected. The environmental risk assessment included in Document II does not indicate a risk for the aquatic environment. As a consequence, a test on aquatic plants is not considered necessary.	
Undertaking of intended data submission [ ]	No studies are planned.	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	*January 2010*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version*	
Remarks		

Section A7
Subsection A7.5.1.1 (01)
Annex Point IIA7.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)

			Official use only
		1 REFERENCE	
	1.1 Reference	**A7.5.1.1/01** [redacted] (2007) 1,2-Benzisothiazolin-3-one: Soil microorganisms: carbon transformation test; [redacted] Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	5.1 Guideline study	Yes, OECD 217	
	5.2 GLP	Yes	
	5.3 Deviations	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	High performance liquid chromatography (HPLC)	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.5.1.1 (01) **INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)**

Annex Point IIA7.4

3.2	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	Not applicable	
3.3	**Testing procedure**		
3.3.1	Soil sample / inoculum / test organism	see Table A7.5.1.1/01-1, see Table A7.5.1.1/01-2	
3.3.2	Test system	see Table A7.5.1.1/01-3	
3.3.3	Application of TS	see Table A7.5.1.1/01-4	
3.3.4	Test conditions	see Table A7.5.1.1/01-5	
3.3.5	Test parameter	Glucose-induced respiration	
3.3.6	Analytical parameter	CO_2	
3.3.7	Duration of the test	28 days	
3.3.8	Sampling	days 0, 7 and 28 for respiration	
3.3.9	Monitoring of TS concentration	No	
3.3.10	Controls	soil without test substance	
3.3.11	Statistics	The respiration rates were statistically analyzed using ANOVA and Bonferroni t-Test or Dunnett's test to determine the statistically significant differences from untreated controls at each sampling interval.	
		4 RESULTS	
4.1	**Range finding test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Effect data	Not applicable	
4.2	**Results test**		

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.5.1.1 (01) **INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)**

Annex Point IIA7.4

	substance		
4.2.1	Initial concentrations of test substance	0 (control), 10.7, 28.7, 100, 317 and 1000 mg BIT/kg soil	**X**
4.2.2	Actual concentrations of test substance	Not applicable	
4.2.3	Growth curves	Not applicable	
4.2.4	Cell concentration data	Not applicable	
4.2.5	Concentration/ response curve	see Figure A7.5.1.1/01-1	
4.2.6	Effect data	At the start of the test, respiration rates ranged from 17.3 to 23.3 mg CO_2 per kg dry soil per hour.	
4.2.7	Other observed effects	none	
4.3	**Results of controls**	see Table A7.5.1.1/01-6	**X**
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 217, Effects on soil microflora respiration transformation. One type of soil, a sandy loam, was used to prepare eighteen individual test chambers with 400 grams of dry soil. Soil moisture contents were adjusted to 22.7% water or 50% of the water holding capacity and acclimated in the dark at approximately 20ºC for 28 days. Three replicates each were treated with BIT at calculated concentrations of 0, 10.7, 28.7, 100, 317 and 1000 mg a.i./kg. Soil samples were collected from each test chamber on Day 0, 7 and 28 for analyses of carbon dioxide production rates.	
5.2	**Results and discussion**	The long-term effects of BIT on carbon tramsformation activity of soil microorganisms were minimal. After 28 days of exposure, the	

Section A7 Subsection A7.5.1.1 (01) Annex Point IIA7.4	Ecotoxicological Profile Including Environmental Fate and Behaviour INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)	
	mean CO_2 production rates were 51% and 44% greater than the untreated controls at the two highest test concentrations. No significant adverse effects were observed.	
5.2.1 EC_{10}	>1000 mg BIT/kg	
5.2.2 EC_{25}	>1000 mg BIT/kg	
5.2.3 EC_{50}	>1000 mg BIT/kg	
5.3 Conclusion	The long term effects of BIT on carbon transformation activity of soil microrganisms were minimal.	
5.3.1 Reliability	(1), reliable without restriction	
5.3.2 Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	***March 2015.***
Materials and Methods	*Applicant's version is accepted with the following remarks:* ▪ *The following deviations were noted:* o *Variation among the controls on day 28 was not within the acceptable range (±15%). While two of the controls showed very similar results for its respiration rate (9.8 and 9.9 CO_2 mg/kg), the variability among the control results is mainly due to the respiration rate value of one single control (14.3 CO_2 mg/kg).* o *Carbon content of microbial biomass is not specified.* ▪ *3.3. According to OECD 217, if the soil was stored, pre-incubation is recommended for a period between 2 and 28 days. For this test, soils were incubated only for one day prior the test.* ▪ *Application of the test substance was made by direct addition to the soils. Normally, the test substance is applied using a carrier.* ▪ *3.3.9. Test substance concentration was not monitored. Therefore, there is no evidence of the actual concentration of BIT during the test.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.1 (01) INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)

Annex Point IIA7.4

	▪
Results and discussion	*Applicant's version is accepted with the following remarks:* ▪ *4.1. Applicant should have performed a preliminary range-finding test, in order to determine the appropriate concentrations of the definitive test, including the EC50 within the range of concentrations tested.* ▪ *4.2. There is a deviation: According to test report, on day 0 comparisons between treatments and controls were not possible due to missing replicates.* *Data provided in test report correspond to calculated CO_2 production rates (Annex V of Doc. IVA), calculated from raw data. Test report should include the raw data (decreases in pressure) used for these calculations.* ***"Table A7.5.1.1/01-6: Respiration rates",** second column title, should read "Measured (mg CO_2/kg dry soil/hour)" instead of "Measured (mg O_2/kg dry soil/hour)".*
Conclusion	*The test was considered valid. According to "**Table A7.5.1.1/01-6: Respiration rates"** and considering the increase in the respiration rates as an effect, the NOEC obtained is 100 mg/kg.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*Although variability among the controls on day 28 was not within the acceptable range (±15%), variability among control replicates at previous intervals and of all other treatment groups was acceptable.*

Table A7.5.1.1/01-1: Microbial sample / Inoculum

Criteria	Details
Nature	loamy sand soil
Sampling site:	Grand Forks County, North Dakota
Geographical reference on the sampling site	Coordinates N 47º 48.166 – W 97º 37.264
Data on the history of the site	Tree farm
Use pattern	Tree farm and no pesticides or fertilizers were applied in the previous year
Depth of sampling [cm]	Top 10-20 cm and sieved to 2 mm
Sand / Silt / Clay content [particle size distribution]	66% sand, 16% silt and 18% clay
pH	7.1
Organic carbon content [% dry weight]	1.4%
Nitrogen content [mg N/100 g]	Not described in report
Maximum water holding capacity [g/100 g dry	45.4%

soil]	
Initial microbial biomass	330 µg/g
Reference of methods	Soil content: USDA Textural Class hydrometer method Microbial Biomass Carbon: Fumigation and Extraction Method by – Vance E.D. (1987) An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. Vol. 19, No. 6, pp.703-707.
Collection / storage of samples	The soil was transported to the laboratory and stored at refrigerated conditions for 80 days then transferred to a large plastic tray, covered with aluminium foil, and placed in a temperature-controlled room to incubate in the dark under aerobic conditions at approximately 20 ºC.
Preparation of inoculum for exposure	Not applicable
Pretreatment	Not applicable

Table A7.5.1.1/01-2: Test organism (*if applicable*)

Criteria	Details
Species	Not applicable
Strain	Not applicable
Source	Not applicable
Sampling site	Not applicable
Laboratory culture	Not applicable
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Not applicable
Pretreatment	Not applicable
Initial cell concentration	Not applicable

Table A7.5.1.1/01-3: Test system

Criteria	Details
Culturing apparatus	11 x 17 inch pyrex glass baking dishes containing 400 grams of dry soil
Number of vessels / concentration	3
Aeration device	Plastic lids had holes drilled into them to allow air circulation
Measuring equipment	[redacted] included plastic cups filled with 40 mL of 1.5 N KOH solution to absorb CO_2 headspace gases.
Test performed in closed vessels	Plastic lids on the glass baking dishes

Table A7.5.1.1/01-4: Application of test substance

Criteria	Details
Application procedure	TS was applied to the soil by direct weight addition in the test chambers
Carrier	Not applicable
Concentration of liquid carrier [% v/v]	Not applicable
Liquid carrier control	Not applicable
Other procedures	Not applicable

Table A7.5.1.1/01-5: Test conditions

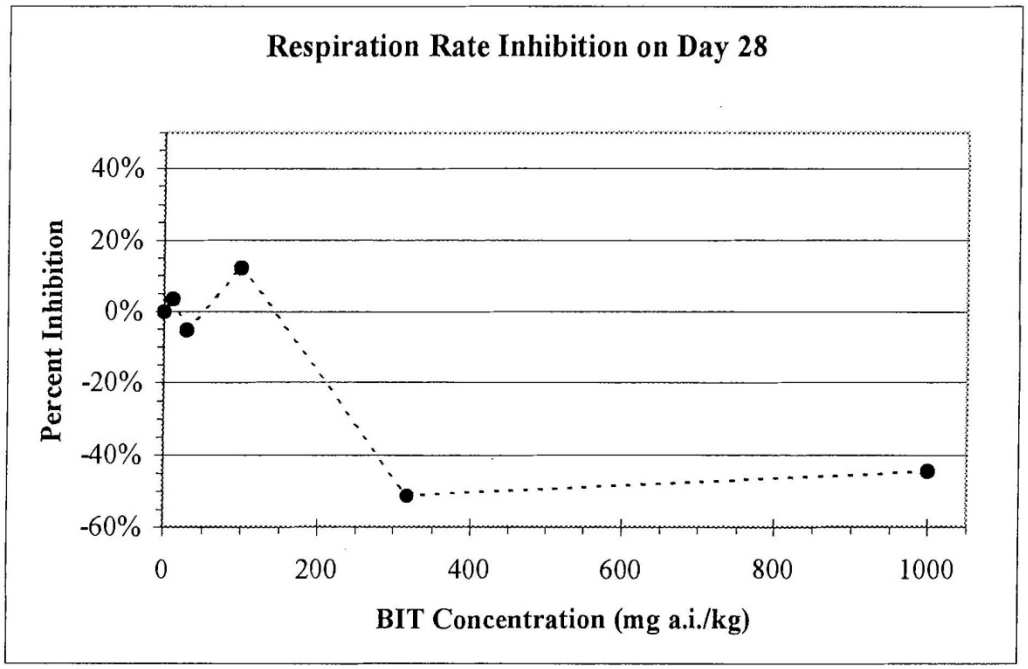
Criteria	Details
Organic substrate	Not applicable
Incubation temperature	19.2 to 22.2 °C
Soil moisture	maintained at 50% of maximum water holding capacity (43.0% to 51.8%)
Method of soil incubation	Individual sub samples
Aeration	Plastic lids had holes drilled into them to allow air circulation

Table A7.5.1.1/01-6: Respiration rates

Test Substance Concentration (nominal) [mg BIT/kg dry soil]	Measured ($mg O_2$/kg dry soil/hour)			% difference to control		
	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28
0 (control)	17.3	11.0	11.3	--	--	--
10.7	18.3	14.3	10.9	106	130	96
28.7	18.8	11.9	11.9	109	108	105
100	23.3	12.9	10.0	135	117	88
317	20.2	10.5	17.1	117	95	151
1000	20.2	10.5	16.4	117	95	145

-- not applicable

Figure A7.5.1.1/01-1: Glucose induced short term respiration


Respiration Rate Inhibition on Day 28
Percent Inhibition
40%
20%
0%
-20%
-40%
-60%
0
200
400
600
800
1000
BIT Concentration (mg a.i./kg)

Section A7
Subsection A7.5.1.1 (02)
Annex Point IIA7.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)

		1 REFERENCE	Official use only
	1.1 Reference	A7.5.1.1/02 [redacted] (2007) 1,2-Benzisothiazolin-3-one: Soil microorganisms nitrogen transformation test; [redacted] Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD 216	
	2.2 GLP	Yes	**X**
	2.3 Deviations	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	High performance liquid chromatography (HPLC)	
3.2	**Reference substance**	Nitrification Inhibitor [redacted]	

Section A7 **Subsection A7.5.1.1 (02)** **Annex Point IIA7.4**		**Ecotoxicological Profile Including Environmental Fate and Behaviour** **INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)**	
		[redacted]	
3.2.1	Method of analysis for reference substance	Not described in report	
3.3	**Testing procedure**		
3.3.1	Soil sample / inoculum / test organism	see Table A7.5.1.1/02-1, see Table A7.5.1.1/02-2	
3.3.2	Test system	see Table A7.5.1.1/02-3	
3.3.3	Application of TS	see Table A7.5.1.1/02-4	
3.3.4	Test conditions	see Table A7.5.1.1/02-5	**X**
3.3.5	Test parameter	Nitrogen transformation by soil microorganisms	
3.3.6	Analytical parameter	Nitrite, nitrate and ammonia measurements	
3.3.7	Duration of the test	28 days	
3.3.8	Sampling	days 0, 7 and 28	
3.3.9	Monitoring of TS concentration	No	
3.3.10	Controls	control without test substance	
3.3.11	Statistics	The mean concentrations of ammonia, nitrite and nitrate were calculated for each test chamber at each sampling interval and each treatment mean was calculated from the three replicates. The mean concentrations were compared to appropriate controls and percent inhibition values were calculated. The mean concentrations were statistically analyzed using ANOVA Dunnett's Test and Tukey Method of Multiple Comparisons to determine statistically significant differences.	
		4 RESULTS	
4.1	**Range finding test**		
4.1.1	Concentration	Not described in report	**X**
4.1.2	Effect data	1000 mg BIT/kg was selected as the test concentration based on the	**X**

Section A7 Subsection A7.5.1.1 (02) Annex Point IIA7.4	Ecotoxicological Profile Including Environmental Fate and Behaviour INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)	
	results from the range-finding test	
4.2 Results test substance		
4.2.1 Initial concentrations of test substance	1000 mg BIT/kg, nominal	**X**
4.2.2 Actual concentrations of test substance	Not applicable	
4.2.3 Growth curves	Not applicable	
4.2.4 Cell concentration data	Not applicable	
4.2.5 Concentration/ response curve	See Figure 7.5.1.1/02-1	
4.2.6 Effect data	see Tables A7.5.1.1/02-6	
4.2.7 Other observed effects	see Tables A7.5.1.1/02-6	
4.3 Results of controls	see Tables A7.5.1.1/02-6	**X**
4.4 Test with reference substance	Performed: Nitrification Inhibitor Formula 2533, Lot Number A6251 contained 2-chloro-6-(trichloromethyl)pyridine coated on a sodium sulfate substrate	
4.4.1 Concentrations	250 mg/kg, nominal	
4.4.2 Results	Nitrification Inhibitor had higher concentrations of ammonia compared with controls but less than BIT; Day 0 = 12.5 mg NH_4^+/kg, Day 7 = 16.0 mg NH_4^+/kg and Day 28 = 5.8 mg NH_4^+/kg. Nitrite concentrations in the Nitrification Inhibitor treated soils ranged from 4.8 to 6.9 mg NO_2^-/kg on Day 0 and were below the LOQ on Days 7 and 28. Nitrate concentrations in the Nitrification Inhibitor treated soils were 69.4 mg NO_3^-/kg on Day 0, 96.9 mg NO_3^-/kg on Day 7, and 166.5 mg NO_3^-/kg on Day 28.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	OECD 216 , Effects on soil microflora ammonia, nitrite and nitrate transformation.	
5.2 Results and	Ammonia: At test day 0, concentrations of ammonia in all alfalfa-amended soils ranged from 11.0 to 18.9 mg NH_4^+/kg and	

Section A7 Subsection A7.5.1.1 (02) Annex Point IIA7.4	Ecotoxicological Profile Including Environmental Fate and Behaviour INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)	
discussion	concentrations in non-amended soils ranged from 0.3 to 1.2 mg NH_4^+/kg. On days 7 and 28, the soils treated with BIT had significantly higher levels of ammonia than the controls in both amended and non-amended soils. Nitrite: At test day 0, concentrations of nitrite in alfalfa-amended controls ranged from 8.2 to 8.4 mg NO_2^-/kg.On days 7 and 28, none of the samples contained measureable amounts of nitrite. The limit of quantitation (LOQ) for nitrite was approximately 3 mg NO_2^-/kg. Nitrate: At test day 0, concentrations of nitrate in all samples ranged from 55.0 to 92.0 mg NO_3^-/kg. There were no statisitically significant treatment related differences. At 1000 mg/kg, BIT transiently inhibited nitrate formation in soil on day 7 as evidenced by increased ammonia concentrations in both amended and non-amended soils and a significant decrease in nitrate concentration in alfalfa-amended soil. The non-amended soils did not show a significant decrease in nitrate concentration. The soil microrganisms recovered by day 28. In amended soils, the nitrate concentration was much less and nitrate concentrations were not statistically significant from the amended controls.	
5.2.1 EC_{10}	alfalfa-amended soil = 833 mg BIT/kg non-amended soil >1000 mg BIT/kg	
5.2.2 EC_{25}	>1000 mg BIT/kg	
5.2.3 EC_{50}	>1000 mg BIT/kg	
5.3 Conclusion	The long term effects of 1,2-Benzisothiazolin-3-one on nitrogen transformation activity of soil microorganisms were minimal. After 28 days of exposure, the mean nitrate concentrations in alfalfa-amended and non-amended soils treated at 1000 mg BIT/kg were 12% and 2% less than the respective untreated controls. The EC_{10} in alfalfa-amended soil was 833 mg BIT/kg and >1000 mg BIT/kg in non-amended soil. The EC_{25} and EC_{50} were estimated to be >1000 mg BIT/kg.	
5.3.1 Reliability	(1), reliable without restriction	
5.3.2 Deficiencies	No	

Evaluation by Competent Authorities	
EVALUATION BY RAPPORTEURMEMBERSTATE	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.1 (02) INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)

Annex Point IIA7.4

Date	*March 2015.*
Materials and Methods	*Applicant's version is accepted with the following remark:* *3.3.4 The moisture contents of the soils ranged from 19.3% to 24.8% (41.9% to54.7% of WHC) throughout the test period, with one exception. The moisture content of test chamber 2 (untreated control) was calculated to be 17.7% (39.1%of WHC) on day 21, just prior to adding water.*
Results and discussion	*Applicant's version is accepted with the following remark:* • *4.1.1 and 4.1.2 On day 28 of the range- finding test, soils treated at nominal concentrations of 0.1, 1.0, 10, 100 and 1000 mg/kg exhibited inhibition of nitrate formation at 18%, 7%, 13%, 0%, and –127%, respectively, when compared with untreated control soil. The increase in nitrate formation at the 1000 mg/kg treatment indicated the test substance may have been used as a nitrogen source; therefore, the study was conducted using both amended and non-amended soils.* • *4.2.1 A geometric series of at least five concentrations should have been used. In addition, these concentrations should have covered the range to determine ECx values.* • *4.3 The variation among the alfalfa-amended control replicates on days 0, 14 and 28 were 28.4%, 11.4% and 6.1%, respectively.*

Measured Concentrations of Nitrate in Soil Samples

Treatment	Test Chamber ID (129E-119-)	Day 0 (mg NO_3^-/kg)	Day 7 (mg NO_3^-/kg)	Day 28 (mg NO_3^-/kg)
Control - Amended	1	55.0	138.0	173.7
	2	66.6	157.7	190.7
	3	90.9	171.9	190.3
	Means:	70.8	155.8	184.9
Reference Inhibitor - Amended	4	59.4	82.6	131.4
	5	67.6	95.5	174.1
	6	81.2	111.6	193.9
	Means:	69.4	96.6	166.5
BIT 1000 mg a.i./kg - Amended	7	71.5	75.9	169.2
	8	76.5	78.8	169.0
	9	92.0	95.0	151.5
	Means:	80.0	83.2	163.3
Control	10	64.2	65.8	81.5
	11	68.3	80.2	94.0
	12	79.3	76.5	100.9
	Means:	70.6	74.2	92.1
BIT 1000 mg a.i./kg	13	65.4	68.5	82.9
	14	70.4	69.5	78.7
	15	78.8	81.9	108.7
	Means:	71.5	73.3	90.1

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.1 (02) INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)

Annex Point IIA7.4

Conclusion	*Adopt applicant's version*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*The variation among the alfalfa-amended controls was greater than the acceptable range specified in the protocol (± 15% of the mean) on day 0; however, the variation among amended and non-amended controls was within the acceptable range at all other intervals during the study. The increased amount of variability at the start of the test did not affect the ability to determine differences between treatments and controls. This deviation had no impact on the results of the study.*

Table A7.5.1.1/02-1: Microbial sample / Inoculum

Criteria	Details
Nature	Sandy loam soil from [redacted]
Sampling site:	
Geographical reference on the sampling site	Grand Forks County, Northwood, North Dakota, USA, N 47º 48.166 and W 97º 37.264
Data on the history of the site	tree farm
Use pattern	Tree farm with no pesticides or fertilizers applied in the previous year
Depth of sampling [cm]	10-20 cm
Sand / Silt / Clay content [particle size distribution]	66% sand, 16% silt and 18% clay
pH	7.1
Organic carbon content [% dry weight]	1.4%
Nitrogen content [mg N/100 g]	Nitrite on day 0 < LOQ; Nitrate on Day 0 = 70.6 mg NO_3^-/kg
Maximum water holding capacity	Mean = 45.4%
Initial microbial biomass	330 µg/g
Reference of methods	Microbial biomass carbon based on a Fumigation and Extraction Method by: Vance, E.D. (1987) An Extraction Method for Measuring Soil Microbial Biomass C. Soil Biol. Biochem., Volume 19, No. 6, pp. 703-707.
Collection / storage of samples	Soil was collected from the top 10-20 cm and sieved to 2 mm.
Preparation of inoculum for exposure	Not applicable
Pretreatment	Not applicable

Table A7.5.1.1/02-2: Test organism (*if applicable*)

Criteria	Details
Species	Not applicable
Strain	Not applicable
Source	Not applicable
Sampling site	Not applicable
Laboratory culture	Not applicable
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Not applicable
Pretreatment	Not applicable
Initial cell concentration	Not applicable

Table A7.5.1.1/02-3: Test system

Criteria	Details
Culturing apparatus	9 x 9 inch Pyrex glass baking dishes with plastic lids each filled with 245 grams of moist soil (equivalent to 200 grams of dry soil)
Number of vessels / concentration	3
Aeration device	Not described
Measuring equipment	Ammonia and nitrogen: Hach DR/890 colorimeter Nitrate and Nitrite: Dionex DX-500 Ion Chromatography System
Test performed in closed vessels	Holes were drilled in the lids to allow circulation of air.

Table A7.5.1.1/02-4: Application of test substance

Criteria	Details
Application procedure	BIT was added to finely gound quartz sand.
Carrier	Finely ground quartz sand
Concentration of liquid carrier [% v/v]	Not applicable
Liquid carrier control	Not applicable
Other procedures	Not applicable

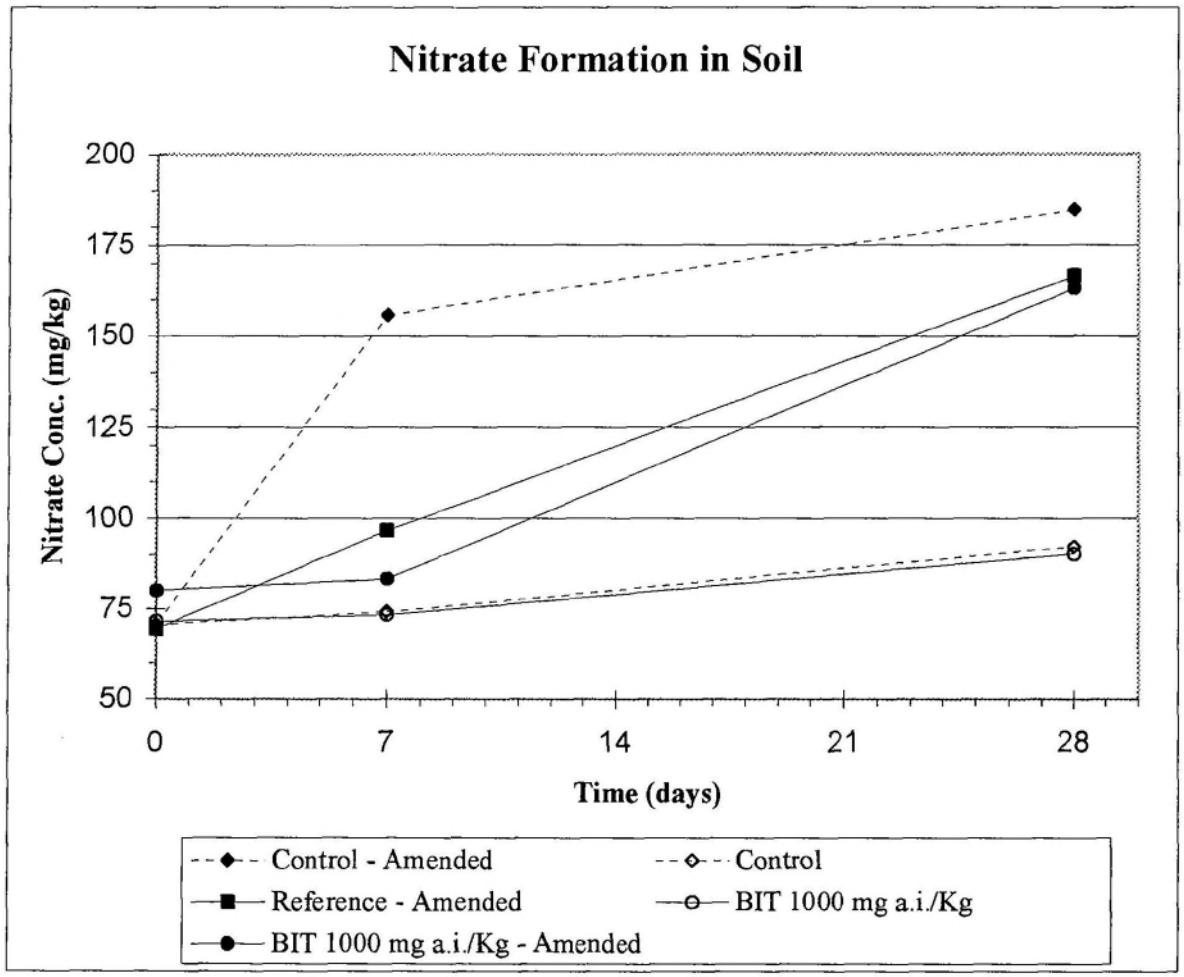
Table A7.5.1.1/02-5: Test conditions

Criteria	Details
Organic substrate	Nine of the test chambers were amended with 1.0 grams of dried, ground alfalfa
Incubation temperature	20 ºC
Soil moisture	The moisture contents of the soils were adjusted to 22.7% water or 50% of the water holding capacity.
Method of soil incubation	All test chambers were incubated under aerobic conditions in the dark at approximately 20 ºC for two days prior to test initiation.
Aeration	yes

Table A7.5.1.1/02-6: Ammonia

Test Substance Concentration (nominal) [mg BIT/kg soil]	Measured Ammonia (mg NH_4^+/kg dry soil/hour)			Measured Nitrate (mg NO_3^-/kg dry soil/day)		
	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28
0, control - amended	14.4	1.5	0.5	70.8	155.8	184.9
Reference Inhibitor - amended	12.5	16.0	5.8	69.4	96.6	166.5
1000 mg BIT/kg - amended	14.0	48.6	45.2	80.0	83.2	163.3
Control	0.7	0.6	0.0	70.6	74.2	92.1
1000 mg BIT/kg	0.5	13.3	25.3	71.5	73.3	90.1

Figure 7.5.1.1/02-1



Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

		1 REFERENCE	Officialuse only
	1.1 Reference	**A7.5.1.2/01** [redacted] (2006) 1,2-Benzisothiazolin-3-one: An acute toxicity study with the earthworm in an artificial soil substrate [redacted] (August 17, 2006), GLP, Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD Method 207	
	2.2 GLP	Yes	
	2.3 Deviations	No	
		3 METHOD	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	not applicable	
3.1.5	Further relevant properties	not applicable	
3.1.6	Method of analysis	High performance liquid chromatography (HPLC) with UV detector	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.5.1.2 **Earthworm, acute toxicity test**

Annex Point IIIA XIII 3.2

3.2	**Reference substance**	Yes, 2-chloracetamide, method of analysis not described.	**X**
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	see Table A7.5.1.2/01-1	
3.3.2	Application of the test substance	Test soil was prepared by mixing BIT with reverse osmosis water and adding it to artificial soil. Moisture content was approximately 35% by weight.	
3.3.3	Test organisms	see Table A7.5.1.2/01-2	
3.3.4	Test system	see Table A7.5.1.2/01-3	
3.3.5	Test conditions	see Table A7.5.1.2/01-4	
3.3.6	Test duration	14 days	
3.3.7	Test parameter	mortality and clinical signs	
3.3.8	Examination	Weight of worms was determined at the start and the end of the test. Time to burrow was observed at test initiation and on Day 7. On days 7 and 14, the contents of each test chamber were removed to determine the number of surviving earthworms.	
3.3.9	Monitoring of test substance concentration	No	
3.3.10	Statistics	The LC_{50}s and 95% confidence intervals were calculated using the Stephan computer program ([redacted]). The Day 7 LC_{50} value was calculated by nonlinear interpolation and the Day 14 LC_{50}value was calculated by the Probit method. Body weights and change in body weights were statistically compared with Dunnett's 2-Tailed Test of Means (α = 0.05) using SAS Version 8 (SAS Institute, Inc. 1999. SAS/STAT User's Guide, Version 8, Cary, North Carolina, USA)	
		4 RESULTS	
4.1	**Filter paper test**	Not performed	
4.2	**Soil test**		
4.2.1	Initial concentrations of	0 (negative control), 28.06, 56.13, 112.25, 224.5, 449 and 898 mg BIT/kg of soil on a dry weight basis	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.5.1.2 **Earthworm, acute toxicity test**

Annex Point IIIA XIII 3.2

	test substance		
4.2.2	Effect data (Mortality)	see Table A7.5.1.2/01-5	
4.2.3	Concentration / effect curve	No	**X**
4.2.4	Other effects	Not applicable	**X**
4.3	**Results of controls**		
4.3.1	Mortality	There were no mortalities in the negative control.	
4.3.2	Number/ percentage of earthworms showing adverse effects	All control worms were normal in appearance and behaviour throughout the test period.	
4.3.3	Nature of adverse effects	Not applicable	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	Chloroacetamide, concentrations not decribed	
4.4.2	Results	14-day LC_{50}: 24.5 mg a.i./kg dry soil with 95% confidence interval of 13 and 50 mg a.i./kg dry soil	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD Method 207, Acute toxicity to the earthworm	
5.2	**Results and discussion**	All control worms survived and were normal in appearance and behaviour throughout the test period. The earthworms showed a strong aversion to the test soils. On Day 0, worms in the negative control and the 28.06 mg BIT/kg treatment group burrowed within approximately ½ hour of being placed on the soil surface at test initiation. Worms in the 56.13 mg BIT/kg group were mostly burrowed at approximately one hour after test initiation. Worms in all of the other groups did not burrow and remained on the soil surface or on the sides of the test chamber above the soil surface. Worms in the 889 mg BIT/kg group were lethargic and some were dead ½ hour after test initiation. Body weights were not determined for the 224.5 mg BIT/kg and higher doses due to insufficient worms or no worms were availablefor final body weight comparisons in	

Section A7 Subsection A7.5.1.2 Annex Point IIIA XIII 3.2	Ecotoxicological Profile Including Environmental Fate and Behaviour Earthworm, acute toxicity test	
	these groups.	
5.2.1 NOEC	28.06 mg BIT/kg dry soil	
5.2.2 LC_0	7-day and 14-day: 28.06 mg BIT/kg dry soil	
5.2.3 LC_{50}	7-day: 278 mg BIT/kg dry soil 14-day: 114 mg BIT/kg dry soil	
5.2.4 LC_{100}	7-day: 449 mg BIT/kg dry soil 14-day: 449 mg BIT/kg dry soil	
5.3 Conclusion	see Table A7.5.1.2/01-7 and see Table A7.5.1.2/01-8	
5.3.1 Other Conclusions		
5.3.2 Reliability	(1), reliable without restriction	
5.3.3 Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*January 2011*
Materials and Methods	*Applicant's version is accepted with the following remark:* *3.2 At [redacted] reference toxicity tests with a reference toxicant, chloroacetamide, are conducted periodically to assess the sensitivity of the test species and test procedures. These studies are conducted under separate protocols, as independent studies. A summary of the results from the most current reference toxicity test is presented in this report.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

Results and discussion	*Applicant's version is accepted with the following remarks:* • *4.2.3: The concentration/effect curve must be included.* • *4.2.4 There were statistically significant effects on final body weight and the change in body weight at the 112.25 mg a.i./kg level when compared to the control group.* • *LC_{50} values should include the correspondent confidence limit intervals:* · *LC_{50}-7-day: 278 mg BIT/kg dry soil (224.5 - 449 mg BIT/kg dry soil)* *LC_{50}-14-day: 114 mg BIT/kg dry soil (98.1 - 132 mg BIT/kg dry soil)*
Conclusion	*Adopt applicant's version*
Reliability	*2*
Acceptability	*Acceptable.*
Remarks	

Table A7.5.1.2/01-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	Reverse osmosis water prepared at laboratory
Holding water different from dilution water	Not applicable
In case of the use of an organic solvent	
Dispersion	Yes, mixing for 20 minutes
Vehicle	Not applicable
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	Not applicable

Table A7.5.1.2/01-2: Test organisms

Criteria	Details
Species/strain	*Eisenia fetida*
Source of the initial stock	[redacted]
Culturing techniques	Not applicable
Age/weight	adult worms with clitella, 0.57 to 0.66 grams
Pre-treatment	24 hours prior to test initiation, worms were placed into artificial soil substrate adjusted to 35% by weight moisture content for the acclimation period.

Table A7.5.1.2/01-3: Test system

Criteria	Details
Artificial soil test substrate	Composition of the test substrate was 20% kaolin clay, 70% sand, 10% sphagnum peat. pH was adjusted to 5.9 by the addition of calcium carbonate. 35% moisture
Test mixture	Test soil was prepared by mixing BIT with reverse osmosis water and adding it to bulk artificial soil with 35% moisture content.
Size, volume and material of test container	One liter glass beakers covered with plastic wrap that was perforated for air exchange
Amount of artificial soil (kg)/ container	750 grams of prepared soil
Nominal levels of test concentrations	0 (negative control), 28.06, 56.13, 112.25, 224.5, 449 and 898 mg BIT/kg of soil on a dry weight basis
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	not described
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7.5.1.2/01-4: Test conditions

Criteria	Details
Test temperature	20 + 2 °C
Moisture content	Day 0: 33.8 to 34.8%, Day 14: 32.8 to 34.0%
pH	Day 0 = 7.0 to 7.4; Day 14 = 7.2 to 7.5
Adjustment of pH	Yes, pH was adjusted to 5.9 by the addition of calcium carbonate.
Light intensity / photoperiod	400-800 lux, 24 h light and 0 h dark
Relevant degradation products	Not applicable

Table A7.5.1.2/01-5: Mortality data

Test Substance Concentration (nominal)[1] [mg BIT/kg artificial soil]	Mortality			
	Number Dead or Missing		Percentage	
	7 d	14 d	7 d	14 d
0 (negative control)	0/40	0/40	0	0
28.06	0/40	0/40	0	0
56.13	7/40	8/40	17.5	20
112.25	7/40	13/40	17.5	33
224.5	8/40	37/40	20	93
449	40/40	40/40	100	100
898	40/40	40/40	100	100
Temperature [°C]	Day 0: 20.5-21.5	Day 14: 20.2-21.0		
pH	Day 0: 7.0-7.4	Day 14: 7.2-7.5		
Moisture content	Day 0: 33.8-34.8	Day 14: 32.8-34.0		

[1] specify, if TS concentrations were nominal or measured

Table A7.5.1.2/01-6: Number affected data

Test Substance Concentration (nominal)[1] [mg BIT/kg artificial soil]	Number Affected			
	Number affected 7 d	14 d	Percentage 7 d	14 d
0 (control)	0/40	0/40	0	0
28.06	0/40	0/40	0	0
56.13	2/40 not found	8/40 not found	5	20
112.25	5/40 not found	13 not found, 6 reduced reaction to mechanical stimuli	12.5	32.5 not found, 15 reduced reaction to mechanical stimuli
224.5	8/40 not found, 12 reduced reaction to mechanical stimuli, 8/40 thin	30/40 not found, 1 reduced reaction to mechanical stimuli, 2/40 thin	20 not found, 30 reduced reaction to mechanical stimuli, 20 thin	75 not found, 2.5 reduced reaction to mechanical stimuli, 5 thin
449	32/40 not found	40/40 not found	80	100
898	40/40 not found	40/40 not found	100	100

[1] specify, if TS concentrations were nominal or measured

Table A7.5.1.2/01-7: Effect data

	14 d [mg BIT/kg soil][1]	95 % C.I.
LC_0	28.06 (m)	Not described
LC_{50}	114 (m)	98.1 – 132 (m)
LC_{100}	449 mg BIT/kg dry soil	Not described

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.5.1.2/01-8: Validity criteria for acute earthworm test according to OECD 207

	fulfilled	Not fulfilled
Mortality of control animals < 10%	yes	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.5.1.3 **TERRESTRIAL PLANT TOXICITY**

Annex Point IIIA XIII 3.4

	Emergence-Day 7	***Emergence-Day 14***	***Emergence-Day 21***	***Survival-Day 21***	***Shoot Dry Weight***
Allium cepa	*7.38±1.06*	*9.00±0.76*	*9.00±0.76*	*8.75±1.28*	*0.185±0.0170*
Avena sativa	*9.50±0.53*	*9.50±0.53*	*9.50±0.53*	*9.50±0.53*	*2.25±0.303*
Brassica rapa	*9.38±0.74*	*9.50±0.76*	*9.50±0.76*	*9.38±0.74*	*4.87±0.839*
Cucumis sativa	*9.88±0.35*	*9.88±0.35*	*9.88±0.35*	*9.88±0.35*	*10.7±0.55*
Lactuca sativa	*9.00±1.07*	*9.13±0.99*	*9.25±1.04*	*9.25±1.04*	*1.12±0.285*
Lycoper-sicon esculentum	*8.13±0.99*	*8.50±0.76*	*8.50±0.76*	*8.25±1.04*	*2.69±0.351*

		1 REFERENCE	Official use only
	1.1 Reference	**A7.5.1.3/01** [redacted] (2006) 1,2-Benzisothiazolin-3-one: A toxicity test to determine the effects of the test substance on seedling emergence of six species of plants; [redacted] (December 13, 2006), GLP, Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD Proposal for Revision of Guideline 208	
	2.2 GLP	Yes	
	2.3 Deviations	No	
		3 METHOD	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	High performance liquid chromatography	
3.2	**Preparation of TS**	see Table A7.5.1.3/01-1	

	solution for poorly soluble or volatile test substances		
3.6.1	TS Concentrations	Nominal (mg BIT/kg dry soil): 0 (negative and solvent controls), 11.1, 33.3, 99.8, 299 and 898 mg for all species and Nominal (mg BIT/kg dry soil): 0 (negative and solvent controls), 0.411, 1.23, 3.70, 11.1 and 33.3 for lettuce (*L. sativa*) Day 0 measured BIT concentrations in stock solutions used to prepare the 11.1, 33.3, 99.8, 299 and 898 mg BIT/kg test soils were 102, 98, 95, 84 and 80% of nominal, respectively.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	see Table A7.5.1.3/01-2	
3.4.2	Test plants	see Table A7.5.1.3/01-3	
3.4.3	Test system	see Table A7.5.1.3/01-4	**X**
3.4.4	Test conditions	see Table A7.5.1.3/01-5	
3.4.5	Test duration	21 days	
3.4.6	Test parameter	seedling emergence, survival, growth (dry weight) and condition	
3.4.7	Sampling	Observations on days 7, 14 and 21 were made to document seedling emergence, i.e., visible plant tissue at the surface of the soil. Observations on day 21 were made to determine the condition of individual seedlings, i.e., necrosis, leaf wrinkle, chlorosis, plant lodging or plant stunting.	
3.4.8	Method of analysis of the plant material	Shoot dry weights were evaluated at test termination. Seedlings were clipped at soil level and the shoots of all living seedlings within a replicate were placed in a labelled bag. The shoots were then dried in an oven and the total dry weight of the replicate was determined.	
3.4.9	Quality control	Yes	
3.4.10	Statistics	Statistical analyses were used to evaluate effects of BIT application on seedling emergence, survival and dry shoot weight. Mean seedling emergence, survival and dry shoot weight of the control and BIT treatment groups were compared with a one-tailed Dunnett's t-test using the Dunnett option of the GLM (general linear model) procedure of SAS version 8 (SAS Institute, Inc. 1999, SAS/STAT User's Guide, version 8, Cary,	**X**

		North Carolina, USA). Dunnett;s test was used to establish the LOEC and NOEC by determining which treatment group differed significantly ($p < 0.05$) from the control group.	
		4 RESULTS	
4.1	**Results test substance**		
4.1.1	Applied initial concentration	Nominal (mg BIT/kg dry soil): 0 (negative and solvent controls), 11.1, 33.3, 99.8, 299 and 898 mg for all species and Nominal (mg BIT/kg dry soil): 0 (negative and solvent controls), 0.411, 1.23, 3.70, 11.1 and 33.3 for lettuce (*L. sativa*) were incorporated into the soil.	
4.1.2	Phytotoxicity rating	see Table A7.5.1.3/01-6	
4.1.3	Plant height	see Table A7.5.1.3/01-6	
4.1.4	Plant dry weights	see Table A7.5.1.3/01-6	
4.1.5	Root dry weights	Not applicable	
4.1.6	Root length	Not applicable	
4.1.7	Number of dead plants	see Table A7.5.1.3/01-6	
4.1.8	Effect data	see Table A7.5.1.3/01-6	
4.1.9	Concentration / response curve	See figures A7.5.1.3/01-1 onions, A7.5.1.3/01-2 oats, A7.5.1.3/01-3 turnips, A7.5.1.3/01-4 cucumber, A7.5.1.3/01-5 lettuce (initial test), A7.5.1.3/01-6 lettuce (final test) and A7.5.1.3/01-7 tomatoes	
4.1.10	Other effects	see Table A7.5.1.3/01-6	
4.1.11		The most sensitive parameter for all six species was dry weight. see Table A7.5.1.3/01-6	**X**
4.2	**Results of controls**		
4.2.1	Number/ percentage of plants showing adverse effects	No effects to onions, oats, turnips, cucumber, lettuce or tomatoes	**X**
4.2.2	Nature of adverse effects	Not applicable	
4.3	**Test with reference**	Not performed	

	substance		
4.3.1	Concentrations	Not applicable	
4.3.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	**OECD Proposal for Revision of Guideline 208 growth test in terrestrial plants with analytical confirmation of dosing solutions.**	
5.2	**Results and discussion**	Effects of soil incorporation of BIT were observed on seedling emergence, survival, growth and condition of the six plant species tested. The most sensitive parameter for all six species was dry weight with EC_{50} values ranging from 18.4 mg BIT/kg for lettuce to 166 mg BIT/kg for oat. The NOEC for tomato dry weight in this study was determined to be less than 11.1 mg BIT/kg, which was the lowest test concentration.	**X**
5.2.1	NOEC	NOEC for tomato dry weight < 11.1 mg BIT/kg dry soil, the lowest BIT concentration, See Table A7.5.1.3/01-7 for other plant species NOEC values.	
5.2.2	EC_{25}	See Table A7.5.1.3/01-7	
5.2.3	EC_{50}	See Table A7.5.1.3/01-7	
5.3	**Conclusion**		
5.3.1	Reliability	(1), reliable without restriction	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*November 2012*

Materials and Methods	*Applicant's version is accepted with the following remarks:* ▪ *3.4.3 Number of seeds used in the study is not optimal in the case of tomato (Lycopersicon esculentum) and cucumber (Cucumis sativa). According to OECD guidelines 208; for theses species one or two, instead of 10 seeds should have been used per container. However, since in all control samples, the validity criteria as stated in the guideline (e.g. seedling emergence, mean survival, exhibition of phytotoxic effects) are fulfilled, the higher number of seeds used for tomato and cucumber does not affect the validity of the study.* ▪ *3.4.4. Test conditions. The reported temperatures and relative humidity show a large variability throughout the test. However, in the control groups, the validity criteria with respect to emergence and survival are fulfilled, which indicates that the large temperature and humidity range did not affect the reliability of the results.*
Results and discussion	*Applicant's version is accepted with the following remarks:* ▪ *5.2. It is not easy to check the validity criteria concerning the control plant with the information provided in this document III. The following table show additional data concerning the negative control plant.* ▪ *No observed sign of toxicity in these negative controls.*

Conclusion	*Based on the results of this study, it can be concluded that 1,2-Benzisothiazol-3(2H)-one may affect the emergence, survival, growth and condition of the six plant species tested. The most sensitive parameter for all six species was dry weight. The lowest EC_{50} value was 18.4 mg/kg for lettuce (L. sativa) and the lowest NOEC was observed for lettuce (L. sativa) dry weight and was determined to be 3.7 mg/kg.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	*Key Study*

Table A7.5.1.3/01-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes, stirring and sonication
Vehicle	Yes, acetone
Concentration of vehicle	Not described
Vehicle control performed	Yes, acetone
Other procedures	Not applicable

Table A7.5.1.3/01-2: Dilution water

Criteria	Details
Source	Not applicable
Alkalinity / Salinity	Not applicable
Hardness	Not applicable
pH	Not applicable
Oxygen content	Not applicable
Conductance	Not applicable
Holding water different from dilution water	Not applicable

Table A7.5.1.3/01-3: Test plants

	Family	Species	Common name	Source (seed)
Monocotyledonae	Liliaceae	Allium cepa	Onion	[redacted]
	Poaceae	Avena sativa	Oat	[redacted]
Dicotyledonae	Brassicaceae	Brassica rapa	Turnip	[redacted]
	Cucurbitaceae	Cucumbis sativa	Cucumber	[redacted]
	Asteraceae	Lactuca sativa	Lettuce	[redacted]
	Solanaceae	Lycopersicon esculentum	Tomato	[redacted]

Table A7.5.1.3/01-4: Test system

Criteria	Details
Test type	greenhouse
Container type	Plastic pots (16 cm diameter by 12 cm deep)
Seed germination potential	provided by seed supplier
Identification of the plant species	provided by seed supplier
Number of replicates	4
Numbers of plants per replicate per dose	10 seeds per replicate
Date of planting	August 25, 2006 and October 12, 2006
Plant density	10 plants/pot
Date of test substance application	test substance was incorporated into the soil prior to seed planting
Height of plants at application	Not applicable
Date of phytotoxicity rating or harvest	7, 14, and 21 days after planting seeds
Dates of analysis	The test was terminated 21 days after seeds were

	planted.

Table A7.5.1.3/01-5: Test conditions

Criteria	Details
Test type	greenhouse
Method of application	soil incorporation
Application levels	Nominal (mg BIT/kg dry soil): 0 (negative and solvent controls), 11.1, 33.3, 99.8, 299 and 898 mg for all species and Nominal (mg BIT/kg dry soil): 0 (negative and solvent controls), 0.411, 1.23, 3.70, 11.1 and 33.3 for lettuce (L. sativa) were incorporated into the soil.
Dose rates	not applicable
Substrate characteristics	sandy loam soil consisting of 65% sand, 18% silt and 17% clay with an organic matter content of 2.2% (organic carbon 1.3%)
Watering of the plants	Seedlings were subirrigated
Temperature	18.88 to 37.76 ºC, initial test 17.20 to 30.53 ºC, repeated test with L. sativa
Thermoperiod	Not applicable
Light regime	6.6 to 16.6 moles photosynthetically active radiation, initial test 7.8 to 18.9 moles photosynthetically active radiation, repeated test with L. sativa
Relative humidity	28.22 to 91.60%, initial test 13.63 to 88.60%, repeated test with L. sativa
Wind volatility	Not applicable
Observation periods and duration of test	Observation periods: 7, 14 and 21 days: the number of emerged plants and condition of emerged plants. Duration: 21 days
Pest control	Seeds were not pretreated with insecticides, fungicides or repellants
Any other treatments and procedures	not applicable

Table A7.5.1.3/01-6:
***Allium cepa* (onion):**

Test Substance Concentration (nominal) [mg BIT/kg]	Number of Emerged Seedlings mean ± SD (% reduction) Day 7	Day 14	Day 21	Seedling Survival mean ± SD (% reduction)	Shoot Dry Weight mean ± SD (% reduction)
Pooled controls	7.38 ± 1.06	9.00 ± 0.76	9.00 ± 0.76	8.75 ± 1.28	0.132 ± 0.0372
11.1	7.25 ± 2.50 (2%)	8.25 ± 1.71 (8%)	8.50 ± 1.29 (6%)	8.25 ± 1.71 (6%)	0.152 ± 0.0340 (-15%)
33.3	5.75 ± 2.22 (22%)	7.75 ± 1.71 (14%)	7.75 ± 1.71 (14%)	7.25 ± 1.50 (17%)	0.106 ± 0.0364 (20%)
99.8	0.25 ± 0.50** (97%)	1.00 ± 0.82** (89%)	1.50 ± 1.29** (83%)	1.25 ± 1.26** (86%)	0.008 ± 0.0071** (94%)
299	0.00 ± 0.00** (100%)	2.00 ± 1.83** (78%)	2.50 ± 1.29** (72%)	1.50 ± 1.00** (83%)	0.005 ± 0.0022** (96%)
898	0.00 ± 0.00** (100%)	0.00 ± 0.00** (100%)	0.00 ± 0.00** (100%)	0.00 ± 0.00** (100%)	0.00 ± 0.0000** (100%)

* Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.05$)
** Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.01$)

***Avena sativa* (oat):**

Test Substance Concentration (nominal) [mg BIT/kg]	Number of Emerged Seedlings mean ± SD (% reduction) Day 7	Day 14	Day 21	Seedling Survival mean ± SD (% reduction)	Shoot Dry Weight mean ± SD (% reduction)
Pooled controls	9.50 ± 0.53	9.50 ± 0.53	9.50 ± 0.53	9.50 ± 0.53	2.25 ± 0.303
11.1	9.75 ± 0.50 (-3%)	10.00 ± 0.00 (-5%)	10.00 ± 0.00 (-5%)	10.00 ± 0.00 (-5%)	2.32 ± 0.893 (-3%)
33.3	9.75 ± 0.50 (-3%)	9.75 ± 0.50 (-3%)	9.75 ± 0.50 (-3%)	9.75 ± 0.50 (-3%)	2.37 ± 0.191 (-5%)
99.8	8.25 ± 1.50 (13%)	8.75 ± 1.50 (8%)	9.00 ± 1.15 (5%)	9.00 ± 1.15 (5%)	1.81 ± 0.089 (20%)
299	6.00 ± 1.63** (37%)	9.00 ± 0.82 (5%)	9.00 ± 0.82 (5%)	9.00 ± 0.82 (5%)	0.46 ± 0.185** (80%)
898	0.75 ± 0.96** (92%)	6.75 ± 0.96** (29%)	7.00 ± 0.82** (26%)	6.75 ± 0.96** (29%)	0.05 ± 0.032** (98%)

* Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.05$)
** Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.01$)

***Brassica rapa* (turnip):**

Test Substance Concentration (nominal) [mg BIT/kg]	Number of Emerged Seedlings mean ± SD (% reduction) Day 7	Day 14	Day 21	Seedling Survival mean ± SD (% reduction)	Shoot Dry Weight mean ± SD (% reduction)
Pooled controls	9.38 ± 0.74	9.50 ± 0.76	9.50 ± 0.76	9.38 ± 0.74	4.87 ± 0.839
11.1	9.25 ± 0.96 (1%)	9.25 ± 0.96 (3%)	9.25 ± 0.96 (3%)	9.25 ± 0.96 (1%)	4.73 ± 0.696 (3%)
33.3	9.00 ± 0.00 (4%)	9.50 ± 0.58 (0%)	9.50 ± 0.58 (0%)	9.50 ± 0.58 (-1%)	3.18 ± 0.870** (35%)
99.8	3.25 ± 1.71** (65%)	3.50 ± 1.29** (63%)	4.50 ± 2.08** (53%)	3.00 ± 2.16** (68%)	0.04 ± 0.048** (99%)
299	0.75 ± 0.96** (92%)	0.75 ± 0.96** (92%)	1.00 ± 0.82** (89%)	0.75 ± 0.96** (92%)	0.01 ± 0.009** (100%)
898	0.00 ± 0.00** (100%)	0.00 ± 0.00** (100%)	0.00 ± 0.00** (100%)	0.00 ± 0.00** (100%)	0.00 ± 0.000** (100%)

* Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.05$)
** Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.01$)

***Cucumbis sativa* (cucumber):**

Test Substance Concentration (nominal) [mg BIT/kg]	Number of Emerged Seedlings mean ± SD (% reduction) Day 7	Day 14	Day 21	Seedling Survival mean ± SD (% reduction)	Shoot Dry Weight mean ± SD (% reduction)
Pooled controls	9.88 ± 0.35	9.88 ± 0.35	9.88 ± 0.35	9.88 ± 0.35	10.7 ± 0.55
11.1	9.75 ± 0.50 (1%)	9.75 ± 0.50 (1%)	9.75 ± 0.50 (1%)	9.75 ± 0.50 (1%)	11.0 ± 0.69 (-2%)
33.3	10.00 ± 0.00 (-1%)	10.00 ± 0.00 (-1%)	10.00 ± 0.00 (-1%)	10.00 ± 0.00 (-1%)	9.9 ± 0.91* (8%)
99.8	8.00 ± 0.82** (19%)	8.75 ± 0.50 (11%)	9.00 ± 0.00 (9%)	8.75 ± 0.50** (11%)	2.6 ± 0.53** (76%)
299	5.00 ± 1.41** (49%)	7.50 ± 1.73** (24%)	7.50 ± 1.73** (24%)	4.25 ± 1.26** (57%)	0.2 ± 0.11** (98%)
898	0.75 ± 1.50** (92%)	3.25 ± 2.63** (67%)	3.25 ± 2.63** (67%)	0.00 ± 0.00** (100%)	0.00 ± 0.00** (100%)

* Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.05$)
** Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.01$)

***Lactuca sativa* (lettuce):**

Test Substance Concentration (nominal) [mg BIT/kg]	Number of Emerged Seedlings mean ± SD (% reduction) Day 7	Day 14	Day 21	Seedling Survival mean ± SD (% reduction)	Shoot Dry Weight mean ± SD (% reduction)
Pooled controls	9.00 ± 1.07	9.13 ± 0.99	9.25 ± 1.04	9.25 ± 1.04	1.12 ± 0.285
0.411	825 ± 2.06 (8%)	825 ± 2.06 (10%)	825 ± 2.06 (11%)	825 ± 2.06 (11%)	0.90 ± 0.335 (20%)
1.23	9.50 ± 1.00 (-6%)	9.50 ± 1.00 (-4%)	9.50 ± 1.00 (-3%)	9.50 ± 1.00 (-3%)	1.02 ± 0.202 (9%)
3.70	9.50 ± 0.58 (-6%)	9.50 ± 0.58 (-4%)	9.50 ± 0.58 (-3%)	9.50 ± 0.58 (-3%)	0.89 ± 0.088 (21%)
11.1	9.75 ± 0.50 (-8%)	9.75 ± 0.50 (-7%)	9.75 ± 0.50 (-5%)	9.75 ± 0.50 (-5%)	0.58 ± 0.135** (48%)
33.3	9.50 ± 1.00 (-6%)	9.50 ± 1.00 (-4%)	9.50 ± 1.00 (-3%)	9.50 ± 1.00 (-3%)	0.45 ± 0.154** (60%)

* Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.05$)
** Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.01$)

***Lycoperscion esculentum* (tomato):**

Test Substance Concentration (nominal) [mg BIT/kg]	Number of Emerged Seedlings mean ± SD (% reduction) Day 7	Day 14	Day 21	Seedling Survival mean ± SD (% reduction)	Shoot Dry Weight mean ± SD (% reduction)
Pooled controls	8.13 ± 0.99	8.50 ± 0.76	8.50 ± 0.76	8.25 ± 1.04	2.69 ± 0.351
11.1	7.75 ± 0.96 (5%)	8.25 ± 0.50 (3%)	8.25 ± 0.50 (3%)	8.25 ± 0.50 (0%)	2.18 ± 0.209** (19%)
33.3	5.00 ± 2.16** (38%)	7.25 ± 1.50 (15%)	7.50 ± 1.29 (12%)	7.50 ± 1.29 (9%)	1.73 ± 0.307** (36%)
99.8	0.00 ± 0.00** (100%)	3.00 ± 2.16** (65%)	4.50 ± 2.08** (47%)	3.50 ± 2.89** (58%)	0.05 ± 0.044** (98%)
299	0.00 ± 0.00** (100%)	1.75 ± 0.50** (79%)	3.25 ± 0.96** (62%)	2.25 ± 0.96** (73%)	0.01 ± 0.006** (100%)
898	0.00 ± 0.00** (100%)	0.00 ± 0.00** (100%)	0.00 ± 0.00** (100%)	0.00 ± 0.00** (100%)	0.00 ± 0.000** (100%)

* Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.05$)
** Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.01$)

Table A7.5.1.3/01-7: Conclusions

Species	21-Day Emergence (mg BT/kg)				21-Day Survival (mg BT/kg)				21-Day Growth (Dry Weight) (mg BIT/kg)			
Monocots:	NOEC	LOEC	EC_{25}	EC_{50}	NOEC	LOEC	EC_{25}	EC_{50}	NOEC	LOEC	EC_{25}	EC_{50}
Allium cepa (onion)	33.3	99.8	26.9	67.6	33.3	99.8	24.3	55.7	33.3	99.8	25.1	42.7
Avena sativa (oats)	299	898	825	>898	299	898	756	>898	33.3	99.8	98.5	166
Dicots:												
Brassica rapa (turnip)	33.3	99.8	59.7	102	33.3	99.8	45.3	79.3	11.1	33.3	29.3	39.0
Cucumbis sativa (cucumber)	99.8	299	297	585	33.3	99.8	272	294	11.1	33.3	40.9	65.1
Lactuca sativa (lettuce)	33.3	>33.3	>33.3	>33.3	33.3	>33.3	>33.3	>33.3	3.70	11.1	3.70	18.4
Lycopersicon esculentum (tomato)	33.3	99.8	87.8	166	33.3	99.8	53.0	110	<11.1	11.1	28.3	40.0

Table A7.5.1.3/01-8: Validity criteria for terrestrial plant toxicity according to EPA OPPTS 850.4150 (vegetative vigor test)

	Fulfilled	Not fulfilled
Adverse effect > 25 % on one or more plant species (EPA)	yes	

Figure A7.5.1.3/01-1: Day 21 Emergence, Survivors and Biomass in Onion exposed to BIT

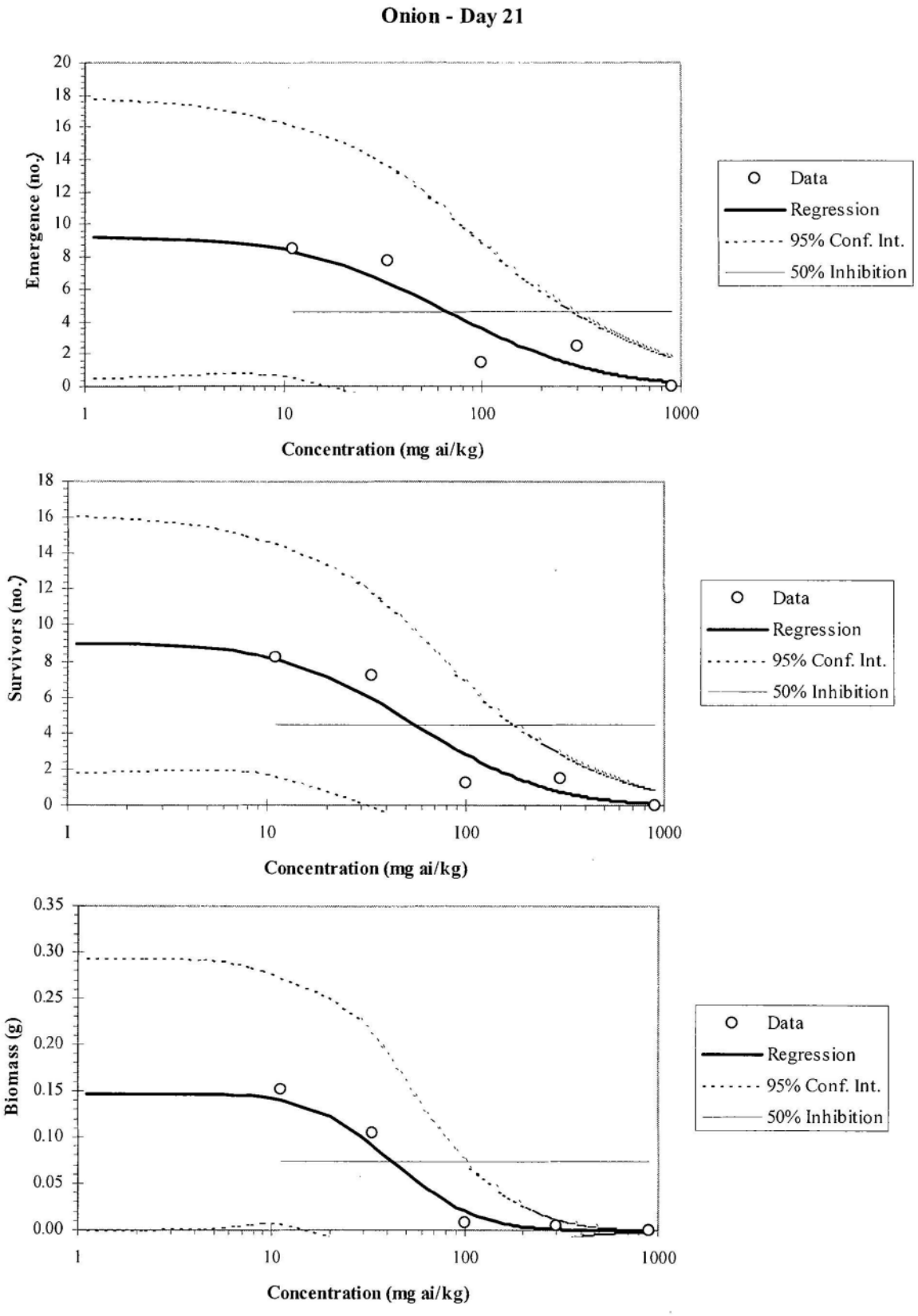


Figure A7.5.1.3/01-2: Day 21 Emergence, Survivors and Biomass in Oats exposed to BIT

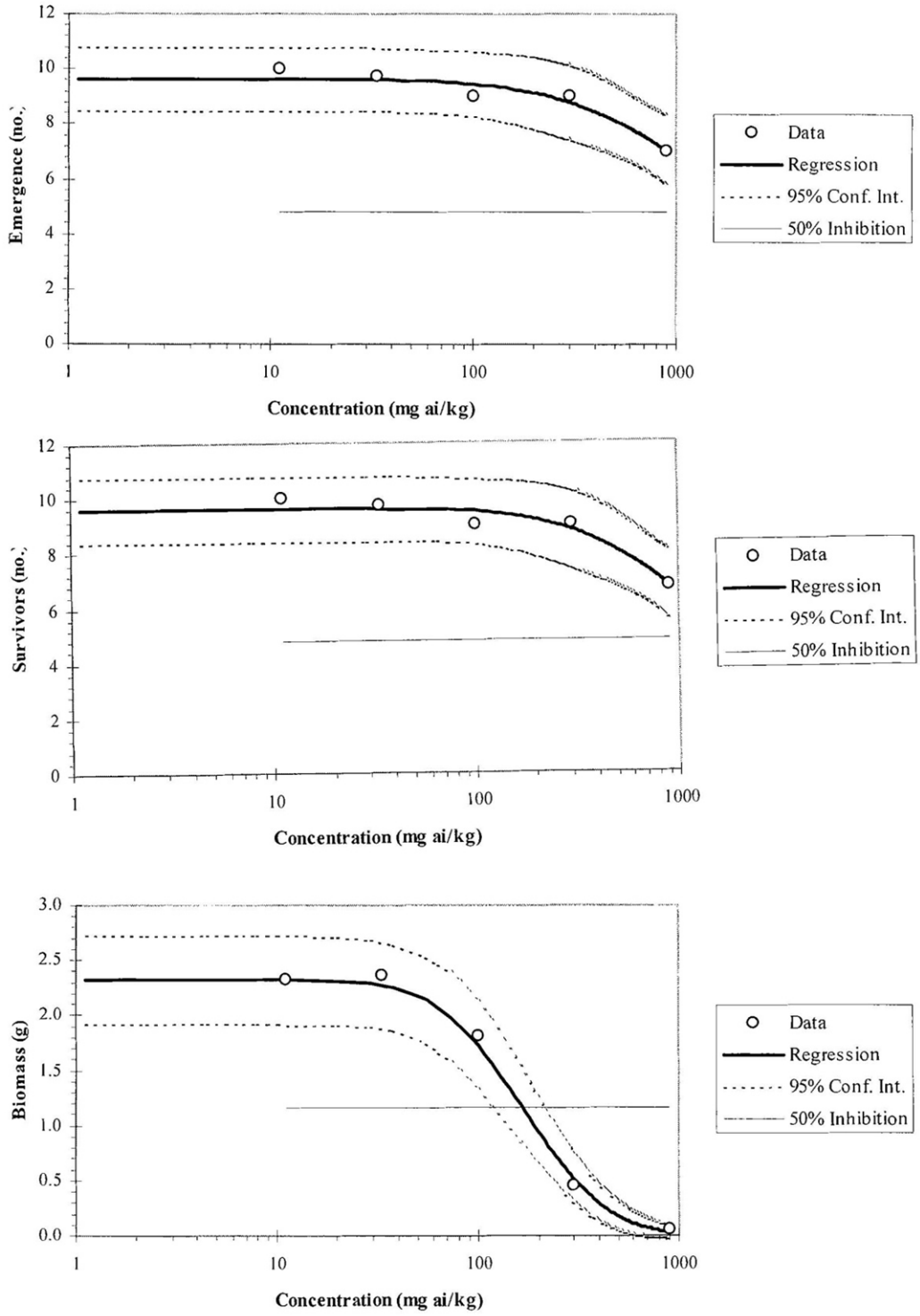


Figure A7.5.1.3/01-3: Day 21 Emergence, Survivors and Biomass in Turnips exposed to BIT

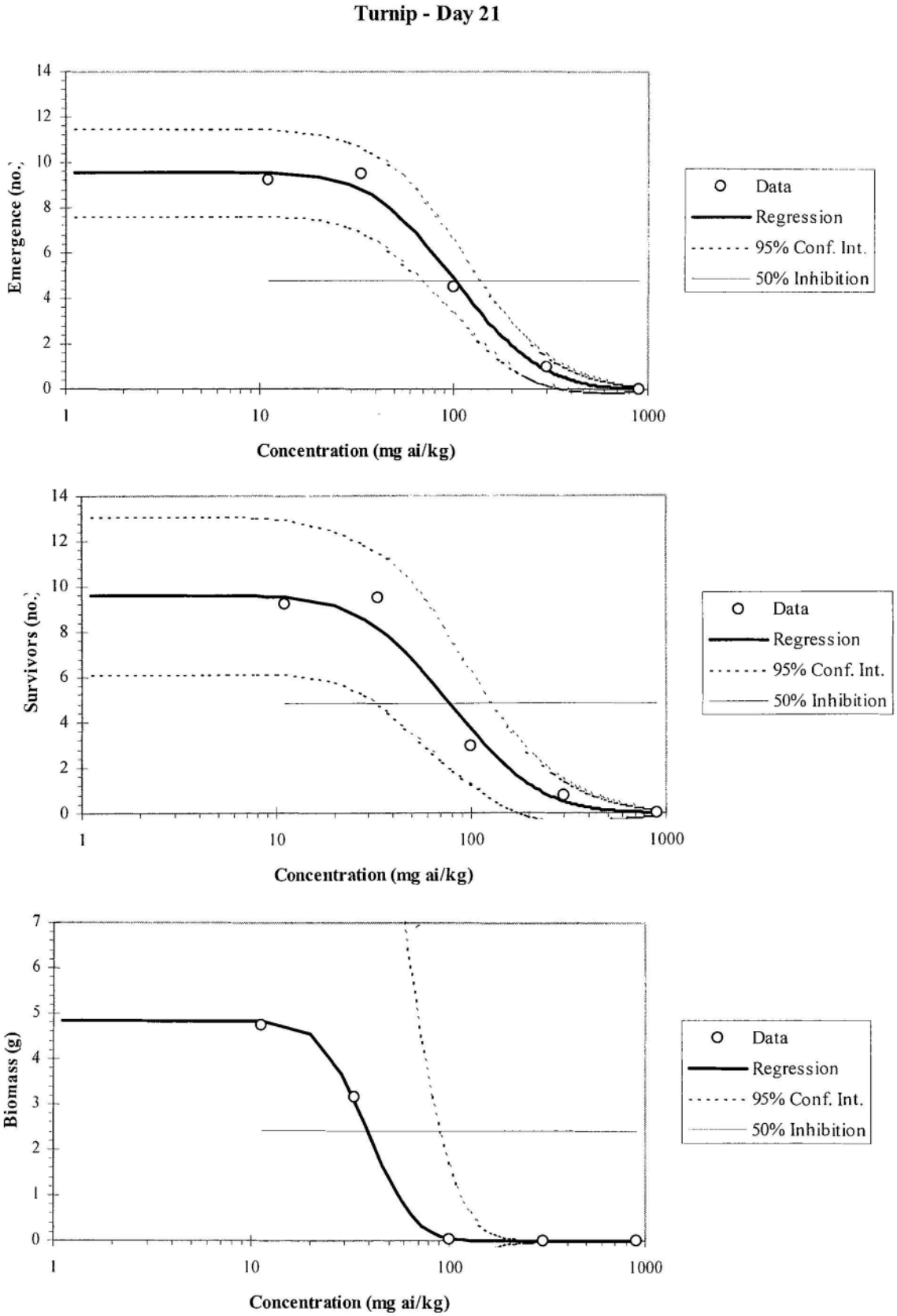


Figure A7.5.1.3/01-4: Day 21 Emergence, Survivors and Biomass in

Cucumber exposed to BIT

Cucumber - Day 21

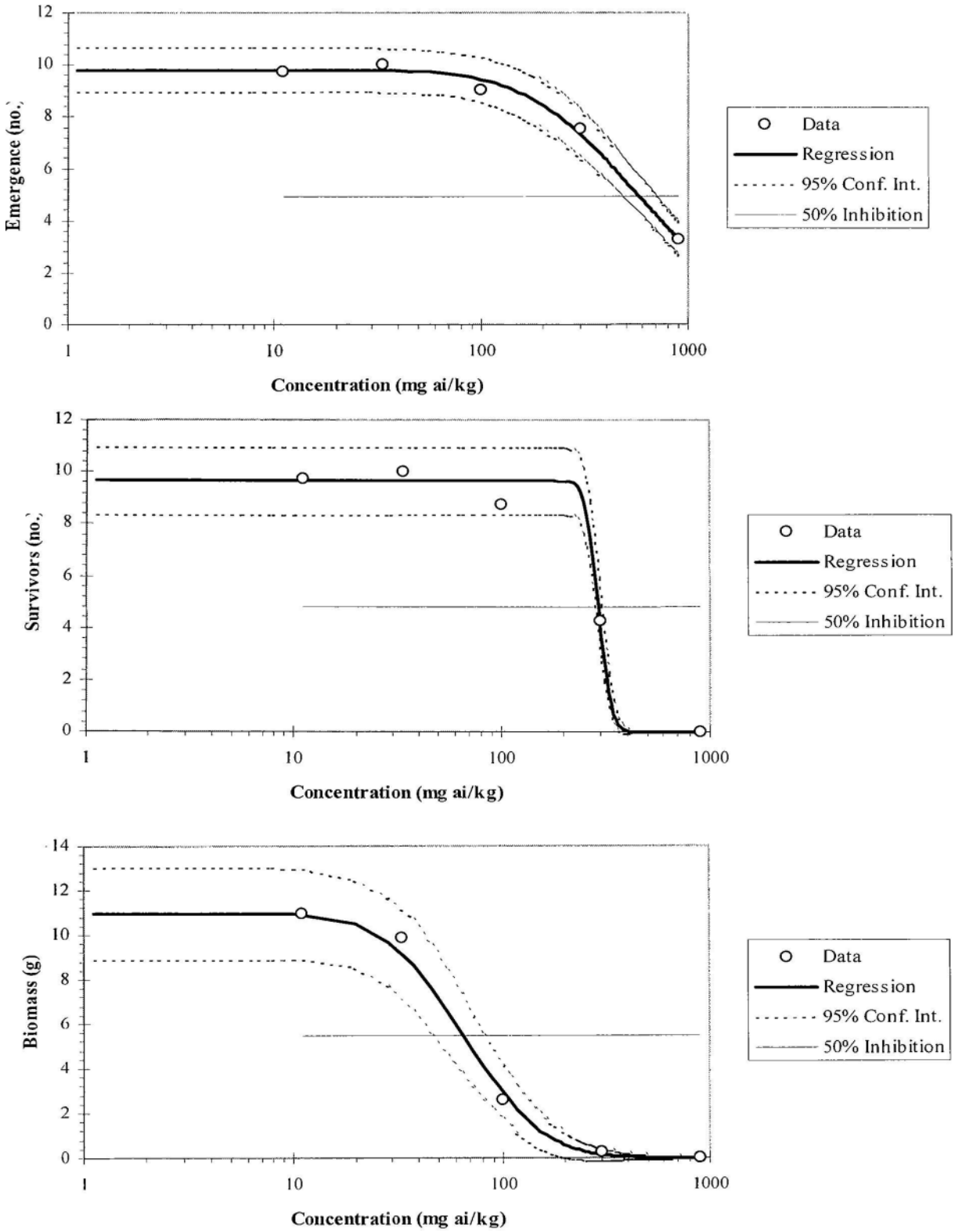


Figure A7.5.1.3/01-5: Day 21 Emergence, Survivors and Biomass in Lettuce (initial trial) exposed to BIT

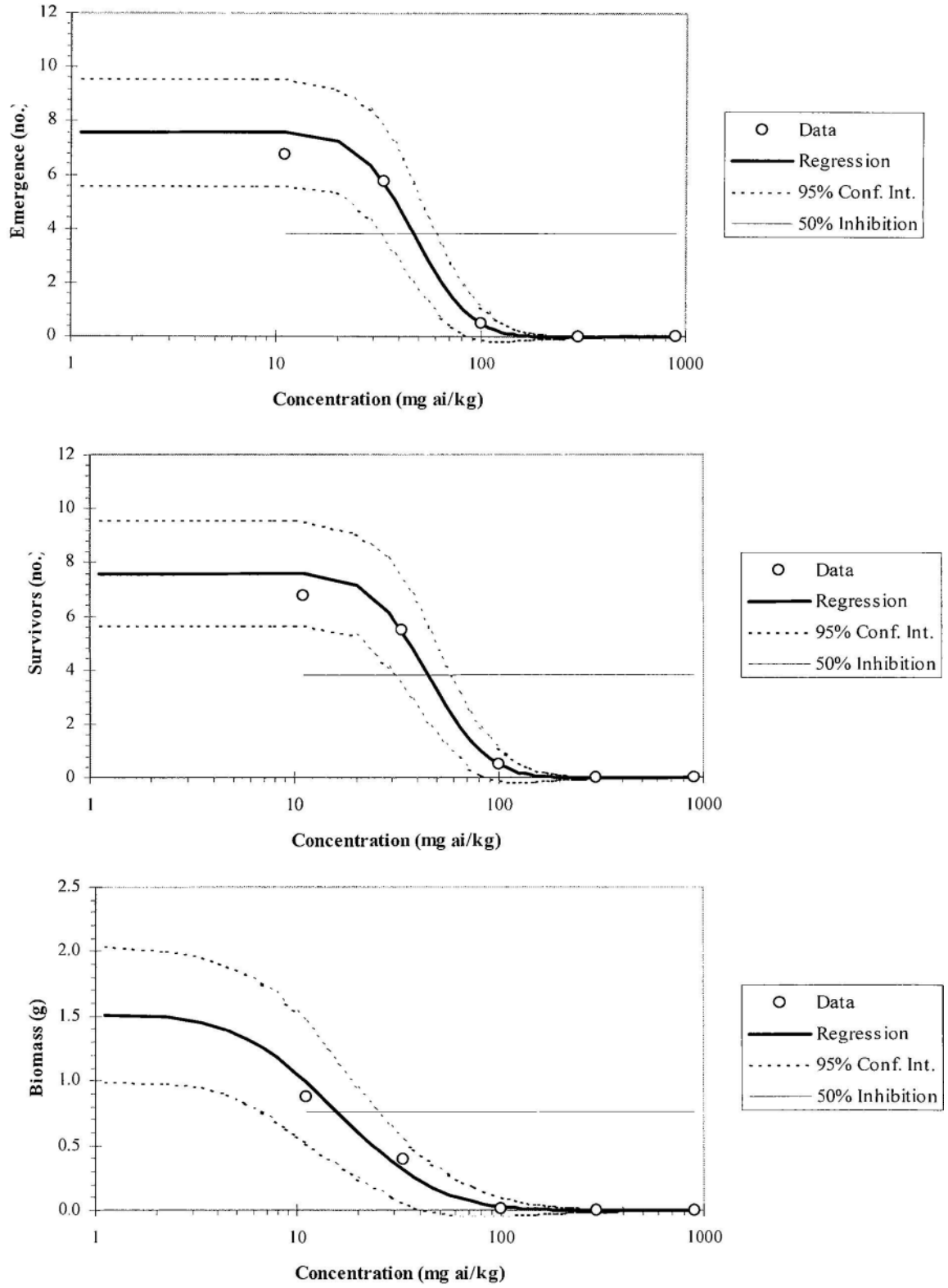


Figure A7.5.1.3/01-6: Day 21 Emergence, Survivors and Biomass in Lettuce (final trial) exposed to BIT

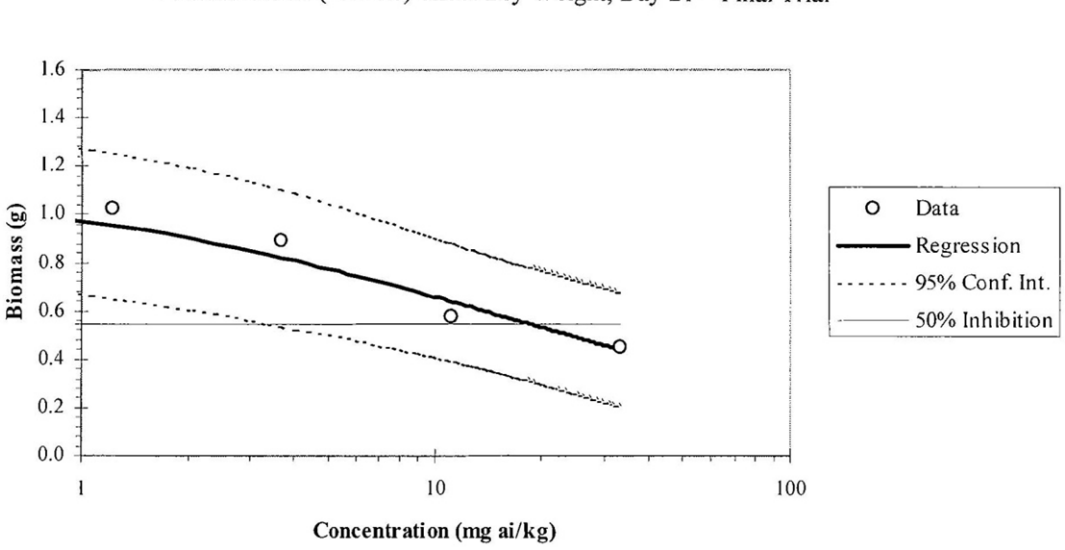
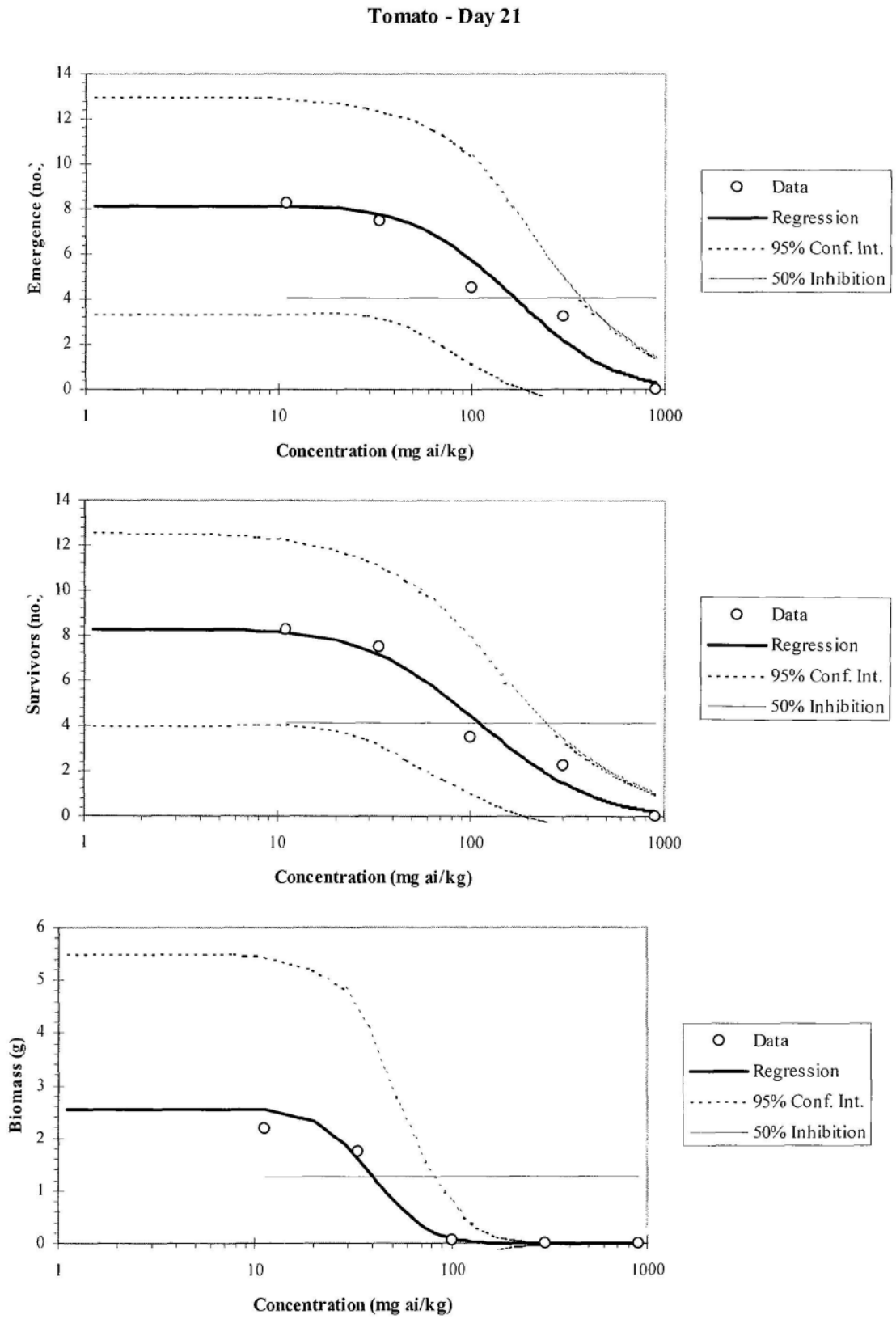


Figure A7.5.1.3/01-7: Day 21 Emergence, Survivors and Biomass in Tomato exposed to BIT



Section A7
Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.2.1
EARTHWORM, CHRONIC TOXICITY TEST

Annex Point IIIA XIII 3.2

		1 REFERENCE	**Official use only**
	1.1 Reference	**A7.5.2/01** [redacted] (2007) 1,2-Benzisothiazolin-3-one: A reproduction study with the earthworm in an artificial soil substrate, [redacted] (January 15, 2007), Unpublished.	
	1.2 Data protection	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	Lanxess Deutschland GmbH	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time in support of the first inclusion into Annex I/IA. Data protection claimed in accordance with the Article 12.1 (c) (ii), as data generated after entry into force of the Directive.	
		2 GUIDELINES AND QUALITY ASSURANCE	
	2.1 Guideline study	Yes, OECD Method 222 and ISO 11268-2	
	2.2 GLP	Yes	
	2.3 Deviations	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis in the diet	High performance liquid chromatography (HPLC)	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.5.2.1 **EARTHWORM, CHRONIC TOXICITY TEST**

Annex Point IIIA XIII 3.2

3.2	**Reference substance**	No	**X**
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	See Table A7.5.1.2/01-1	
3.3.2	Application of the test substance	Test soil was prepared by mixing the appropriate amount of BIT in deionised water with dry artificial soil to which cow manure was added. Additional deionized water was added to the dry artificial soil to achieve a moisture content of approximately 35% by weight. Test soil components were mixed for a total of 20 minutes in order to achieve a homogeneous state. Negative control soil was prepared in the same manner as the treated soil but with only the addition of water.	
3.3.3	Test organisms	See Table A7.5.2.1/01-2	
3.3.4	Test system	see Table A7.5.2.1/01-3	
3.3.5	Test conditions	see Table A7.5.2.1/01-4	
3.3.6	Duration of the test	56 days: adult exposure for 28 days and cocoons / juveniles exposure for 28 days	
3.3.7	Test parameter	Mortality, growth and reproduction	
3.3.8	Examination/ Observation	After 28 days of adult exposure, mortality and growth (percent weight change) were evaluated. After 56 days, the number of juvenile worms was assessed (reproduction).	
3.3.9	Monitoring of test substance concentration	No	
3.3.10	Statistics	Differences between the BIT treatment groups and the control group were evaluated to assess potential effects on body weight and change in body weight using the Dunnett's 2-tailed test (p = 0.05) in SAS version 8.2 (SAS Institute, Inc. 1999. SAS/STAT User's Guide, Version 8.2, Cary, North Carolina, USA). Prior to conducting Dunnett's test, the data were tested for homogeneity of variance and normal distribution. Differences between the mean numbers of juveniles produced in the treatment groups and the control group were determined using Dunnett's 1-tailed test (p = 0.05). The Jonckheere-Terpstra Test for Trend (p = 0.05) was also used to evaluate the numbers of juveniles produced.	
		4 RESULTS	

Section A7 **Ecotoxicological Profile Including Environmental Fate and Behaviour**

Subsection A7.5.2.1 **EARTHWORM, CHRONIC TOXICITY TEST**

Annex Point IIIA XIII 3.2

4.1	**Filter paper test**	Not performed	
4.2	**Soil test**		
4.2.1	Initial concentrations of test substance	0 (control), 1.3, 2.5, 5.0, 10, 20 and 40 mg BIT/kg dry soil.	
4.2.2	Effect data (Mortality)	see Table A7.5.2.1/01-5, there was no treatment-related mortality of adult earthworms	**X**
4.2.3	Concentration / effect curve	None	
4.2.4	Other effects	No effects upon adult earthworm weights. There were no statistically significant effects on numbers of juveniles produced in the 1.3, 2.5, 5.0, 10, 20 and 40 mg BIT/kg dry soil treatment groups, however, the decrease in the mean number of juveniles at the 40 mg BIT/kg level indicated a possible treatment-related effect.	
4.3	**Results of controls**		
4.3.1	Mortality	1.3%	
4.3.2	Number/ percentage of earthworms showing adverse effects	See Table A7.5.2.1/01-6, one earthworm was not found and was presumed dead	
4.3.3	Nature of adverse effects	None	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD Method 222 and ISO 11268-2, Earthworm reproduction test	
5.2	**Results and discussion**	BIT did not affect mortality and adult earthworm weight. BIT produced no effects upon adult earthworm weights. There were no statistically significant effects on numbers of juveniles produced in the 1.3, 2.5, 5.0, 10, 20 and 40 mg BIT/kg dry soil treatment groups, however, the	**X**

Section A7	Ecotoxicological Profile Including Environmental Fate and Behaviour
Subsection A7.5.2.1	EARTHWORM, CHRONIC TOXICITY TEST
Annex Point IIIA XIII 3.2	

		decrease in the mean number of juveniles at the 40 mg BIT/kg level indicated a possible treatment-related effect.	
5.2.1	NOEC	20 mg BIT/kg dry soil	
5.2.2	LC_{10}	> 40 mg BIT/kg dry soil	
5.2.3	EC_{50}	> 40 mg BIT/kg dry soil	
5.2.4	LC_{100}	no concentration caused 100% mortality	
5.3	**Conclusion**	see Table A7.5.2.1/01-7 and see table A7.5.2.1/01-8	
5.3.1	Other Conclusions		
5.3.2	Reliability	(1), reliable without restriction	
5.3.3	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*January 2011*
Materials and Methods	*Applicant's version is accepted with the following remark:* *3.2 A reference toxicity test was conducted with carbendazim in 2005 (as cited in Doc. IV-A). The LC_{50} value for the mortality of the adult earthworms exposed to carbendazim for 28 days was 5 (4-8) mg a.i./kg dry soil. The EC_{50} value for reproduction was calculated to be 1.85 (1.792-1.913) mg a.i./kg dry soil. The NOEC was determined to be 1 mg a.i./kg dry soil, and the LOEC, 2 mg a.i./kg drysoil.*
Results and discussion	*Applicant's version is accepted with the following remark:* *4.2.2 and 5.2 The test concentrations should also included the EC_{50} value.*
Conclusion	*Applicant's version is adopted.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.5.2.1/01-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	deionized water prepared at laboratory
Holding water different from dilution water	Not applicable
Dispersion	BIT was mixed with artificial soil for 20 minutes to assure homogeneity
Vehicle	Not applicable
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	Not applicable

Table A7.5.2.1/01-2: Test organisms

Criteria	Details
Species/strain	Eisenia fetida
Source of the initial stock	[redacted]
Culturing techniques	Earthworms were from synchronous cultures (individuals not differing in age by more than four weeks) maintained in moist peat moss and fed saturated alfalfa and/or cow manure
Age/weight	0.48 to 0.63 grams weight at initiation, worms had well developed clitella
Pre-treatment	Eight days prior to test initiation, earthworms (with clitellum) were selected and placed in a glass container containing bedding. The worms were held under the environmental conditions to be used during testing. Two days prior to the test, the earthworms were removed from the container and divided into five one-liter beakers containing artificial soil substrate adjusted to a moisture content of approximately 35% by weight for the acclimation period. Earthworms were fed cow manure throughout the acclimation period

Table A7.5.2.1/01-3: Test system

Criteria	Details
Artificial soil test substrate	Composition of the artificial soil was 20% kaolin clay, 70% sand, 10% sphagnum peat moss and 35% moisture. One gram of cow manure/100 g soil was added to the mixture.

Test mixture	1.3, 2.5, 5.0, 10, 20, 40 mg BIT/kg dry soil
Size, volume and material of test container	1 L glass beakers
Amount of artificial soil (kg)/ container	750 g prepared artificial soil
Nominal levels of test concentrations	1.3, 2.5, 5.0, 10, 20, 40 mg BIT/kg dry soil
Number of replicates/concentration	4 for BIT groups and 8 for negative control
Number of earthworms/test concentration	40 for BIT groups and 80 for negative control
Number of earthworms/container	10
Light source	fluorescent bulbs
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7.5.2.1/01-4: Test conditions

Criteria	Details
Test temperature	20 ± 2 °C
Moisture content	Day 0 = 33.6 to 34.0 % Day 56 = 34.6 to 36.2 %
pH	Day 0 = 7.0 to 7.2; Day 56: 7.1 to 7.3
Adjustment of pH	Not applicable
Light intensity / photoperiod	400 to 800 lux, 16 h light and 8 h dark
Relevant degradation products	Not applicable

Table A7.5.2.1/01-5: Mortality data

Test Substance Concentration (nominal)[1] [mg BIT/kg artificial soil]	Mortality	
	Number Dead or Missing Day 28	Percentage Day 28
0 (control)	1/80	1.25
1.3	0/40	0
2.5	0/40	0
5.0	0/40	0
10	1/40	2.5
20	0/40	0
40	0/40	0
Temperature [°C]	20 ± 2 °C	
pH	7.1 to 7.3	
Moisture content	34.6 to 36.2 %	

[1] specify, if TS concentrations were nominal or measured

Table A7.5.2.1/01-6: Number affected data

Test Substance Concentration (nominal)[1] [mg BIT/kg artificial soil]	Number Affected		
	Worm weights (grams/replicate) Day 28		Mean Replicate Reproduction Day 56
	Mean change	% change	Mean number of juvenile worms
0 (control)	0.101	10.1	104
1.3	0.095	9.5	98.8
2.5	0.090	9.0	99.8
5	0.080	8.0	108
10	0.111	11.1	100
20	0.070	7.0	102
40	0.093	9.3	87.5
Temperature [°C]	20 ± 2 °C		
pH	7.1 to 7.3		
Moisture content	34.6 to 36.2 %		

[1] specify, if TS concentrations were nominal or measured
* Statistically significant ($p \leq 0.05$) reduction in the number of juvenile worms produced as compared to control.

Table A7.5.2.1/01-7: Effect data

LOEC (number of juveniles)	40 mg BIT/kg dry soil (n)
NOEC (number of juveniles)	20 mg BIT/kg dry soil (n)
EC_{50} (reproduction)	>40 mg BIT/kg dry soil (n)

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.5.2.1/01-8: Validity criteria for acute earthworm test according to OECD 222

	fulfilled	Not fulfilled
Mortality of control animals < 10%	yes	

Section A7 **Subsection A7.5.2.2** **Annex Point IIIA XII.2.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **Biological Sewage Treatment – Anaerobic biodegradation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	For the in can application (PT 6), as well as for Metal working fluid preservatives (PT13), a long term toxicity of BIT to terrestrial plants is not required as the terrestrial compartment is not the major compartment of concern.	
Undertaking of intended data submission [ ]	No further studies planned	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*January 2011.*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version*	
Remarks		

Section A7 **Subsection A7.5.3** **Annex Point IIIA XII.2.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON BIRDS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	For the in can application, an acute or 8-day study on birds is not required because the terrestrial organisms are not target organisms.	
Undertaking of intended data submission [ ]	No further studies planned	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*January 2011.*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version*	
Remarks		

Section A7 **Subsection A7.5.3.1.3** **Annex Point IIIA XII.2.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON BIRDS: EFFECTS ON REPRODUCTION**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	For the in can application or the metalworking fluid preservatives, a reproduction study on birds is not required because the terrestrial organisms are not target organisms.	
Undertaking of intended data submission [ ]	No further studies planned	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Janaury 2011.*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version.*	
Remarks		

Section A7 **Subsection A7.5.4.1** **Annex Point IIIA XII.2.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ACUTE TOXICITY TO HONEYBEES AND OTHER BENEFICIAL ARTHROPODS, FOR EXAMPLE PREDATORS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	Tests on honeybees are not required for the in can applicationor the metalworking fluid preservatives.	
Undertaking of intended data submission [ ]	No further studies planned.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE FI	
Date	*January 2011.*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version*	
Remarks		

Section A7 **Subsection A7.5.5** **Annex Point IIIA XII.2.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **BIOCONCENTRATION, TERRESTRIAL**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	Section A7.5.5.1 Bioconcentration in earthworms: The potential of BIT bioconcentration in earthworms is very low. based on the partition coefficient.	
Undertaking of intended data submission [ ]	No further studies planned	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*January 2011.*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version*	
Remarks		

Section A7 **Subsection A7.5.6** **Annex Point IIIA XII.2.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON OTHER TERRESTRIAL NON-TARGET ORGANISMS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	For the in can application or the metalworking fluid preservatives, further tests are not required as the terrestrial compartment is not the major compartment of concern.	
Undertaking of intended data submission [ ]	No further studies planned	
Evaluation by Competent Authorities		

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*January 2011.*
Evaluation of applicant's justification	*Accept the applicant's version*
Conclusion	*Accept the applicant's version*
Remarks	

Section A7 **Subsection A7.5.7** **Annex Point IIIA 13.3**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON MAMMALS: ACUTE ORAL TOXICITY, SHORT TERM TOXICITY, EFFECTS ON REPRODUCTION**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Officialuse only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	Tests with mammals are summarised in the Toxicological section (Section A6). The summaries are not repeated in the current section, please refer to section A6.	
Undertaking of intended data submission [ ]	-	
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	*January 2011.*	
Evaluation of applicant's justification	*Accept the applicant's version*	
Conclusion	*Accept the applicant's version*	
Remarks		

Section A8 Measures to be adopted to Protect Man, Animals and the Environment

Subsection (Annex point)		Official use only
8.1 (IIA, VIII 8.1)	**Recommended methods and precautions concerning handling, use, storage, transport or fire**	
	Precautions during handling: Avoid dust, keep packing tightly closed and clean. Precautions during storage: Must be marked, palletised and shrink-wrapped for transportation. In case of fire remove product. Store in such a way that the material is prevented from drying out. Packaging for use: HM-HDPE open top drums, with polythene liner. Suitable extinguishing media: Use foam, carbonic acid, powder or water mist. Special protective equipment: **Firefighters should be equipped with breathing apparatus.** Control Limits: Material corrodes with metals such as steel, copper and zinc. Other Information: The compound should not be in contact with oxidising agents. Avoid contact with oxidising materials and acids. Respiratory Protection: Dust respirator P2. Hand Protection: PVC/synthetic (nitrile) rubber gloves. Eye Protection: Always use goggles or face visor etc. Skin Protection: Disposable dress on top of normal working clothes. Always use gloves/and boots made of nitrile rubber. General Protection: Keep workplace clean. Replace drum lids promptly after use, to avoid excess moisture loss to remaining contents. Material must not get too dry.	
8.2	**In case of fire, nature of reaction products, combustion gases, etc.**	

Section A8	Measures to be adopted to Protect Man, Animals and the Environment	
(IIA, VIII 8.2)		
	By fire CO and CO_2 are developed and harmful or poisonous gases like SOX, NOX, NH_3 could be generated.	
8.3 (IIA, VIII 8.3)	**Emergency measures in case of an accident**	
	First Aid Measures: Inhalation: Symptoms are sneezing and coughing. By prolonged inhalation risk of allergy. Remove the affected person to fresh air and seek medical attention. Skin contact: Wash skin immediately with water, using soap if available. Remove contaminated clothing. Seek medical attention if symptoms persist. Risk of sensitisation. Eye contact: Wash immediately with eye wash solution and/or water. Seek medical attention. Ingestion: Immediately rinse mouth; give litre of water or milk to drink. Do not induce vomiting. Seek medical attention.	
8.4 (IIA, VIII 8.4)	**Possibility of destruction or decontamination following release in or on the following:**	
	Do not contaminate any lakes, streams, ponds, groundwater or soil.	
8.4.1 (a) air	No environmental hazards have to be specially mentioned. No special measures are proposed.	
8.4.2 (b) water, including drinking water	The contaminated water may be neutralised (detoxified) by applying alkaline 5% sodium bisulphite solution. Take care to dispose of wash water appropriately.	
8.4.3 (c) soil	The contaminated area may be treated by washing with alkalinesodium bisulphate solution.	
8.5 (IIA, VIII 8.5)	**Procedures for waste management of the active substance for industry or professional users**	
8.5.1 Possibility of re-use or recycling (IIA, VIII 8.5.1)	**No specific information given**	
8.5.2 Possibility of neutralisation of effects (IIA, VIII 8.5.2)	**Collected waste may be neutralised (detoxified) by applying alkaline 5% sodium bisulphite solution.**	
8.5.3 Conditions for controlled discharge	Disposal of product: Sweep up and place in suitable containers for subsequent	

Section A8		Measures to be adopted to Protect Man, Animals and the Environment	
	including leachate qualities on disposal (IIA, VIII 8.5.3)	decontamination. Collected waste may be neutralised (detoxified) by applying alkaline 5% sodium bisulphite solution. The contaminated area may also be treated by washing with alkaline sodium bisulphate solution – take care to dispose of wash water appropriately. Follow relevant local, state, provincial, federal or national laws and regulations. Do not contaminate any lakes, streams, ponds, groundwater or soil. Keep unnecessary people away, isolate hazard area and deny entry. The compound should not be in contact with oxidising agents. Avoid contact with oxidising materials and acids. Disposal of containers: Treat polythene liners containing residues of product as waste preferably for incineration. The drums may re-cycled after first rinsing with alkaline 5% sodium bisulphite solution and then water.	
8.5.4	Conditions for controlled incineration (IIA, VIII 8.5.4)	**No specific information given**	
8.6 (IIA, VIII 8.6)		**Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms**	
		No specific information given	

Evaluation by Competent Authorities	
EVALUATION BY RAPPORTEURMEMBERSTATE	
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	*Accepted.*
Remarks	

Section A9 Classificationand Labelling

Annex Point IIA, IX

	CLASSIFICATION AND LABELLING	Official use only
Classification	Xn; R22 – harmful if swallowed, Xi; R38-41 – irritant to skin, risk of serious damage to eyes R43 - may cause sensitization by skin contact N; R50 – very toxic to aquatic organisms	
Symbols		
R phrases	R22, R38, R41, R43, R50	
S phrases	S2, S24, S26, S37/39, S61	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEURMEMBERSTATE
Date	*May 2020*
Materials and Methods	
Results and discussion	
Conclusion	*See updated classification according to Regulation (EC) No 1272/2008 below*
Reliability	
Acceptability	
Remarks	

Classification	GHS07; H302 – Harmful if swallowed. GHS06; H330 – Fatal if inhaled. GHS05; H318 – Causes serious eye damage. GHS07; H317 - May cause an allergic skin reaction. GHS09; H400, H410 – Very toxic to aquatic life.
Symbols	
H phrases	H302, H330, H318, H317, H400, H410

P phrases	P102, P262, P305+P351+P338, P280, P273+P502